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MINUTES OF THE 44th GENERAL ASSEMBLY OF THE EUROPEAN ASSOCIATION FOR THE STUDY OF DIABETES

held in Rome, Italy on 10 September 2008 at 18:15

Present:	**E. Ferrannini**	**(President)**
	G. Spinas	**(Honorary Treasurer)**
	M. Stumvoll	**(Honorary Secretary)**
	E. Gale	**(Editor-in-Chief, Diabetologia)**
	V. Jörgens	**(Executive Director)**
	and 42 members	

The President welcomed everyone to the 44th General Assembly.

1. MINUTES 43rd GENERAL ASSEMBLY 2007

Since there were no comments, the minutes were approved unanimously and officially signed as a correct record.

2. REPORTS

a) President

The President reported on the joint project with EPA, ESC and EASD to prepare a statement concerning cardiovascular risk in diabetes in patients with severe mental illness.
The President confirmed that the Foundation was continuing to flourish. At present, 16 EFSD programmes are in place and meetings have been scheduled in Rome with potential new partners.

b) Honorary Treasurer

The Honorary Treasurer reported that the positive balance was mainly due to an increase in the number of registered delegates and an increase in the interest accrued. The expenditure in 2007 increased due to the increase in the amount spent on the salaries and the expenditure for the Annual Meeting. It had been decided to transfer 1.7 million to the Foundation. Dr. Spinas said that of the Foundation's total income more than 90% was spent on research.

Dr. Spinas expressed his thanks to Drs Grüßer and Jörgens for their support and advice and to the team in Düsseldorf in general and to Mrs Klee and Ms Deparade in particular for the precise handling of the accounts.

The President thanked the Honorary Treasurer for his diligence and asked if there were any questions. There were none.

c) Honorary Auditors

The President asked the Honorary Auditor, Dr. Tiedge, to formally discharge the accounts. He confirmed that the accounts had been checked carefully and were in perfect order. Dr. Ferrannini asked for the vote to accept the accounts.

The accounts were accepted unanimously (one abstention).

d) Honorary Secretary

Dr. Stumvoll reported that he had restructured some of the groups and the committee; there are now 40 reviewers to score 8 topics. Of the 2498 abstracts received,

1402 had been accepted. This number was then divided into 264 orals and 1138 posters. He said the Programme Committee was already in place for the Annual Meeting in Vienna and it would convene later that day.

Dr. Stumvoll closed his report by thanking all members of the EASD staff, in particular Mrs H. Goliberzuch and M. Toledo, for their outstanding help and support with the organisation of the EASD Annual Meetings.

Dr. Ferrannini thanked Dr. Stumvoll for his diligence and asked if there were any questions. There were none.

e) Editor-in-Chief, Diabetologia

Dr. Gale reported that a total of 1769 articles had been submitted to Diabetologia in 2007, which was a slight increase over 2008. The median time from receipt to first decision was 17 days. The acceptance rate for all article types combined fell to 17.4%. The impact factor rose to 5.8.

He expressed his thanks to the referees and associates and said their work was much appreciated. He also thanked his team in Bristol.

Dr. Ferrannini thanked Dr. Gale for the excellent work he had done. He said a sub-committee had been appointed by the Executive Committee to evaluate potential candidates for the position of Editor-in-Chief. There were no questions.

f) Chair, Postgraduate Education Sub-committee

Dr. Nolan reported that the usual postgraduate courses had taken place in Jena, Loipersdorf and Wroclaw. The course planned for Belgrade has been postponed until 2009. A course is being planned by Dr. Mankovsky in Ukraine.

Regarding web-based education, 2 pilot sessions on standard topics were held in Düsseldorf in 2008. Further web-casts are planned including hypertension and diabetes, diabetes and the heart and type 2 in adolescents and children.

Dr. Nolan thanked the team in Düsseldorf, especially Mrs. M. Hata and Mr. B. Carey, for their friendly assistance.

Dr. Ferrannini thanked Dr. Nolan for his valuable work. There were no questions.

3. ELECTIONS

a) President 2008-2011

The council's election of Dr. U. Smith was unanimously approved with one abstention.

b) Vice President 2008-2011

The Council's election of Dr. A.J.M. Boulton was unanimously approved with one abstention.

c) Editor-in-Chief extension 2009-2010

The extension of the Editor-in-Chief's term of office until 2010 was unanimously approved with one abstention.

d) Honorary Auditors 2008-2011

The Council's election of Drs. K. Paterson (UK) and C. Tack (The Netherlands) was approved with 1 abstention.

c) Council Members 2009-2012

The following Council Members were unanimously (1 abstention) elected by the General Assembly:
J.-M. Boavida (P), C.-G. Östenson (S), A. Pfeiffer (D) and N. Wareham (UK).

4. STUDY GROUPS

Dr. Stumvoll said that the Study Group Forum which had been held on 7 September had been well attended and no problems had arisen.

5. HONORARY MEMBERSHIP

Drs. J.-P. Assal and R. Unger were unanimously elected by the General Assembly.

6. MEMBERSHIP FEE

The General Assembly unanimously approved the proposal that applications for membership of EASD starting on 1 July would run for 1½ years.

7. ANY OTHER BUSINESS

There was no other business.

Dr. Ferrannini thanked the out-going Vice President, Dr. U. Smith, for his dedication and presented him with the Albert Renold Medal. Dr. Smith expressed his thanks for the confidence that had been shown in him when he was elected President.

Dr. Smith warmly thanked Dr. Ferrannini for his outstanding service to the EASD as President and handed over the Albert Renold medal.

Dr. Ferrannini also thanked the outgoing members of the Council:
Drs. K. Dahl Jørgensen, B. Mankovsky and S. Sasson.

Dr. Ferrannini thanked the industry for their support. He also expressed his sincere gratitude to the Local Organising Committee for their outstanding contribution to the organisation of the 44th EASD Annual Meeting. He again thanked Dr. Stumvoll and the members of the Programme Committee for their hard work with regard to the scientific programme. The President warmly thanked the EASD team in Düsseldorf for their kind and efficient help.

Dr. Ferrannini brought the General Assembly to a close at 18:35.

Agenda for the 45th General Assembly of the European Association for the Study of Diabetes

to be held in the Basch Hall at the Messe Wien, Vienna, Austria on 01 October 2009 at 18:15

1. Minutes of the 44th General Assembly, Rome, Italy 2008

2. Reports

a) President	Dr. U. Smith
b) Honorary Treasurer	Dr. G. Spinas
c) Honorary Auditors	Dr. K. Paterson Dr. C. Tack
d) Honorary Secretary	Dr. M. Stumvoll
e) Editor in Chief, Diabetologia	Dr. E. Gale
f) Chair, Postgraduate Education Sub-committee	Dr. J. Nolan

3. Elections

a) Vice President 2009-2012	in place of Dr. C. Boitard
b) Editor-in-Chief 2010-2013	in place of Dr. E. Gale
c) Honorary Treasurer Extension 2009-2010	Dr. G. Spinas
d) Chair, PGESC Extension 2009-2010	Dr. J. Nolan
e) Council Members 2010-2013	in place of Dr. F. Ashcroft (UK) Dr. F. Bosch (E) Dr. V. Petrenko (LT) Dr. A. Siebenhofer (A)

4. Study Groups

5. Honorary Membership

6. Any other business

45th EASD Annual Meeting of the European Association for the Study of Diabetes

Vienna, Austria, 30 September – 2 October 2009

Abstracts

Index of Oral Presentations

Index of Poster Sessions

OP 1 GLP-1 in the treatment of type 2 diabetes mellitus

1

One-year exenatide treatment improves beta cell response in metformin treated patients with type 2 diabetes which is sustained after 5 weeks discontinuation of treatment

M.C. Bunck[1], A. Mari[2], A. Corner[3], B. Eliasson[4], R.M. Shaginian[5], Y. Wu[6], P. Yan[6], R.J. Heine[1,7], U. Smith[4], M.-R. Taskinen[3], H. Yki-Jarvinen[3], M. Diamant[1];
[1]Department of Endocrinology / Diabetes Center, VU University Medical Center, Amsterdam, Netherlands, [2]Institute of Biomedical Engineering, Padua, Italy, [3]Department of Medicine, Helsinki University Central Hospital, Finland, [4]Lundberg Laboratory for Diabetes Research, Sahlgrenska University Hospital, Goteborg, Sweden, [5]Eli Lilly, Houten, Netherlands, [6]Amylin Pharmaceuticals, Inc., San Diego, United States, [7]Eli Lilly, Indianapolis, United States.

Background and aims: Recently we reported exenatide (EX) improved hyperglycaemic clamp derived measures of ß-cell function, compared to insulin glargine (IG) in metformin (MET) treated patients with type 2 diabetes. Here we report the effect of EX treatment on the ß-cell response to a more physiological stimulus, i.e. a mixed meal.
Material and methods: In this investigator-designed study, we compared the effects of both EX and IG treatment on prandial ß-cell function during an 8 hour, high fat, double mixed meal test. Sixty-nine MET treated patients with T2D (pre-treatment (PT) age 58±8y; HbA1c 7.5±0.8%; BMI 31±4kg/m2 [mean±SD]) were randomised to EX (n=36) or IG (n=33). Meal tests were performed at PT, after 51-weeks on-treatment and again after 5-weeks off-treatment on 59 patients (EX: n=30; IG: n=29) who received a breakfast at t=0h and lunch at t=4h both containing approximately 50g fat, 75g carbohydrates, and 35g protein.C-peptide deconvolution was used to calculate insulin secretion rates (ISR), and a mathematical model to obtain the dose-response relating ISR to glucose concentrations (bGS=glucose sensitivity=dose-response slope; ISR@Gref=ISR at reference glucose level [Gref]) and a time-dependent potentiation factor expressing a relative potentiation of the dose-response relation during the meal (POT; [time]-to-basal potentiation ratio).
Results: After 1-year treatment, EX resulted in a greater upward shift in ISR-gref than IG (between group geometric LS mean ratio±SE: 1.41±0.17, P=0.007 (ISR@7.0mM); 1.32±0.16, P=0.025 (ISR@7.5mM) and 1.26±0.15, P=0.060 (ISR@8.0mM)). Additionally, EX treatment increased POT after both breakfast and lunch meals compared to IG (between group geometric LS mean ratio±SE: 1.22±0.11; P=0.026 and 1.19±0.11; P=0.054, for [170-190min]-to-basal and [240-480min]-to-basal respectively). Both Ex and IG treatment equally improved bGS (median[IQR] change from PT: EX: 7.9[2.2-27.5], P<0.001; IG: 12.8[5.6-29.3], P<0.001, between group comparison P=0.544). After the 5-week off-drug period, ISRgref remained improved in both the EX and IG treated patients without a significant difference between the two treatment groups (geometric LS mean±SE ratio to PT for EX and IG respectively: 1.52±0.18, P=0.003 and 1.47±0.19, P=0.106 (ISR@7.0mM); 1.45±0.15, P=0.004 and 1.46±0.16, P=0.053 (ISR@7.5mM); 1.41±0.14, P=0.007 and 1.45±0.15, P=0.039 (ISR@8.0mM)). All other measures returned to PT values.
Conclusion: In this study, 1-year of EX therapy, compared to IG, significantly improved both the prandial ISR over a range of glucose concentrations and the potentiation of the ISR to glucose dose-response relation. The beneficial effects on the dose-response relation were sustained after a 5-week off-treatment period in both the EX and IG treated groups.
Supported by: Amylin Pharmaceuticals, Inc., Eli Lilly

2

A switch from twice-daily exenatide to once-daily liraglutide further improves glycaemic control in patients with type 2 diabetes on oral agents

J. Buse[1], G. Sesti[2], W.E. Schmidt[3], E. Montanya[4], Y. Xu[5], C. Chang[5], L. Blonde[6], J. Rosenstock[7];
[1]Endocrinology, University of North Carolina School of Medicine, Chapel Hill, United States, [2]Magna Graecia University of Catanzaro, Italy, [3]St Josef-Hospital, Ruhr University Medical Facility, Bochum, Germany, [4]Hospital Unversitari Bellivtge-IBIDELL, University of Barcelona, Spain, [5]Novo Nordisk Inc, Princeton, United States, [6]Ochsner Medical Center, New Orleans, United States, [7]Dallas Diabetes and Endocrine Center, Dallas, United States.

Background and aims: Liraglutide is a once-daily human glucagon-like peptide-1 (GLP-1) analogue in development for the treatment of type 2 diabetes. A 26-week randomised trial (LEAD-6) showed that, on a background of metformin and/or sulphonylurea (SU), liraglutide was more effective than exenatide in improving glycosylated haemoglobin (HbA_{1c}) and homeostasis model assessment index of beta-cell function (HOMA-B), with less hypoglycaemia. This extension compared the effect of switching from exenatide to liraglutide treatment versus continued liraglutide treatment.
Materials and methods: In total, 389 patients completing LEAD 6 entered the 14-week non-randomised extension phase (97% of these completed the extension). Patients switched from exenatide 10 µg twice daily to liraglutide 1.8 mg once daily (OD) (following 0.6 mg OD for 1 week and 1.2 mg OD for 1 week), or continued liraglutide 1.8 mg OD.
Results: Patients switching from exenatide to liraglutide further improved glycaemic control from week 26: HbA_{1c} decreased by 0.32% (p<0.0001), 57% of patients reached HbA_{1c} target <7.0% by week 40, and fasting plasma glucose (FPG) significantly decreased by 0.9 mmol/L (p<0.0001). Significant improvements in beta-cell function were seen, with HOMA-B increasing by 14.5% (p≤0.001). Also, significant additional reductions in body weight (BW) and systolic blood pressure (SBP) occurred in the exenatide-to-liraglutide group, as well as the liraglutide-to-liraglutide group (Table). Furthermore, switching from exenatide to liraglutide was characterised by a decreased rate of minor hypoglycaemia (2.6 vs 1.3 events/patient/year for Wk 0-26 and Wk 26-40, respectively). Patients continuing liraglutide treatment reported 0.7 events/patient/year during the extension phase (vs 1.9 events/patient/year; Wk 0-26). Lower rates of nausea were observed during the extension when compared to the main trial. The proportion of subjects reporting nausea reduced from 28% and 25.5% for the exenatide-to-liraglutide group and the liraglutide-to-liraglutide group, respectively, during the main trial to 3.2% and 1.5%, respectively, during the extension phase. No pancreatitis occurred.
Conclusion: Switching from exenatide to liraglutide is well tolerated and provides additional benefits in overall glycaemic control, and beta-cell function

	Liraglutide	Exenatide	*P*-value	Liraglutide to liraglutide	*P*-value	Exenatide to liraglutide	*P*-value
	Wk 0-26 (n=233)	Wk 0-26 (n=231)	Week 26	Wk 26-40 (n=200)	Wk 26-40	Wk 26-40 (n=186)	Wk 26-40
Diabetes duration, yrs±SE	8.5±0.4	7.9±0.4.9	NA	8.3±0.4	NA	7.7±0.4	NA
Baseline weight, kg±SE	93.1±1.3	93.0±1.3	NA	91.1±1.4	NA	91.0±1.3	NA
Baseline HbA_{1c}, %±SE	8.2±0.1	8.1±0.1	NA	7.0±0.1	NA	7.2±0.1	NA
Change in HbA_{1c}, %±SE	-1.1±0.1	-0.8±0.1	***	-0.1±0.04	NS	-0.3±0.04	***
% with HbA_{1c}<7.0%	54[#]	43[#]	*	61[†]	NA	58[†]	NA
Change in FPG, mmol/L±SE	-1.6±0.2	-0.6±0.2	***	-0.2±0.1	NS	-0.9±0.2	***
Change in weight, kg±SE	-3.2±0.3	-2.9±0.3	NS	-0.4±0.2	*	-0.9±0.2	***
Change in HOMA-B, %±SE	32.1±6.8	2.7±6.8	***	-1.8±4.8	NS	14.5±4.4	***
Change in SBP, mmHg±SE	-2.5±1.2	-2.0±1.2	NS	-2.2±0.9	*	-3.8±0.8	***

Wk 0-26=ITT population in randomised trial. Wk 26-40=ITT population in non-randomised extension. Wk 26-40 comparison=Baseline was Wk 26 (LOCF) value. NS, non-significant; NA, not applicable (statistical significance not determined); [#]data are from week 26; [†]data are from week 40. *p<0.05; **p≤0.001; ***p<0.0001.

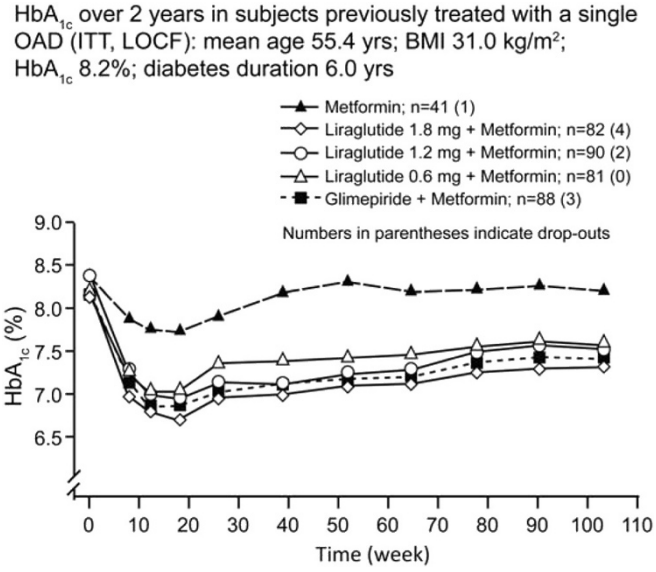
while also reducing BW and SBP. Maintaining liraglutide treatment achieves durable glycaemic control, weight loss and SBP reductions with minimal levels of hypoglycaemia and nausea.
Supported by: Novo Nordisk

3

Long-term sustained glycaemic control with liraglutide and glimepiride (both plus metformin), with added benefits of weight loss and less hypoglycaemia with liraglutide: 2-year LEAD-2 data

A. Frid[1], K. Hermansen[2], M. Nauck[3], N. Shah[4], T. Tankova[5], I. Mitha[6], M. Zdravkovic[7], D. Matthews[8];
[1]Öresund Diabetes Team AB, Lund, Sweden, [2]University Hospital, Aarhus, Denmark, [3]Diabeteszentrum, Bad Lauterberg im Harz, Germany, [4]KEM Hospital, Mumbai, India, [5]Medical University, Sofia, Bulgaria, [6]BenMed Hospital, Benoni, South Africa, [7]Novo Nordisk, Bagsvaerd, Denmark, [8]OCDEM, Oxford, United Kingdom.

Background and aims: Liraglutide is a once-daily (OD) human GLP-1 analogue. In 1091 patients with T2D, 6 months liraglutide compared with glimepiride (both combined with metformin; met) led to similar glycaemic control, but added benefits of lower weight, reduced hypoglycaemia and improved SBP with liraglutide. Here we report data from the planned 1.5-year extension.
Materials and methods: Subjects were randomised (2:2:2:1:2) to liraglutide OD (0.6, 1.2 or 1.8mg) +met, met alone [met], or met+glimepiride (4mg OD) [glim]. Met dose was 1.5-2g/day. First 6 months double-blind, double-dummy; then open-label.
Results: 89% (780 subjects) of 26-wk completers entered the extension; 529 (68%) of them completed 2 yr. Prior to trial entry, subjects were previously treated with 1 or 2 OADs. HbA_{1c} in subjects using 1 OAD before trial entry (35% of patients, baseline HbA_{1c} 8.2%) was improved and sustained for 2 yr. For these patients (ITT, LOCF) after 2 yr, HbA_{1c} was 7.38%, 7.44%, 7.74% for liraglutide 1.8, 1.2, 0.6mg, 7.49% for glim and 8.12% for met alone, respectively ($p>0.05$ for liraglutide 1.8mg vs glim) (Fig). In the total population (ITT, LOCF), the proportion reaching $HbA_{1c}<7.0\%$ after 2 yr was 31.1%, 29.9%, 19.7% for liraglutide 1.8, 1.2, 0.6mg, 23.5% for glim and 10.8% for met alone ($p<0.0001$ liraglutide 1.8mg vs glim). Providing comparable HbA_{1c} improvement to glim but bringing more subjects to target, liraglutide led to significant and sustained weight loss (1.8mg: -2.91kg; 1.2mg: -3.03kg; 0.6mg; -2.07kg) whereas glim resulted in weight gain (0.7kg, $p<0.0001$ vs all liraglutide doses). Estimated mean waist circumference was significantly reduced by 1.8-2.8cm from baseline to 2 yr with liraglutide vs an increase with glim (+0.2cm, $p\leq0.0001$). With liraglutide, hypoglycaemia incidence was similar to met alone and 10-fold less than glim (events/subj yr: 0.15 [each liraglutide dose]; 0.16 [met]; 1.60 [glim], $p<0.0001$ vs all liraglutide doses). Odds-ratio for achieving composite endpoint of $HbA_{1c}<7.0\%$+no weight gain+no hypoglycaemia for liraglutide 1.8mg was 4.9 vs glim, 3.8 vs met alone ($p<0.001$; $p=0.0002$). Although not significant between groups, visceral adipose tissue area was reduced by mean of 18 and 13cm^2 with liraglutide 1.2 and 1.8mg, respectively and increased by 9 and 2cm^2 with met alone and glim, measured by single-slice abdominal CT scan.
Conclusion: Liraglutide OD in combination with met for 2 yr brought comparable improvement in HbA_{1c} as glim in combination with met. However, hypoglycaemia with liraglutide was only a tenth of that with glim and sustained ~3kg weight loss was obtained during the 2 yr in contrast to a small weight gain with glim.



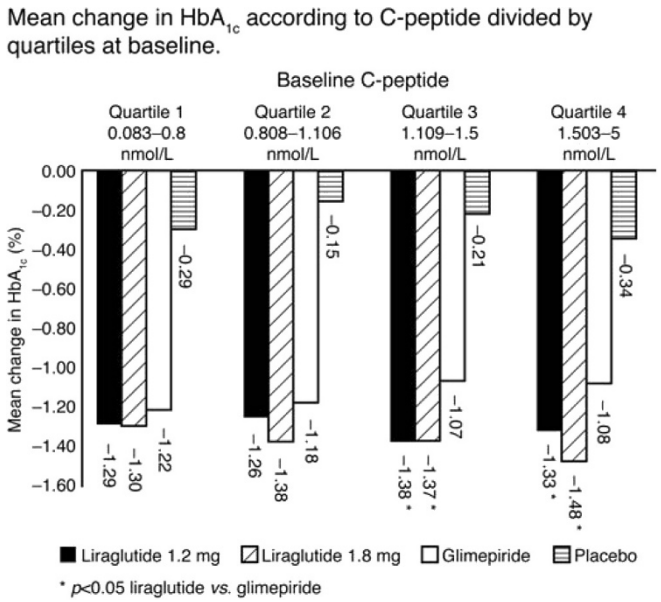
Supported by: Novo Nordisk

4

Liraglutide is associated with a significantly greater improvement in glycaemic control than glimepiride in patients with highest baseline beta cell function

D.R. Matthews[1], A. Falahati[2], A.D. Toft[2], J. Meier[3];
[1]NIHR Oxford Bomedical Research Centre, OCDEM, The Churchill Hospital, Oxford, United Kingdom, [2]Novo Nordisk, Bagsvaerd, Denmark, [3]Ruhr University, Bochum, Germany.

Background and aims: Treatments for T2D with beneficial effects on beta-cell function include glucagon-like peptide (GLP)-1 agonists and sulphonylureas (SUs). Liraglutide, a once-daily human GLP-1 analogue, promotes glucose-dependent insulin release and preserves beta-cell function as well as being associated with weight loss and less risk of hypoglycaemia than SUs. This report compares the efficacy (HbA_{1c}) of liraglutide (1.2 mg or 1.8 mg/day) and glimepiride in patients with T2D according to baseline beta-cell function.
Materials and methods: A meta-analysis was performed across six randomized controlled 26-week trials (3967 patients). An ANCOVA model adjusted by quartile of beta-cell function at baseline (as defined by HOMA-B and C-peptide) assessed the impact of beta cell functional capacity on response to treatment modality.
Results: After 26 weeks' treatment, liraglutide reduced HbA_{1c} from baseline significantly more than glimepiride ($p<0.01$), with reductions of 1.4%, 1.3% and 1.1% for 1.8 mg, 1.2 mg and glimepiride, respectively. Liraglutide reduced HbA_{1c} significantly across all quartiles of HOMA-B and C-peptide (≥1.3%). Reductions of HbA_{1c} with liraglutide were significantly greater than those with glimepiride in 2nd quartile (0.23% [SE=0.12], 0.28% [0.11] for 1.2 mg and 1.8 mg vs glimepiride) and 4th quartile (0.42% [0.11], 0.40% [0.11] for 1.2 mg and 1.8 mg vs glimepiride) from baseline HOMA-B ($p<0.05$), and in 3rd and 4th quartiles of baseline C-peptide (Figure). The greatest improvements in HbA_{1c} occurred in patients with the greatest baseline beta-cell function (Q4) taking liraglutide, whereas no effect of baseline quartile could be seen with glimepiride (Figure).
Conclusion: Liraglutide was effective across the continuum of beta-cell activity. Since treatment with liraglutide was even more effective in subjects with the best beta-cell function at baseline, this might imply enhanced clinical benefits when liraglutide is initiated early.



Supported by: Novo Nordisk

5

The DPP-4-inhibitor sitagliptin improves glucose tolerance in type 2 diabetes mellitus involving GLP-1 dependent and GLP-1 independent pathways

G. Kutscherauer, A. Bedorf, M. Nicolaus, B. Göke, J. Schirra;
Department of Internal Medicine II, Campus Großhadern, University of Munich, Germany.

Background and aims: Inhibitors of the enzym Dipeptidyl-peptidase-4 (DPP-4) are currently used for treatment of type 2 diabetes mellitus (T2DM). DPP-4-inhibitors prevent the degradation of the insulinotropic gut hormones GLP-1 and GIP, thereby enhancing plasma levels of the bioactive peptides. Both, GLP-1 and GIP are postprandially (PP) released and stimulate insulin secretion via specific receptors on the B-cell. However, the importance of the incretin hormones for the efficacy of DPP-4-inhibitors has never been demonstrated. Moreover, because several studies indicated a failure of synthetic GIP to stimulate insulin secretion in T2DM, a defect of the GIP action in T2DM has been suggested. To evaluate the contribution of endogenous GLP-1, we examined the effect of the specific DPP-4 inhibitor Sitagliptin on glucose tolerance and insulin excursions after an oral glucose tolerance test (OGTT) in patients with T2DM with and without a GLP-1 receptor blockade using exendin(9-39) (Ex-9).
Materials and methods: Double-blind, placebo-controlled, four-arm-cross-over study in 24 patients with T2DM (61±7 y, HbA1c 6.2±0.2, BMI 27.7±0.9). On four days separated by a wash-out period of at least one week blood glucose and plasma insulin concentrations were measured during the four hours after ingestion of an OGTT (75g). In a randomized cross-over fashion, oral treatment with placebo or 100 mg Sitagliptin was combined with an intravenous background infusion of saline or Ex-9 at 900 pmol/kg/min. The oral medication was given the day before and 60min before ingestion of the OGTT. The dose of Ex-9 used here blocks the insulinotropic effect of GLP-1 in human by at least 95%. The insulin-to-glucose ratio (IGR) was calculated as a measure of glucose sensitivity. Analysis of PP incremental means by two-way RM ANOVA followed by Student-Newman-Keuls multicomparison t-test (see table).
Results: Ex-9 significantly increased PP blood glucose (P=0.004 vs saline iv) and decreased PP plasma insulin excursions (P<0.001 vs saline iv) with and without Sitagliptin. Sitagliptin decreased the mean PP blood glucose excursion (P<0.001 vs placebo po) and PP glucose peak (P<0.001 vs placebo po) with and without Ex-9 iv. In spite of the lower PP glycemia, Sitagliptin increased PP plasma insulin (P=0.008), and this again was not dependent on background infusion. IGR was significantly increased under Sitagliptin during saline iv (diff. 13.4±3.5 µU/mg, P<0.001 vs placebo) and such increment of IGR under Sitagliptin, albeit lower, was detectable during Ex-9, too (diff. 7.7±2.0, P=0.012 vs placebo).

Table: Postprandial glycemia and plasma insulin excursions after an OGTT in T2DM

	Plac po + saline iv	Sita po + saline iv	Plac po + Ex-9 iv	Sita po + Ex-9 iv
Glucose, PP mean (mg/dl)	76.8±4.8	63.0±4.5*	83.9±5.2#	76.5±5.9*#
Glucose, PP peak (mg/dl)	156±5	126±6*	167±6#	158±7*#
Insulin(µU/ml)	31.7±5.5	35.2±5.6*	25.6±4.1#	28.9±4.7*#
Insulin-Glucose ratio (µU/mg)	46.2±7.3	59.6±8.5*	35.6±6.2#	43.3±7.1*#

Mean±SEM. *P<0.05 vs placebo po, same iv infusion; #P<0.05 vs saline iv., same oral treatment (2-way ANOVA followed by SNK test)
Conclusion: In patients with T2DM, endogenous GLP-1 significantly reduces postprandial glycemia by stimulating insulin secretion. Acute DPP-4 inhibition by Sitagliptin improves glucose tolerance by enhancing endogenous GLP-1 action. However, Sitagliptin is still active after blockade of the GLP-1 receptor. We suggest, that GIP accounts for the GLP-1 independent pathway of Sitagliptin.
Supported by: MSD

6

Incidence of acute pancreatitis in exenatide initiators compared to other antidiabetic drug initiators: a retrospective, cohort study

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Background and aims: Post-marketing cases of acute pancreatitis in patients treated with exenatide BID have been reported. Using a large, geographically diverse US healthcare insurance claims database, we conducted a retrospective, cohort study to estimate the absolute and relative risk of acute pancreatitis in patients who initiated exenatide relative to those who initiated other antidiabetic drugs. This represents an interim analysis of claims data to be further validated through medical records review.
Materials and methods: Eligible patients were enrolled in the health care plan for at least 9 continuous months (Sep 1, 2004 to Dec 31, 2007) and had no history of chronic or acute pancreatitis. Propensity scores for exenatide initiation were developed from baseline characteristics (proxies for diabetes mellitus (DM) severity, cardiovascular disease (CV), known acute pancreatitis risk factors, and empirically-derived variables), which were obtained from 9 months of claims data prior to cohort entry. Cases were patients with a primary diagnosis of acute pancreatitis (ICD-9 577.0) associated with an emergency room visit or hospitalisation during follow-up. We used multivariable Poisson regression to estimate the incidence rate (IR), rate ratio (RR), and 95% confidence intervals (CI) comparing periods of current, recent, and past use of exenatide to periods of current, recent, and past use of other antidiabetic drugs.
Results: Initiators of exenatide (N= 25,719; 80% age 40-64 y) were more likely to be female (56% vs 49%) and had a higher baseline prevalence of claims for type 2 DM (84% vs 59%), obesity (16% vs 8%), and CV risk factors (hyperlipidaemia 77% vs 54%; hypertension 64% vs 49%) than initiators of other antidiabetic drugs. In addition, exenatide initiators were more likely to use multiple DM drugs (hypoglycaemic agents: biguanides 60% vs 20%; insulin-release stimulant type 48% vs 18%; insulin-response enhancer 46% vs 13%; insulins 24% vs 9%, lipotropics 61% vs 38% , ACE inhibitors 39% vs 27%) than other antidiabetes drug initiators. Compared to other antidiabetic drugs, the RR of acute pancreatitis for exenatide was: 0.9 [CI 0.6-1.3] for current use, 0.9 [CI 0.4-2.1] for recent use, and 1.4 [CI 0.9-2.3] for past use.
Conclusion: These data suggest that use of exenatide was not associated with an increased rate of acute pancreatitis compared to other antidiabetic drugs.

OP 2 Prediction and prevention of type 2 diabetes mellitus

7

The diabetes risk score outperforms fasting plasma glucose and glucose tolerance tests: combined results from the Inter99 and Botnia Studies

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Background and aims: Improved identification of subjects at high risk for development of Type 2 diabetes (T2D) would allow more aggressive preventive interventions to be targeted towards those individuals who could benefit most. The aim of this study was to validate a multi-marker panel that predicts 5 year risk of T2D on an independent population, and to compare the performance to a combined clinical model of fasting plasma glucose and glucose tolerance.
Materials and methods: The Diabetes Risk Score (DRS) was based on a 7-biomarker panel developed from the Inter99 cohort, a Danish longitudinal population-based study of middle-aged participants. The test was performed on an independent population, the Botnia cohort, a Finnish family-based study designed to identify genetic factors associated with the development of T2D. All 1993 Botnia participants with outcomes and available baseline specimens were tested. Reclassification analysis was used to consider the utility of the DRS relative to plasma glucose tests. In idealized current clinical practice, patients with impaired fasting glucose would be further stratified by 2hr OGTT. Glucose test results were categorized into Low (NFG), Medium (isolated IFG) and High (IFG/IGT) risk groups. The DRS results were applied to reclassify the glucose categories using three bins: Low (score =4=7.3). To increase the statistical power of this analysis, subjects in the Botnia cohort who converted to diabetes at any time during the study over 15.5 years were considered converters.
Results: The DRS stratified the Botnia population more accurately than glucose measures. As shown in the table, DRS identified 7% of the population as high risk, of which 30.7% converted. Using glucose measures, 6% of subjects had both IFG and IGT, of which only 19.8% converted. DRS assigned low risk to 68.4% of subjects, of which 2.1% converted; by contrast, 81.7% of subjects had normal fasting glucose of which 4.5% converted. The overall net reclassification index (NRI) was highly significant (0.341, p<0.0001). The NRI in each of the glucose strata were also significant; DRS showed the greatest improvement among intermediate risk subjects with isolated IFG.
Conclusion: DRS assigned Botnia subjects to low, medium and high risk groups with more accuracy than the combined results of fasting plasma glucose and oral glucose tolerance tests. These results provide independent validation of a panel of serum biomarkers developed in the Inter99 study for assessing the risk of developing type 2 diabetes.

Risk Group by Glucose Measures		Risk Group by DRS: Low (< 4)	Medium (≥ 4 - 7.3)	High (≥ 7.3)	All DRS Groups	NRI (p-value)
Low: FPG < 6.1 mM	No. of Obs.	1286	314	28	1628	0.431 (<0.0001)
	% of Pop.	64.5%	15.8%	1.4%	81.7%	
	Conv. Rate	2.2%	11.5%	35.7%	4.5%	
Medium: FPG ≥ 6.1 mM & OGTT < 7.8 mM	No. of Obs.	65	127	52	244	0.631 (0.0012)
	% of Pop.	3.3%	6.4%	2.6%	12.2%	
	Conv. Rate	0.0%	7.9%	21.2%	8.6%	
High: FPG ≥ 6.1 mM & OGTT ≥ 7.8 mM	No. of Obs.	12	52	57	121	0.504 (0.0156)
	% of Pop.	0.6%	2.6%	2.9%	6.1%	
	Conv. Rate	0.0%	5.8%	36.8%	19.8%	
All Glucose Groups	No. of Obs.	1363	493	137	1993	0.341 (<0.0001)
	% of Pop.	68.4%	24.7%	6.9%	100.0%	
	Conv. Rate	2.1%	9.9%	30.7%	6.0%	

DRS, Diabetes Risk Score; FPG, Fasting Plasma Glucose; OGTT, 2 hr Oral Glucose Tolerance Test; NRI, Net Reclassification Index; % of Pop., Percentage of Population; Conv. Rate, Conversion Rate

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8

The impact of body fat distribution and ectopic fat in determining different glucose tolerance categories

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Background and aims: In the pre-diabetic state impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are both risk factors for the development of type 2 diabetes and cardiovascular disease. However, they differ in their impact on these diseases. So far it is unknown whether visceral fat and ectopic fat in the liver, important determinants of type 2 diabetes and cardiovascular disease, and their humoral products, the adipokine adiponectin and the hepatokine fetuin-A, differ between these conditions and, thus, may explain the different risk.
Materials and methods: In 330 individuals at risk for type 2 diabetes we determined glucose tolerance status by means of a 2 hr 75 g oral glucose tolerance test. Total body fat and visceral fat were precisely quantified by magnetic resonance (MR) tomography. Liver fat was measured by ^{1}H-MR spectroscopy.
Results: A total of 210 individuals were found to have normal glucose tolerance (NGT), 41 IFG, 43 IGT and 36 IFG + IGT. Between the four categories of fasting glycemia and glucose tolerance total body fat was not different (p=0.51) after adjustment for age and gender. However, a small but continuous increase in adjusted visceral fat content was found from NGT to IFG+IGT (means±SE, NGT: 3.07±0.10; IFG: 3.11±0.21; IGT: 3.61±0.21; IFG+IGT: 3.84±0.23 kg, p=0.03). A large difference among the categories was found for liver fat content. There was a continuous increase in liver fat content from NGT (4.73±0.42%) to IFG (5.86±0.92%) to IGT (8.65±0.92%) and IFG+IGT (11.11±1.01%) after adjustment for age and gender (p<0.0001). Circulating levels of the adipokine adiponectin also differed among the categories, although the differences were small (NGT: 13.67±0.46; IFG: 14.47±1.00; IGT: 11.76±0.99; IFG+IGT: 10.80±1.13 μg/dl, p=0.08). In contrast, there was a larger difference in the circulating hepatokine fetuin-A among the categories (NGT: 265±3; IFG: 260±8; IGT: 284±7; IFG+IGT: 286±9 μg/dl, p=0.01). Next we investigated the predictive effect of total body fat, visceral fat and liver fat on determining the glucose tolerance categories. In a forward stepwise regression analysis liver fat was the strongest determinant of the categories (p<0.0001) followed by age (p=0.0005) and gender (p=0.11). In another analysis including age, gender, adiponectin and fetuin-A, age was the strongest determinant of the categories (p<0.0001) followed by fetuin-A (p=0.016) and adiponectin (p=0.02).
Conclusion: Among total body fat, visceral fat and ectopic fat in the liver, liver fat strongly and continuously increases when fasting glycemia and glucose tolerance move from NGT to IFG, IGT and IFG+IGT. Furthermore, liver fat is predicting the glucose tolerance category independently of total- and visceral fat. In addition, among the humoral products of fat and liver, fetuin-A was a stronger predictor of the categories than adiponectin. Thus, liver fat may represent an important target in the treatment of pre-diabetes and in the prevention of type 2 diabetes.
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9

Use of multiple metabolic and genetic markers to improve the prediction of type 2 diabetes: the EPIC-Potsdam study

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Background and aims: Precise prediction of the risk of type 2 diabetes is essential for early and cost-effective prevention. We evaluated whether metabolic factors and single nucleotide polymorphisms (SNPs) can improve diabetes risk prediction in a population-based, prospective study beyond an established prediction model based on age, anthropometric, diet, and lifestyle variables (German Diabetes Risk Score).

Materials and methods: A case-cohort study within the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study comprising 27,548 participants was designed. We randomly selected a subcohort of 2,500 individuals from the 26,444 participants with blood of whom 1,991 were diabetes-free at baseline and had anamnestic, anthropometrical, lifestyle, and metabolic data. Of the 801 incident type 2 diabetes cases identified in the cohort during 7 years of follow-up 582 remained for analyses after exclusions. Prediction models were compared by receiver operator characteristics (ROC) and reclassification was evaluated by the integrated discrimination improvement (IDI).
Results: Prediction by the risk score (ROC-AUC: 0.8448) was improved by inclusion of plasma glucose (ROC-AUC: 0.8636, p<0.001) and HbA1c (ROC-AUC: 0.8833, p<0.001). ROC-AUC was further improved with HDL-cholesterol, triglycerides, γ-glutamyltransferase, and alanine-aminotransferase (0.8974, p=0.0028), which was confirmed by improved risk classification (relative IDI: 5%), but additional information on adiponectin or hs-CRP resulted not in meaningful improvements. Information on diabetogenic SNPs (preliminary set of 17 variants) beyond these characteristics resulted in slight improvement in discrimination (ROC-AUC: 0.9053, p=0.0183) and risk classification (relative IDI: 4%). Parental history was associated with higher diabetes risk not explained by genetic variants evaluated, and this information provided a small improvement of risk prediction beyond the German DRS (relative IDI: 5%).
Conclusion: Metabolic markers, in particular plasma glucose and HbA1c, substantially improve risk prediction of type 2 diabetes beyond non-invasive risk factors. Seventeen SNPs slightly improved the prediction beyond these characteristics.
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10

Prevention of type 2 diabetes development by an IKKβ inhibitor
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Background and aims: High doses of anti-inflammatory drugs like aspirin and salicylates have been shown to improve insulin and glucose metabolism in insulin resistant and diabetic patients through unspecific inhibition of the IKKβ and NFkB pathways. Based on this, we have investigated the effects of a specific IKKβ-inhibitor on β-cell survival and the prevention of development of type 2 diabetes in the gerbil *Psammomys obesus* (*P. obesus*) fed a high energy diet.
Materials and methods: We investigated the development of body weight, blood glucose, HbA_1C, plasma insulin and pancreatic insulin content in P. obesus fed a high energy diet, and treated with a specific IKKβ-inhibitor (60 mg/kg; n=10) or vehicle (n=12) for one month. The effects of IKK-β inhibitor on β-cells were also studied using INS-1 cells and primary islets from *P. obesus,* exposed to interleukin-1β (IL-1β) followed by analysis of reactive oxygen species (ROS), insulin secretion and cell death.
Results: Vehicle treated animals developed diet induced diabetes during the 28 days on high energy diet with a significant increase in blood glucose (3,4±0,1mM vs. 15,5±1,3; p<0,001) and HbA_1C (4,8±0,1% vs. 6,4±0,3%; p<0,001). To compensate for the hyperglycemia the vehicle treated animals displayed a significant increase in plasma insulin until day 21 where hyperinsulinemia was declining, most likely caused by accelerated β-cell destruction (day 1: 1301±197pM vs. day 21: 6782±889; p<0,001 and day 28: 5578±708pM). Animals treated with the IKKβ-inhibitor showed no sign of diet induced diabetes during the 28 days on high energy diet. No increase in HbA_1C was observed and only a minor increase in plasma glucose and a non-significant increase in plasma insulin levels were detected. The animals treated with the IKKβ-inhibitor also had significantly lower levels of blood glucose (day 28: IKKβ-inh 5,7±0,9mM vs. vehicle 15,5±1,3mM; p<0,001), HbA_1C (day 28: IKKβ-inh. 5,0±0,2% vs. vehicle 6,4±0,3; p<0,001) and plasma insulin (day 21: IKKβ-inh. 3741±783pM vs. vehicle 6782±889; p<0,05), as compared to vehicle treated animals despite no difference in body weight (day 28: IKKβ-inh. 219±6,5g and vehicle 217±5,8g). Total pancreatic insulin was measured at day 28. The levels of pancreatic insulin stored in the IKKβ-inhibitor treated animals was more than three-fold that observed in the vehicle treated animals (day 28: IKKβ-inh. 26±7,2μg vs. vehicle 7±1,5μg; p<0,05). In primary islets and β-cells the IKKβ-inhibitor reversed IL-1β induced ROS production and diminished insulin production and β-cell death.
Conclusion: We have shown that development of diet induced hyperinsulinemia and hyperglycemia in *P. obesus* was prevented by treatment with a specific IKKβ-inhibitor. The IKKβ-inhibitor also prevented cytokine induced loss of insulin production and β-cell death in primary islets and β-cells possible by the inhibited production of ROS. This was also reflected *in vivo* since treated animals had a much larger insulin reserve compared to the exhausted β-cells in vehicle treated animals. These data support the use of anti-inflammatory drugs in prevention of type 2 diabetes. In conclusion, we have shown that an inhibitor of the NFkB pathway was able to prevent type 2 diabetes development in *P. obesus* by reversing insulin resistance and protecting β-cell function.

11

Dapagliflozin prevents the development of diabetes in male ZDF rats
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Background and aims: Dapagliflozin (Dapa), a potent and selective SGLT2 inhibitor, is currently under investigation as a novel agent for the treatment of type 2 diabetes. The present study was designed to examine whether treatment with Dapa for 5 weeks can prevent the development of diabetes in a model of rapid beta cell failure, the male ZDF rat.
Materials and methods: Male ZDF rats at 6 weeks of age were dosed *q.d. p.o.* for 5 weeks in the following groups: 1) age-matched lean littermates + vehicle (N=6; Lean), 2) ZDF + vehicle (N=8; ZDF), 3) ZDF + Dapa (1 mg/kg, N=8; Dapa), and 4) ZDF + rosiglitazone (10 mg/kg, N=8; Rosi) as a positive control. Oral glucose tolerance tests (OGTT, 2 g/kg) were undertaken 24 hr post final dose, after an overnight fast at the end of the 5 week treatment. Hemoglobin A1c (HbA1c) and Echo MRI were used to evaluate glycemic control and body composition, respectively, in a separate cohort (N=5) treated identically. One way ANOVA followed by Dunnett's or Tukey-Kramer test were performed for statistical analyses.
Results: In the ZDF group at 5 weeks, fasting plasma glucose (FPG) levels were 272 ± 40 mg/dL. At this time point, Dapa (80 ± 4 mg/dL; p< 0.01) and Rosi (100 ± 4 mg/dL; p<0.01) treated rats both had significantly lower FPG. HbA1c was significantly reduced with Dapa (4.6 ± 0.1%; p<0.001) and Rosi (4.1 ± 0.1%; p<0.001) compared to ZDF (7.8 ± 0.4%); both Dapa and Rosi groups were similar to Lean (4.1 ± 0.1%). In an OGTT, both Dapa and Rosi groups displayed significantly reduced glucose area under the curve (ΔAUC glucose; mg/dL*min) vs. ZDF (ZDF: 32847 ± 3995; Dapa: 11938 ± 1014, p<0.001; Rosi: 17526 ± 808, p<0.001). ΔAUC glucose in Dapa was not significantly different from Lean. Fasting insulin levels were 1.0 ± 0.1 ng/mL in Lean, and were lower in Dapa vs. Rosi (2.6 ± 0.2 ng/mL vs. 4.1 ± 0.4 ng/mL, respectively; p<0.05). Dapa fasting insulin levels were similar to ZDF (2.1 ± 0.3 ng/mL). The incremental change in insulin concentration in response to glucose challenge at 30 minutes ($\Delta Insulin_{30min}$) was greater in Dapa (7.2 ± 1.2 ng/mL, p<0.001) compared to ZDF (1.1 ± 0.4 ng/mL). Dapa $\Delta Insulin_{30min}$ was significantly different from Rosi (3.3 ± 0.9; p<0.05). Dapa treatment did not change body weight (BW, 393.4 ± 5.3 g) or fat mass (FM, 120.0 ± 3.5 g) vs. ZDF (365.2 ± 11.9 g and 111.9 ± 3.8 g, respectively), unlike Rosi treatment which increased BW (488.0 ± 15.1 g, p<0.001) and FM (219.2 ± 8.5 g, p<0.001) vs. ZDF.
Conclusion: These results suggest that once-daily dosing with Dapa prevented the development of diabetes, was body weight neutral, and preserved beta cell function in a rodent model of type 2 diabetes.

12

Liraglutide, a once-daily human GLP-1 analogue, reduces the prevalence of prediabetes in obese subjects over 20 weeks: a randomized placebo-controlled trial
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Background and aims: Prediabetes is a strong predictor of type 2 diabetes with an estimated prevalence in Europe of 10-20%. Liraglutide reduces

HbA_{1c} by 1.0-1.5% and weight by 2-3 kg with doses up to 1.8 mg in patients with type 2 diabetes. This double-blind, placebo-controlled trial with open-label orlistat comparator was the first to investigate the effect of liraglutide in obese non-diabetic subjects. We have previously reported on body weight, the primary outcome. This abstract focuses on prediabetes status, a secondary endpoint.

Materials and methods: Subjects (18-65 years, BMI 30-40 kg/m^2), of which 175 had prediabetes based on 2003 ADA criteria, were randomized equally to 1 of 4 liraglutide doses (1.2, 1.8, 2.4 or 3.0 mg once daily s.c.), placebo once daily s.c. or orlistat (120 mg 3x daily orally) for 20 weeks. Prediabetes was defined as IGT or IFG (IGT: 2h glucose 7.8-11 mmol/L in OGTT; IFG: FPG 5.6-6.9 mmol/L). All subjects were advised and encouraged to reduce daily energy intake by 500 kcal and to increase physical activity. www.clinicaltrials.gov ID NCT00422058.

Results: In the ITT population (561 of 564 randomized subjects) with LOCF, the estimated mean placebo-subtracted weight loss from randomization was 2.1 kg [95% CI 0.6-3.6] (liraglutide 1.2 mg) to 4.4 kg [2.9-6.0] (liraglutide 3.0 mg). Mean weight loss with liraglutide 3.0 mg was 7.2 kg. Weight loss from screening, including 2-week run-in, ranged from 4.1 ± 3.9 kg with placebo to 9.1 ± 5.2 kg with liraglutide 3.0 mg (Table). Prediabetes at baseline was 31% and the incidence over 20 weeks was reduced by about 90% with liraglutide 1.8-3.0 mg. In a post hoc analysis, liraglutide-treated subjects had significantly greater odds of having normal glucose tolerance at Week 20 compared to those treated with placebo or orlistat (p<0.01 all doses, Table). The estimated odds were between 4 and 38 for liraglutide compared with 1.5 for placebo or orlistat. The number of subjects needed to be treated to prevent one case of prediabetes was 3-6 with liraglutide 1.2-3.0 mg, compared to 24 with orlistat. Compared to placebo, more events of nausea and vomiting of mild to moderate intensity occurred with liraglutide, mostly within the first 4-6 weeks.

	Liraglutide 1.2 mg	**Liraglutide 1.8 mg**	**Liraglutide 2.4 mg**	**Liraglutide 3.0 mg**	**Placebo**	**Orlistat**
Change from baseline to Week 20						
Body weight from screening (kg)[1]	**-6.7 ± 4.0**	-7.1 ± 5.8	-7.9 ± 5.0	-9.1 ± 5.2	-4.1 ± 3.9	-5.5 ± 4.3
Prediabetes to normal[2] status	**18/26 (69%)**	26/27 (96%)	23/26 (88%)	24/25 (96%)	13/28 (46%)	9/22 (41%)
Normal[2] to prediabetes status	**3/51 (6%)**	0/44 (0%)	1/46 (2%)	2/53 (4%)	11/48 (23%)	10/53 (19%)
Estimated odds ratio[3]						
Versus placebo	3.0 [1.1; 8.1]	26.1 [3.8; 181]	10.7 [2.4; 47]	12.5 [2.9; 55]	–	–
Versus orlistat	3.0 [1.1; 8.3]	26.3 [3.7; 185]	10.8 [2.4; 48]	12.6 [2.9; 56]	–	–

[1]Values are the mean +/- SD
[2]FPG <5.6 mmol/L or <7.8 mmol/L in OGTT
[3]Estimated mean OR [95% CI] for having normal glucose tolerance

Conclusion: Liraglutide 1.2-3.0 mg once daily for 20 weeks produced weight loss, reduced the incidence of prediabetes and reversed existing prediabetes in obese subjects. Treatment with liraglutide normalizes raised glucose levels and may delay the onset of type 2 diabetes.

Supported by: Novo Nordisk A/S

OP 3 Diabetes education and management

13

Cost effectiveness of the DESMOND structured education programme for patients newly diagnosed with type 2 diabetes

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Background and aims: DESMOND is a structured education programme for patients newly diagnosed with Type 2 diabetes. The programme meets criteria laid down by the National Institute for Health and Clinical Excellence in the UK. Previously reported results from the RCT demonstrated improvements in some bioemedical and lifestyle measures, notably smoking and body weight, and in some health scores including illness beliefs and depression scores. This analysis assesses the within-trial and long-term cost-effectiveness of DESMOND compared to usual care.

Materials and methods: The DESMOND trial was a multi-site cluster randomised controlled trial enrolling 824 people newly diagnosed with Type 2 diabetes. 12 month follow-up data including biomedical, lifestyle and NHS resource use data were collected on 749 participants. The analysis here uses the described data from the 12 month period of the DESMOND trial and the Sheffield 2 Diabetes Model to estimate the long-term outcomes, costs and benefits and cost-effectiveness of the DESMOND intervention. The estimated long-term costs and benefits of DESMOND, including its impact on use of therapies, cardiovascular events and quality-adjusted life years (QALYs) were aggregated to obtain the incremental cost per QALY. The effects of the intervention were assumed to have some sustained effect up to 3 years. Uncertainty around key parameters, such as weight changes, smoking rates and durability of effects, was explored using probabilistic sensitivity analysis (PSA) and alternative scenarios.

Results: The main drivers of the results were a 1.26 kg greater weight loss and favourable changes in smoking status with the DESMOND intervention. The resulting estimated lifetime additional costs, based on the cost of the intervention in the trial, are £218 and there is an increase in QALYs of 0.0406 and an incremental cost effectivenss ratio (ICER) of £5,369. PSA results indicate that there is an 81% likelihood that DESMOND is cost-effective compared to usual care at a UK £ 20,000 cost/QALY willingness to pay threshold. The analysis was then repeated using the current real-world cost of delivering the DESMOND intervention in a typical primary care trust which is £76 per patient compared to £203 in the trial. In this case the lifetime incremental cost is only £91 resulting in an ICER of £2,241 and the PSA indicates that there is an 87% likelihood that DESMOND is cost-effective at £20,000 cost/QALY threshold. A sensitivity analysis, based around the real world cost of DESMOND and more conservative assumptions regarding the durability of effects, resulted in an ICER of £2,647 and an 80% likelihood that DESMOND is cost effective.

Conclusion: The long-term model suggests that DESMOND, given as a one off intervention, is very cost effective even with modest assumptions around how long effects are sustained. Further model-based economic analyses can be undertaken when evidence becomes available on the effectiveness of DESMOND as an on-going intervention as recommended by NICE.

Supported by: Diabetes UK

14

Economic impact of modular outpatient diabetes education programme

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Background and aims: The economic impact of a health intervention can be described by evaluation of savings with following methods: participant surveys, time value of money-analysis, extrapolation from published cost estimates, cost-benefit analyses, cost-utility studies and return on investment. Our aim was to evaluate the economic impact of our outpatient diabetes

modular education program for metabolic syndrome and diabetes, using a novel cost-utility assessment method in the context of the Austrian and German health care system and its outcomes as currently applied in diabetes treatment practice (as shown by FQSD, "Forum Qualitätssicherung Diabetes").

Materials and methods: We used the mathematical diabetes mellitus simulation model PROSIT-Danube A which is based on Markov modelling of natural course of the disease while joining cost data to the disease model. This cost-utility analysis determines the best way of spending given health care budget. It relates cost to healthy years gained (e.g. quality-adjusted life years, QALY, and/or healthy years equivalents) as benefits converted into monetary terms.

To ensure high quality epidemiological data necessary for correct input parameters, we used independent, benchmarking-based data of FQSD. Here, for benchmarking purposes 14 biggest diabetes care centres from Austria and Germany have been selected. Selection criteria for voluntarily registered centres included biggest available numbers of individuals per centre assessed with annual basic information sheets as acquired for 2 years, care for mainly adult outpatients with metabolic syndrome / type 1 and type 2 diabetes and the availability of HbA_{1c} assay reference ranges.

The simulation of effects of our diabetes structured education system was used in comparison: our subgroup treated with structured modular patient education (95 % of structured diabetes education, 35% hypertension and nephropathy prevention module), against all other patients (36% diabetes education, used data of patients with documented microalbuminuria) as found within an international benchmarking study: n = 209 / 10.325 patients, initial age 49 / 60 yrs, sex 56 / 53 % female, duration of diabetes 19 / 10 yrs, smoker 14 / 13%, HbA_{1c} 6,6 / 7,1%, blood pressure (systolic) 128 / 138 mmHg, blood pressure (diastolic) 72 / 80 mmHg, blood pressure (MAP) 91 / 99 mmHg, BMI 26 / 30 kg/m², anti-hypertensive treatment 78 / 52 %, ACE inhibitors 50 / 40%.

Results: The cost of QALY in our cost-utility calculation was 2.300 EUR while the cost per QALY in all other patients reached 2.587 EUR (both discounted values by 3.5%). Similarly, the calculated discounted cost of lifeyear was 2.277 EUR in our group versus 2.532 EUR in other centres. This finding confirms clinical findings on effective nephropathy prevention with modular diabetes education including hypertension module where necessary. Structured patient education for competent treatment proved to be cost-effective.

Conclusion: This PROSIT-based cost-utility analysis shows that structured modular ambulatory education in diabetes brings a considerable return on investment in terms of cost reduction for QALYs. Education provides benefits converted to monetary terms. Financial efficacy of patient education should be perceived by insurance and health care providers in the current health care plans of the EU.

15

Validating a diabetes specific quality of life measure in an English speaking population: the Irish Dose Adjustment for Normal Eating (DAFNE) Study

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Background and aims: The Irish DAFNE Study is evaluating a structured education programme for people with Type 1 diabetes in Ireland. Quality of life is an important outcome of the study. We chose the Diabetes-Specific Quality of Life Scale (DSQOLS) as our main measure. The instrument was originally developed in Germany to evaluate the Düsseldorf insulin treatment and training programme. It contains 77 items including 10 individual treatment goal items, 10 satisfaction with treatment success items and 57 diabetes-related distress items (additional items were included to assess impact of hypoglycaemia on quality of life). The scale was altered slightly from the original, with input from people with diabetes, to reflect linguistic differences and more culturally appropriate scale-items. A subset of participants completed both DSQOLS and the Audit of Diabetes-Dependent Quality of Life (ADDQoL) tool. The ADDQoL was developed in England and consists of 20 items.

Materials and methods: Reliability coefficients (Cronbach's α) were computed for all psychometric subscales. Internal consistency of subscales and correlation between subscales (Pearson's correlation) were measured.

Results: A total of 42 individuals (55% female, mean age 39 years, mean diabetes duration 16 years, mean HbA1c 8.6%) completed the DSQOLS and the ADDQoL scales. The Cronbach's α for the 8 DSQOLS sub-scales were; Social relations (11 items), 0.9; Leisure time flexibility (6 items), 0.89; Physical complaints (9 items), 0.86; Worries about future (5 items), 0.87; Diet restrictions (9 items), 0.91; Daily hassles (6 items), 0.82; Fear of hypoglycaemia (11 items), 0.95 and Daily burdens and restrictions (57 items), 0.97. Cronbach's α for Average-Weighted Impact (AWI) of the ADDQoL was 0.83. In ADDQoL the overall QoL item, the future QoL item and AWI score were significantly correlated (r = .387, .341 and .324 respectively). In DSQOLS all subscales were significantly correlated to each other (r between 0.637 and 0.861). Subscales from both instruments that were significantly correlated included; Dependence (ADDQoL) and Leisure time flexibility (DSQOLS, $r = 0.363$, $p < 0.05$), Dependence (ADDQoL) and Social relations (DSQOLS, $r = 0.403$, $p < 0.01$). Holidays/ Leisure (ADDQoL) were significantly correlated to Leisure time flexibility, Physical complaints, Worries about future, Fear of hypoglycaemia and Daily burden and restrictions (DSQOLS, r = 0.393, 0.363, 0.358, 0.343 and 0.355 respectively, $p < 0.05$).

Conclusion: A high degree of internal consistency was achieved by both instruments. High inter and intra-correlations of the subscales were suitably reported. In the absence of a global QoL score from DSQOLS it was difficult to compare the 2 instruments at baseline. However future measurements within the Irish DAFNE Study will enable the responsiveness and sensitivity to change of these 2 quality of life scales to be assessed and compared.

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16

Depression and sense of coherence are associated with food intake and compliance with dietary guidance in type 1 diabetes

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Background and aims: In type 1 diabetes, appropriate insulin dosing and compliance with dietary recommendations are important in achieving good metabolic control. As psychological disturbances are common in patients with type 1 diabetes, we aimed to study the associations between psychological and dietary factors. Additionally, we studied which factors are associated with compliance with dietary guidance received in health care.

Materials and methods: A total of 527 participants [46% men, mean age 47 (range 17-84) years, diabetes duration 30 (1-62) years] in the Finnish Diabetic Nephropathy Study were included in this cross-sectional study. Beck Depression Inventory (BDI) and sense of coherence test were used to study depression and sense of coherence (SOC), respectively. Data on food consumption and dietary guidance were collected using a questionnaire. Compliance with dietary guidance was assumed if patient reported following instructions always or most of the time.

Results: Overall rates of depression (score ≥16) and weak SOC (score <60) were 13% and 18%, respectively, and 9% of patients were both depressed and had weak SOC. A total of 86% of depressed and 87% of non-depressed patients had received dietary guidance (ns). These patients did not differ in compliance with dietary guidance (53% vs. 60%, p=0.27). Depressed males consumed less fresh vegetables, bread and coffee, but more fried foods compared to males without depression (p<0.05, all). Female patients with depression consumed less dairy products and fish (p<0.05, both) compared to females without depression. Of patients with weak and strong SOC, 89% and 87% had received dietary guidance (ns), but of these 48% and 61%, respectively, reported adhering to the dietary guidance often or always (p<0.05). Male patients with weak SOC consumed more soft drinks and fried foods than males with strong SOC did (p<0.05, all). Additionally, they reported more frequently using no spread on bread compared with males with strong SOC (22% vs. 5%, p<0.01). Females with weak SOC consumed less fish, cooked vegetables, and fruits and berries, but more soft drinks compared to females with strong SOC (p<0.05, all). In a logistic regression analysis, strong SOC, dietary guidance given by a physician (but not dietitian or nurse), longer diabetes duration and adherence to any special dietary regimen were associated with good self-reported compliance with dietary guidance when adjusted for gender.

Conclusion: Depression and weak SOC were associated with less prudent food choices such as infrequent consumption of fish and vegetables. Strong SOC is associated with good compliance with dietary guidance. Dietary guidance from physicians may improve the compliance. Our results suggest that

psychological well-being may play an important role in modifying dietary habits among patients with type 1 diabetes.
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17

Effects of group-based lifestyle rehabilitation on glycaemic control, physical fitness and risk factors for cardiovascular disease in patients with type 2 diabetes

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Background and aims: Current guidelines recommend education, physical activity and change in diet for type 2 diabetes patients, yet composition and organization of non-pharmacological care are still controversial. We investigated the effectiveness of a new group-based lifestyle rehabilitation programme in a Health Care Centre in primary care versus conventional individual counselling in an Outpatient Clinic.
Materials and methods: We randomized 143 adult type 2 diabetes patients to either the intervention group consisting of an education programme delivered by a multi-disciplinary team, a supervised exercise programme, and a cooking course, or the control group receiving individual counselling with a diabetes nurse specialist and a dietician. Outcome measures at 6 months follow-up included HbA1c (primary outcome), blood pressure, lipid profile, anthropometric variables, and physical fitness.
Results: Baseline characteristics were comparable, 22 patients did not complete 6 months follow-up, and 121 patients were included in the intention-to-treat analysis. 64% completed 75% of the exercise programme, and 76% attended 5 of 6 education sessions. In the control group 90% completed 75% of the counselling sessions. After 6 months HbA1c decreased from 7.8 ±0.8% to 7.5% ±1.0% in the intervention group and from 7.8 ±0.8% to 7.2 ±0.9% in the control group (p=0.054). In the two groups combined the overall decrease in HbA1c was significantly lower after 6 months compared to baseline (-0.4%, p<0.01). Similarly, there was a reduction in systolic blood pressure (-5 mmHg, 95% CI (-7.8 to -3.0)), weight (-2 kg, 95% CI (-2.7 to -1.4)), and waist circumference (-2 cm, 95% CI (-2.6 to -1.3)) after 6 months in the two groups combined, but there were no differences between the groups. There was no difference in the lipid profile between the two groups although there were a small overall decrease in the level of p-cholesterol (-0.3 mmol/L, 95% CI (-0.4 to -0.1)) and p-triglycerides (-0.2 mmol/L, 95% CI (-0.5 to -0.02)). Muscle strength in legs improved by 30% during the exercise programme in the intervention group (132 to 169 kg, 95% CI (27.6 to 46.7)). No improvement in muscle strength in the control group after 6 months.
Conclusion: Despite an improvement in muscle strength the new multi-disciplinary rehabilitation programme for type 2 diabetes patients did not improve glycemic control more than the conventional individual counselling. Likewise, there were equal effects on blood pressure, lipids, weight, and waist circumference in both groups. These results suggest that a multi-disciplinary rehabilitation programme in a Health Care Centre in primary care setting is a comparable alternative to the conventional individual counselling in an outpatient setting.
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18

Motivational enhancement therapy with and without cognitive behaviour therapy to treat type 1 diabetes: the long-term outcomes of a randomised controlled trial

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Background and aims: Psychological treatments can lead to improvements in glycaemic control in the short term (12 months). We conducted a follow up of a cohort from a randomised controlled trial, in which we reported that motivational enhancement therapy (MET) with cognitive behavioural therapy (CBT) improved glycaemic control in type 1 diabetes compared to usual care at 12 months by half percent reduction in HbA1c but MET alone compared to usual care did not. We aimed to test whether this beneficial effect was maintained at 36 months.
Materials and methods: In the original study, 1659 patients were screened from 8 diabetes centres in London and Manchester, United Kingdom of which 507 were eligible. The baseline sample consisted of 344 adults with type 1 diabetes with glycated haemoglobin between 8.2 and 15%, and without complications or severe comorbid disease. They had been randomised to either nurse delivered MET (4 sessions over 2 months), MET plus CBT (12 sessions over 6 months) or usual diabetes care. Participants were contacted for informed consent to the longterm follow up. The main outcome was the 36 month change in glycated haemoglobin.
Results: To date 6 diabetes centres have been followed up (n=253) and data on the primary outcome is currently available on 115 original participants, giving a preliminary response rate of 45.4%. In the preliminary analysis, the mean baseline HbA1c was 9.37%, and the mean 36 month HbA1c was 8.72%, representing an average decrease in HbA1c of 0.65% (95% CI 0.41 to 0.90, p=0.001) from baseline to 36 months (table 1). Using ANCOVA regression modelling, there was no difference between MET plus CBT versus usual care at 36 months (baseline adjusted mean difference in HbA1c 0.11% (95% confidence interval -0.42 to 0.65) and nor between MET versus usual care (baseline adjusted mean difference in HbA1c 0.03% (-0.54 to 0.61).
Conclusion: Adjusting for baseline HbA1c, the 3 groups are very similar at months. The effectiveness of MET plus CBT at 12 months had disappeared at 36 months. There may be a selection bias in early respondents as the responders on average have improved their glycaemic control.

Table 1: HbA1c at baseline and at 36 months by intervention (n=115)

Group	Baseline HbA1c % mean (SD)	36 month HbA1c % mean (SD)
Usual care (n=42)	9.32 (0.86)	8.65 (1.39)
MET (n=41)	9.28 (0.67)	8.74 (1.16)
MET plus CBT (n=32)	9.56 (1.27)	8.79 (1.33)

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OP 4 Diabetes and the heart

19

Myocardial function and myocardial lipid accumulation in insulin resistant and type 2 diabetic women

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Background and aims: Impaired glucose metabolism is an independent risk factor for cardiovascular disease and heart failure. Non-invasive quantification of myocardial lipid accumulation by localized ^{1}H MR spectroscopy has recently revealed possible links between myocardial steatosis, myocardial function and impaired glucose metabolism in type 2 diabetic patients. Insulin resistance is associated with enhanced ectopic lipid accumulation in skeletal muscle and liver. Therefore, the aim of the present study was to assess relations between insulin resistance and myocardial lipid accumulation and function in non-diabetic women.
Materials and methods: ^{1}H magnetic resonance imaging and localized ^{1}H single voxel spectroscopy were used to measure left ventricular dynamic parameters and myocardial lipid accumulation in cardiac septum of non-diabetic insulin sensitive (IS: n=6, 50±5 years, BMI 25±2 kg/m^2) and insulin resistant (IR: n=7, 50±6 y., 26±2 kg/m^2) women as well as females with type 2 diabetes mellitus (T2DM: n= 6, 51±8 y., 28±2 kg/m^2; HbA1c 9.2±1.9%). In non-diabetic women, insulin sensitivity was assessed by oral glucose tolerance test and subsequent calculation of the Clamp-Like Index (CLIX; IR: 4.5±0.4 vs. IS: 9.7±0.7, p<0.0001). Data are given as mean±SD.
Results: As to myocardial function, we could not find any difference between the study groups regarding left ventricular ejection fraction (IS: 69.3±2.5; IR: 70.5±2.8, T2DM: 68.8±1.7%), end-systolic volume (IS: 35±12; IR: 30±13; T2DM: 27±12 ml) and cardiac output (IS: 4.9±0.8, IR: 4.8±1.2, T2DM: 4.4±0.5 l/min), whereas end-diastolic volume (IS 113±19; IR 98±28; T2DM 84±16 ml) and stroke volume (IS 79±10; IR 68±18; T2DM 56±8 ml) tended to be lower in IR (IS vs. IR: ns) and was significantly decreased in T2DM compared to IS (T2DM vs. IS: p< 0.02). Myocardial lipid content was not different between IS and IR (p= 0.7), but tended to be increased (p= 0.2) in T2DM group (IS: 0.43±0.25; IR: 0.38±0.17, T2DM: 0.68±0.36 %)
Conclusion: Our results suggest that increased myocardial lipid content and impaired myocardial function are not linked to insulin resistance per se, but might develop after the manifestation of type-2 diabetes.
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20

Glucosamine increases hexosamine biosynthesis and O-linked N-acetylglucosamine in the heart, and leads to metabolic alterations similar to those seen in diabetic cardiomyopathy

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Background and aims: Increased hexosamine biosynthesis pathway (HBP) flux and O-linked attachment of N-acetyl-glucosamine (O-GlcNAc) on nucleocytoplasmic proteins have both been implicated in cardiovascular complications associated with diabetes. Therefore, the goal of this study was to determine the impact of increases in HBP flux and O-GlcNAc levels - induced by glucosamine (GlcN) - on cardiac function and metabolism.
Methods: Hearts were perfused with 0; 0.05; 0.1; 1; 5 and 10 mM GlcN, metabolic fluxes were determined by using ^{13}C-NMR isotopomer analysis. UDP-GlcNAc, a product of HBP and a precursor of O-GlcNAc was assessed by HPLC and protein O-GlcNAc, AMPK and ACC phosphorylation and membrane asociated FAT/CD36 was assessed by immunoblot analysis.
Results: GlcN had no effect on cardiac function or on cardiac ATP content at any concentration; however, 0.1 mM GlcN significantly increased both UDP-GlcNAc and O-GlcNAc by 40-50% (p<0.05, Vs 0mM GlcN). This increase in O-GlcNAc was associated with a significant increase in palmitate oxidation (46±4 vs 67±2%) and a concomitant decrease in lactate (30±3% vs 14±1%; p<0.05) and pyruvate (8±2% vs 3±1%) oxidation. Higher concentrations of GlcN had no additional effect on substrate utilization; however, UDP-GlcNAc and O-GlcNAc levels reached levels ~2-fold higher than 0 mM GlcN at 1 GlcN and did not increase further. GlcN had no effect on AMPK or ACC phosphorylation. On the other hand GlcN at every concentrations increased membrane associated FAT/CD36 levels in a dose dependent manner.
Conclusion: GlcN significantly increased O-GlcNAc levels and altered fatty acid and carbohydrate oxidation in a similar manner to that seen with diabetes at least in part by increasing membrane associated FAT/CD36 levels. These data suggest that changes in O-GlcNAc levels may contribute to the shifts in cardiac substrate utilization seen in diabetes.
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21

Rats with experimental diabetic cardiomyopathy develop diastolic heart failure that is ameliorated by the direct renin inhibitor, aliskiren

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Background and aims: Heart failure is a common cause of morbidity and mortality in diabetic patients that frequently manifests in the absence of impaired left ventricular systolic function. In contrast to the strong evidence base for the treatment of systolic heart failure, the benefit of the treatment of heart failure with preserved left ventricular function (HFPEF) with ACE inhibitors and ARBs is unclear. In the setting of recent studies demonstrating the effects of direct renin inhibition in diabetic nephropathy, we sought to determine the effectiveness of this strategy in the syndrome of HFPEF due to diabetes. Furthermore, in light of the recently described pathogenetic effects of the (pro)renin receptor [(P)RR], we assessed its expression in the hearts of diabetic animals treated with and without aliskiren.
Materials and methods: Using a hemodynamically-validated rodent model of diabetic cardiomyopathy that develops HFPEF, the diabetic (mRen-2)27 transgenic rat (Ren-2), we randomized animals to receive either vehicle or aliskiren (10mg/kg/day via osmotic mini-pump) for 6 weeks. Cardiac function was assessed by *in-vivo* cardiac catheterization with pressure-volume loop acquisition. Following this, animals were killed, and cardiac tissue was collected for histological assessment of fibrosis (picrosirius red staining) and myocyte hypertrophy (cross sectional area). (P)RR expression was assessed by real-time PCR, Western blot analysis and immunohistochemistry.
Results: Despite preserved systolic function, diabetic rats developed diastolic dysfunction in association with cardiac fibrosis, and cardiomyocyte hypertrophy. Aliskiren treated diabetic animals demonstrated improved chamber compliance (0.032 ± 0.006 v 0.057 ± 0.006 mmHg/μL) and early active relaxation (Tau, 8.75 ± 0.40 v 9.98 ± 0.29 msec) when compared to untreated diabetic counterparts (p<0.05). Aliskiren reduced collagenous matrix in diabetic animals (2.38 v 1.40 % proportional area picrosirius red staining, p<0.05), reduced LV mass by 52% (p<0.01 versus untreated diabetic rats) and normalized cardiomyocyte diameter (22.35 v 15.68 μm, p<0.01). (P)RR was predominantly localized to cardiac myocytes by immunohistochemistry. Expression of both (P)RR mRNA and protein were increased with diabetes and reduced to baseline by aliskiren (control, diabetes, diabetes+aliskiren: 1.13 ± 0.04, 3 ± 0.2, 1.15 ± 0.07 AU, respectively; p<0.01 control versus diabetes and p<0.05 diabetes+aliskiren versus diabetes).
Conclusion: Direct renin inhibition with aliskiren prevented the development of structural and functional changes of HFPEF in the diabetic Ren-2 rat. The biological significance of modulation in (P)RR expression by diabetes and aliskiren is uncertain. However, in the setting of its purported pathogenetic role in diabetic nephropathy, its upregulation in the diabetic heart suggests that it may also be involved in the development of diabetic cardiomyopathy.
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22

Ablation of fatty acid transporter CD36 protects against western type diet-related cardiac dysfunction following pressure overload in mice: modeling diabetic cardiomyopathy

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Background and aims: An unbalance in cardiac substrate supply, with increased utilization of long-chain fatty acids (LCFA) over glucose, is regarded to contribute to the functional and structural changes in diabetic cardiomyopathy (DCM). In the hypertrophied heart an unbalance in substrate utilization develops, with increased glucose utilization over LCFA. We investigated whether manipulation of cardiac substrate utilization by i) a western-type diet (WTD) to increase LCFA supply, ii) fatty acid translocase CD36 ablation to decrease LCFA supply, or iii) a combination of both, influences the development of cardiac hypertrophy and failure during pressure overload.
Materials and methods: Twelve weeks old male wildtype (WT) and CD36$^{-/-}$ mice (KO) were started on chow or WTD (42% fat, 29% sucrose; t=0). Ten weeks after initiation of the diet, groups were subdivided in sham-operated mice and mice that underwent aortic constriction (AC) to induce pressure overload (t=10). Diet exposure was continued throughout the study. Six weeks after surgery, mice (n=5-8 for each group) were sacrificed (t=16). Using echocardiography, cardiac systolic function (fractional shortening; FS%) was determined at t=0, 10 and 16. For statistical analysis two-way ANOVA with Bonferroni post-tests were performed.
Results: WT and KO mice on either diet displayed similar cardiac function parameters at t=0 and t=10, suggesting that 10 weeks of WTD, CD36 ablation or a combination of both, does not influence cardiac function. At t=16, heart weight (HW) and FS% from sham operated mice where alike, suggesting that CD36 ablation, WTD or a combination of both, do not affect cardiac function. However, differing responses where seen in mice that underwent AC (table 1). Whereas WTD resulted in significant cardiac hypertrophy and systolic dysfunction after AC in WT mice, it had protective effects with respect to cardiac remodeling and functional changes in KO mice.

	WT		KO	
	chow	WTD	chow	WTD
	AC versus sham	AC versus sham	AC versus sham	AC versus sham
Heart weight (% alteration)	+46 % *	+ 81 % *	+ 100 % **	+ 18 %
FS% (% alteration)	no change	- 35 %	- 27 %	no change

Table 1: Effect of WTD versus chow on heart weight and function in KO and WT mice 6 weeks after aortic constriction. *: p<0.05, **: p<0.001

Conclusion: In contrast to the effects observed in WT mice, exposure of KO to a WTD protected against the adverse effects of pressure overload on cardiac remodeling and function. These data provide the first evidence of an interaction between CD36 and nutrient supply to modulate cardiac function in compromised hearts in a model for DCM. These findings may provide therapeutic targets for ameliorating cardiac outcome in patients with DCM.
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23

Predictors of glucose lowering therapy intensification among patients with diabetes hospitalized with acute myocardial infarction

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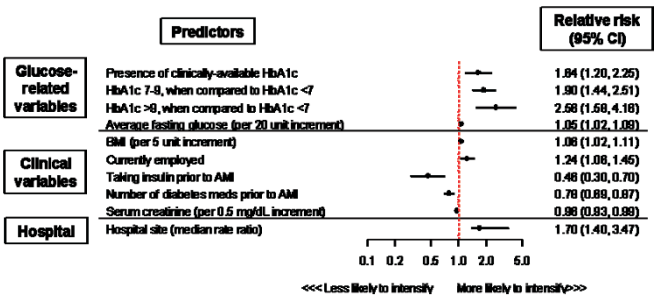
Background and aims: Poor glucose control is common among patients (pts) with diabetes (DM) hospitalized for acute myocardial infarction (AMI). Thus, AMI hospitalization may present an opportunity to modify glucose management, but rates and predictors of glucose therapy intensification (GTI) after AMI have not been described.

Materials and methods: TRIUMPH is a prospective registry of AMI pts from 26 US centers. GTI was defined as an increased dose or addition of a new oral antihyperglycemic drug, initiation of insulin, or increased total daily insulin dose by ≥20% from baseline. Changing one oral agent to another was not counted as GTI. Independent predictors of GTI were identified using hierarchical Poisson regression (with hospital as a random effect). Candidate variables were those associated with GTI in univariate analysis (p<0.1 level), or those potentially related to DM severity and management. Since practice patterns vary across institutions, we evaluated the association of hospital site with GTI using the median rate ratio (MRR), which describes how likely two identical pts would receive GTI at one hospital as compared with another.
Results: Between 2005-07, TRIUMPH enrolled 2755 consecutive AMI pts of whom 724 (26%) had type 2 DM. Of pts with DM, 505 (70%) had HbA_{1c} measured clinically: HbA_{1c} level was <7% in 177 pts (35%), 7-9% in 196 pts (39%), and >9% in 132 pts (26%). Overall, 234 pts with DM were discharged with GTI (32%); these pts had fewer DM meds (mean 0.8 vs 1.1, p<0.001) and less frequent insulin therapy (15% vs 37%, p<0.001) on admission, higher initial (272 vs 205 mg/dL, p<0.001) and average fasting glucose (171 vs 148 mg/dL, p<0.001), more HbA_{1c} assessment during hospitalization (80% vs 64%, p<0.001), and higher HbA_{1c} levels (9.1% vs 7.7%, p<0.001). Independent predictors of GTI are listed in the Figure. MRR was 1.61, suggesting significant variability in GTI prescription across institutions.
Conclusion: Nearly two-thirds of AMI pts with DM have suboptimal or poor long-term glycemic control, but only a minority have GTI prior to discharge. Key among the independent predictors of GTI are hospital-based factors (HbA_{1c} assessment and hospital site), with >60% variation in GTI across hospitals likely reflecting the lack of clinical consensus for managing DM after AMI. These findings highlight the need to determine the relationship between different strategies of post-AMI glucose management and patient outcomes.



Supported by: sanofi-aventis USA

24

Secretory products of epicardial fat induce insulin resistance and impair cardiomyocytes function

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Background and aims: Epicardial fat (EF) is a visceral adipose tissue around the heart, which is not separated by a fascia. EF is a source of inflammatory cytokines which may interact with myocardium and coronary arteries. Increasing evidence suggests that the EF may play a role in the pathogenesis of cardiovascular diseases and special forms of cardiomyopathy.
The aim of this study is to analyse the secretion of EF compared to subcutaneous fat (SF) and its impact on the function of primary rat cardiomyocytes.
Materials and methods: 7 weeks old guinea pigs were fed with a high energy (HE) diet for 6 month. As a control we used a low energy (LE) diet. At the end of the feeding study, glucose tolerance tests (GTT) and an echocardiography (Echo) were performed. After sacrifice, we collected EF and SF and generated fat explants and conditioned media (CM). For analysis of cytokine secretion antibody arrays were used.
To analyse the effects of EF and SF on cardiomyocytes we incubated primary rat cardiomyocytes with CM or control media. Subsequently, we analysed insulin-stimulated signaling at the level of Akt Ser473-phosphorylation. Furthermore, functional alteration in cell shortening and Ca2+-transient fluxes were measured using a Contractility and Fluorescence system.

Results: Animals receiving a HE diet are characterized by reduced insulin sensitivity as demonstrated by GTT. Moreover, echo tests showed that these animals were characterised by a decreased left ventricular function of the heart at the level of the ejection fraction and the fractional shortening ($p< 0.05$). Analysis of CM showed that the two diets induced a different secretion pattern of various cytokines. For example, the secretion of Activin A was 4-fold higher in CM from HE compared to LE animals. Comparing different fat depots from one animal, differences in the secretion pattern could also be found. The secretion of Activin A was 10-fold higher in EF.
Treatment of cardiomyocytes with CM (EF) from HE animals results in a significantly decreased phosphorylation of Akt after stimulation with insulin compared to CM (EF) from LE animals ($p< 0.05$). Cardiomyocytes which were incubated with CM from SF also showed a decrease in the phosphorylation of Akt, but no significant difference between HE and LE explants could be found.
Contractility and Ca2+-transient experiments showed that steady-state shortening amplitude and intracellular Ca2+ peak after incubation with CM in cardiomyocytes are reduced. Furthermore, much less cardiomyocytes which were incubated with CM are able to contract after electrical stimulus compared to control media. Long term incubation of CM showed no further impairment when compared to short term incubation.
Conclusion: In summary, HE feeding induces starting insulin resistance and impaired left ventricular function. Isolated EF from HE animals was characterized by a different secretion pattern as compared to LE but also to SF. CM generated from EF causes dysfunction of primary rat cardiomyocytes since these cells are characterized by impaired insulin signaling, reduced contractility and Ca2+-influx. Our results suggest that the secretion of various cytokines by EF with its direct effect on the myocardium might be involved in obesity-related cardiomyopathy.

OP 05 Genes, autoimmunity and type 1 diabetes

25

Predictors of associated autoimmune diseases in families with type 1 diabetes. Results from the type 1 diabetes genetics consortium (T1DGC)
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Background and aims: The T1DGC is an international effort aimed at the study of the genetics and pathogenesis of type 1 diabetes (T1D). Families with at least 2 affected siblings have been included in order to find genes associated with risk/protection for T1D. The aim of our study was to find predictors of the presence of associated autoimmune diseases (AAID) in the T1DGC dataset available on the 1st October 2008.
Materials and methods: Clinical information was obtained using questionnaires at each of the participating centres, anti-GAD and anti-IA2 (RIA) were measured in serum from the affected siblings (Bristol, UK; Southbank, AUS and Aurora, CO, US) and HLA-genotyping was performed (PCR-based, sequence-specific oligonucleotide probe system) centrally (Oakland, CA, US; Melbourne, Australia and Malmö, Sweden). Presence of AAID was defined by the information obtained from the questionnaires. Statistical analysis was performed using R. Differences between groups were analysed using Wilcoxon-Mann-Whitney's test and chi-squared. To assess the predictors of AAID, a multivariate logistic regression analysis was performed on the affected siblings, including age of onset, time since diagnosis, antibody positivity and risk and protective HLA haplotypes as independent variables. We describe the results obtained from the families where DRB1-DQA-DQB1 haplotypes could be unequivocally estimated. High-risk and protective haplotypes were defined as the 4 most susceptible and protective, respectively, according to a previous report from the T1DGC.
Results: Information from 3310 families was available at the time of analysis and unambiguous HLA haplotypes were identifiable in 1864 families (8327 participants). 12.9% of all the participants and 13.1% of the T1D affected siblings had at least 1 AAID. Among the latter, AAID were distributed as follows: 63.5% thyroid, 19.44% celiac, 7.13% psoriasis, 6.7% vitiligo, 5.8% rheumathoid arthritis, 3.67% inflammatory bowel and 2,37% other diseases). Age of diabetes onset was similar, but current age (23.5 vs 18.4 (mean difference 4.0-6.3) years, p= 2.2e-16) and time since diagnosis (13.0 vs 8.8, (mean difference 3.2-5.2) years, p=2.2e-16) were higher in the siblings with AAID. Anti-GAD positivity was similar whereas anti-IA2 positivity was less frequent in subjects with AAID (39.9 vs 49.6%, p=0.0001). Risk and protective DRB1-DQA1-DQB1 haplotype distributions were similar in the group of siblings with T1D with and without AAID. In the logistic regression analysis, only age of onset (p = 6.3e-6) time since diagnosis (OR: 1.05 (CI 1.04-1.06) per year, p = 2e-16) and a-GAD positivity (OR:1.35 (CI 1.09-1.67) p=0.005) were significant predictors of AAID.
Conclusion: In the current study, the older the onset of T1D and the longer the duration of follow-up, the higher the risk of AAID. Furthermore, the positivity of a-GAD was also independently associated with AAID once adjusted for duration of T1D.
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26

Prognostic relevance of autoantibody-based screening in the general population - results from the Karlsburg type 1 Diabetes Risk Study after 14 years
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Background and aims: The cumulative risk for progression to Type I Diabetes mellitus (T1DM) as well as the prognostic relevance of T1DM-related

autoantibodies (AAb) and genetic markers like the HLA-DQB1 has been examined in several studies in first-degree relatives of patients. The aim of the study was to assess the cumulative T1DM risk in the general population without first-degree heredity using a combined screening for established AAbs against Glutamate decarboxylase (GADA), Protein tyrosine phosphatase (IA-2A) and Insulin (IAA) as well as HLA-DQB1 genotyping.
Materials and methods: 14,742 schoolchildren (7,508 female, 7,234 male, age 6-17 years) were enrolled in the Karlsburg Type I Diabetes Risk Study and tested for GADA, IA-2A and IAA using radio-ligand binding assays. Determination of all 3 AAbs was possible in 13,165 subjects. Children who tested positive for at least one AAb in the screening (cut-off at the 98th percentile) were invited for follow-up testing (cut-off at the 99th percentile). Children which were positive in the 1st follow-up were repeatedly tested in the further study course. HLA-DQB1 genotyping was performed at 1st follow-up by PCR and DNA hybridization with specific probes. All HLA-DQB1 alleles other than the T1DM-associated alleles *0302 and *02 and the dominant protective allele *0602 were considered as neutral. Two-sided X^2 statistics and Kaplan-Meier survival analysis were used for statistical analysis (SPSS 15.0).
Results: 4.03% (530/13,165) subjects were tested positive for at least one AAb in the initial screening, with 3.6% (474/13,165) of them having singular AAbs and 0.43% (56/13,165) having multiple AAbs. At the first follow-up, 1.18% (156/13,165) schoolchildren were tested positive for at least one AAb. 0.92% (n=121) had singular and 0.27% (n=35) multiple AAbs. 65/114 HLA-DQB1-typed children with singular AAbs had at least one diabetes-associated HLA-DQB1 allele, compared to 33/35 with multiple AAbs (p<0.001). Up to now, 24 subjects (0.18%) progressed to T1DM. Two of these children were initially tested positive for one AAb but had diabetes-associated HLA-DQB1 alleles. 20/24 of these probands had multiple AAbs, of those 19/20 had diabetes-associated HLA-DQB1 alleles and 1/20 has neutral alleles. 2/24 children who became diabetic were initially AAb-negative but were found to have multiple AAb at manifestation. The cumulative 10-year-risk for progression to T1DM was 1.2% for subjects with singular AAbs compared to 65.1% for subjects with multiple AAbs (p<0.001). No child with the dominant-protective HLA-DQB1 allele *0602 became diabetic. The cumulative 10-year-risk for developing T1DM is significantly higher for subjects with diabetes-associated HLA-DQB1-alleles (30.3%) compared to children with neutral alleles (3.7%, p=0.012).
Conclusion: The results of this study demonstrate that the T1DM risk for subjects from the general population is significantly associated with multiple AAb positivity and with the detection of diabetes-associated HLA-DQB1 alleles. It can be concluded that the prognostic value of a combined testing for diabetes-associated AAbs and immunogenetic markers in the general population is comparable to results shown for first-degree relatives.
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27

Genome-wide mapping of variants associated with type 1 diabetes nephropathy
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Background and aims: Both environment and genetic factors are likely to play a role in the development of diabetic nephropathy (DN). The genetic factors related to DN have not been fully identified in earlier studies. High-throughput genomewide association methods may provide a useful tool in the determination of genetics of DN.
Materials and methods: This study uses genome-wide association (GWA) analysis in a case-control study setting. The Finnish Diabetic Nephropathy Study (FinnDiane) is a nationwide, comprehensive large-scale multi-center study of patients with type 1 diabetes (T1D). We initiated a GWA scan of 4000 T1D patients in May 2008. Our aim is to genotype altogether 1500 T1D cases with DN (micro- or macro-albuminnuria or ESRD) and 2500 normoalbuminuric controls with T1D duration of at least 16 years. So far approximately 1900 patients have been genotyped (1037 cases and 859 controls).
The GWA scan is performed on an Illumina platform (Illumina 610Quad chip) including approximately 599.000 probes targeting multiallelic SNPs, situated both within and outside of genes. To ensure high data quality, we excluded SNPs with low genotyping rate, low minor allele frequency or significant deviation from Hardy-Weinberg equilibrium within controls. Unique individuals with high genotyping rate, correct sex and no close relatives were accepted. This resulted in 950 cases and 809 controls at 551953 SNPs passing the quality control in this preliminary analysis. Plink software was used for data handling and analysis. Association was calculated for each SNP with allelic Fisher's test, which assumes the association to be additive.
Results: 8 SNPs with p-values smaller than 10-5 were found in 5 different chromosomal locations (1q, 4q, 6q, 12q, 18q with p-values 2.3x10^-7 - 9.3x10^-6). Further putative SNPs include 53 SNPs with p-value smaller than 10^-4 and 613 SNPs with p-value smaller than 10^-3.
Conclusion: These preliminary data suggest that there might be new genomic regions related to DN, although the p-values did not survive rigorous correction for multiple testing. Nevertheless, the power of GWA is known to increase rapidly with the sample size. Thus we hope that the ongoing genotyping of more patients will give more convincing results that survive also replicative analysis.
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28

A genome-wide SNPxSNP search for epistasis identifies gene-gene interaction in type 1 diabetes
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Background and aims: Common complex diseases, such as type 1 diabetes, are influenced by multiple genetic and environmental risk factors. Many new risk loci have been identified in the last two years, all with relatively small risk ratios. There is no doubt that additional risk loci with independent effects remain to be identified in order to explain the full genetic component in the susceptibility to type 1 diabetes. However there is also a possibility that gene-gene interactions may contribute to disease risk. Epistatic effects have been identified between classic loci, such as the *HLA* genes and *INS* as well as recently *PTPN22*. However, so far no genome-wide search for epistatic effects has been conducted. Using genome-wide association data we conducted a SNPxSNP search for epistasis in order to address the question of gene-gene interactions that affect the risk of type 1 diabetes.
Materials and methods: In phase 1 of the study 600 Danish cases and controls were analysed for 50,000 SNPs genotyped on Affymetrix 50K arrays. Logistic regression was used to analyse all pair-wise combinations of SNPs for deviation from a log-additive risk model. SNP pairs with lowest p-values were prioritised for replication in phase 2 and genotyped using TaqMan assays in a larger case-control material consisting of 1500 Danish cases and 1850 blood donor controls. All pair-wise combinations were again analysed for epistasis using logistic regression. Expression profiles of selected transcripts were analysed using low density arrays (Applied Biosystems) in a preparation of human islets from 9 donors before and after cytokine stimulation, and in lymphocytes from 9 male type 1 diabetic patients and 8 healthy male controls. Relative expression was analysed using the ΔΔCt method and significance of expression level differences was evaluated using paired t-tests in islets and f- and t-tests in lymphocytes.
Results: In phase 1 we identified three SNP pairs that were prioritised for replication (p<3e-08). One of the interactions was replicated in phase 2 using the same log-additive model (p=0.02, OR=1.23). The two interacting SNPs are located in an intron of *IRF2* on chromosome 4 and downstream *XCL1* on chromosome 1. None of the SNPs identified through epistasis were significant in a single marker analysis. IRF2 was found to be significantly upregulated after cytokine stimulation in human islets (p=0.002). Furthermore a trend towards a lower expression of XCL1 was found in lymphocytes from diabetic individuals compared to healthy controls (p=0.07).
Conclusion: We have identified and replicated an epistatic effect of two SNPs that were associated with the risk of type 1 diabetes. Furthermore functional effects of the corresponding gene IRF2 was demonstrated in a model system of human type 1 diabetes. IRF2 and XCL1 have previously been implicated in human immune-mediated diseases. Their exact genetic and functional involvement in the pathogenesis of type 1 diabetes remains to be elucidated and replicated in independent studies.
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29

Strong association between *SLC30A8* gene variant and ZnT8 autoantibody specificity during disease progression in two independent cohorts of children with newly diagnosed type 1 diabetes

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Background and aims: The zinc transporter, ZnT8, is newly established as a major autoantigen in type 1 diabetes and the common non-synonymous variant, rs13266634, of the SLC30A8 gene was recently found to determine the ZnT8 autoantibody (ab) specificity in a Caucasian and Japanese type 1 diabetes population. The aim of this study was to explore the association between the rs13266634 variant and the ZnT8 autoantibody specificity (arginine versus tryptophan consisting epitope) during the first year after disease diagnosis in two independent cohorts of children with newly diagnosed type 1 diabetes. Furthermore, we investigated the association of ZnT8 autoantibodies with age of onset and with disease progression (loss of residual beta-cell function).
Materials and methods: The Hvidøre cohort consists of 275 children aged<16 years from 22 paediatric centres in 18 countries; the Danish cohort consists of 130 children aged<16 years from 4 paediatric centres. All patients were newly diagnosed with type 1 diabetes. A 90-minutes Boost-test was carried out in each patient at 1, 6, 12 months to characterize the residual beta-cell function. Genotyping was done at Steno Diabetes Center. ZnT8_arg and trp ab determination was done by RIA assay at UMAS, Malmø.
Results: The CC genotype of the rs13266634 variant was highly associated with ZnT8-arg ab 1, 6 and 12 months after onset in both cohorts (with TT as ref group: log est.:1.5, 1.5, 1.3 corresponding p-values: <0.0001, Hvidøre cohort; log est.:1.9, 1.8, 2.2 corresponding p-values: 0.003, 0.004, 0.0002, Danish cohort). By contrast the TT genotype was associated with ZnT8-trp ab 1, 6 and 12 months after onset in both cohorts (with CC as ref group: log est.:1.6, 1.5, 1.5 corresponding p-values: <0.0001, Hvidøre cohort; log est.:2.2, 2.2, 1.4 corresponding p-values: <0.0001 for 1 and 6 months and p=0.006 for 12 month, Danish cohort). We found an allele dosage effect on the ZnT8 ab titers; CC carriers presented app. twice the level of ZnT8-arg ab compared to CT carriers (1 mth est.:1.6 vs 0.9, respectively) and TT carriers presented also higher level of ZnT8-trp ab compared to CT carriers (1 mth est.:1.6 vs 0.6, respectively). The youngest age group (0-4 yrs) had significantly lower ZnT8-arg ab level compared with the older age group at 1 month and at 12 months (12 mth: est.:-0.7 p=0.02). Loss of beta-cell function did not associate significantly with ZnT8 ab levels.
Conclusion: The strong genetic determination of the ZnT8 ab specificity is sustained the first 12 months of disease progression in two independent cohorts of children with new onset type 1 diabetes. Furthermore, there was a clear allele dosage effect on the ZnT8 ab titer which was maintained during the progression of the disease. The youngest patients continued to exhibit the lowest level of ZnT8-arg ab during first year after disease diagnosis but ZnT8 ab did not associate with loss of beta-cell function. These data strongly suggest a humoral involvement of ZnT8 ab in the progression of type 1 diabetes with no direct effect on the residual beta-cell function.

30

Gene expression differences in pancreas and brain between diabetes-prone BB/OK rats and their diabetes protected congenic BB.6S derivatives

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Background and aims: Congenic BB.6S rats are characterized by a drastically reduction of diabetes frequency by 94% compared with their parental BB/OK rat strain. To increase the chance of identification of the appropriate protective gene(s), the expression of 31,000 genes in pancreas and brain of BB/OK and BB.6S rats was studied using GeneChip microarray analysis.
Materials and methods: For the hybridization of Affymetrix GeneChips we use pooled samples of 20 individual rats (BB/OK 10M:10F; BB.6S 10M:10F) with an age of 8 days following the protocol described in the Affymetrix GeneChip Expression Analysis Technical Manual at the Interdisziplinäres Zentrum für klinische Forschung Leipzig (IZKF), Medizinische Fakultät der Universität Leipzig. In addition one gene was sequenced.
Results: By pair-wise comparisons between BB/OK and BB.6S rats, a set of 35 genes was identified in brain and pancreas representing genes whose level of expression importantly differed between BB/OK and BB.6S rats. The genes were located on 14 chromosomes (2, 3, 6, 7, 8, 9, 11, 12, 13, 15, 16, 18,19). Only one gene mapped on chromosome 6q32 within the region which was exchanged in the congenic strain BB.6S by that of SHR rats. According to the function of genes, 8 out of 36 were involved in ion binding, 8 in protein binding, 6 were enzymes, 5 hypothetical proteins 3 were involved in cell communication, 2 in protein transport and particularly 1 gene plays a role in cancer, one in ion transporter and one was unknown protein. The gene on chromosome 6q32 was a hypothetical protein which was sequenced. A SNP was found between BB and SHR as well as their congenic BB.6S.
Conclusion: The data clearly show that the chromosomal exchange in BB.6S rats led to gene expression differences between BB/OK and BB.6S rats which were mainly located on other chromosomes than 6. Only one gene on chromosome 6 characterized as hypothetical protein was differently expressed between BB/OK and BB.6S. The sequence of this gene was different between BB/OK and BB.6S so that it could be a candidate for diabetes protection.
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OP 6 Inflammation, insulin action and type 2 diabetes

31

Mice lacking lipocalin-2 are protected from developing insulin resistance associated with aging and obesity

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Background and aims: Obesity is characterized as a low-grade inflammation of the adipose tissue and increased circulating concentrations of pro-inflammatory cytokines. The "inflamed" adipose tissue can secrete a large amount of pro-inflammatory adipokines / cytokines that act as mediators for obesity-related metabolic syndrome. Previous studies have shown that the expression and production of lipocalin-2, a pro-inflammatory adipokine, are elevated in obese animal and human subjects. In this study, we aim to evaluate whether lipocalin-2 deficiency can protect the mice from developing systemic insulin resistance.
Methods and results: C57 wildtype and lipocalin-2 deficient mice (Lcn2-KO) were given free access to high fat diet from the age of 4 weeks for the induction of dietary obesity. In addition, genetic obesity was induced by cross-breeding male C57BL/6J db/+ mice with female Lcn2-KO mice to generate the leptin receptor $(Lepr)^{-/-}$/lipocalin-$2^{-/-}$ double knockout mice (DKO). Deficiency of lipocalin-2 in mice prevents age-associated decline in insulin sensitivity and the development of insulin resistance induced by high fat diet and genetically obesity. In diet-induced obesity, lower fasting blood glucose was consistently observed in Lcn2-KO mice (5.6±1.18 mmol/L vs that in C57 wildtype mice, 8.4±1.51 mmol/L, $p<0.05$). HOMA-IR index was increased by 77.93% in C57 wildtype after 18-weeks high fat diet feeding. On the other hand, in mice under normal diet, at the age of 21-week, the fasting glucose levels of Lcn2-KO mice were significantly lower than those of C57 mice (3.62±0.35 mmol/L in Lcn2-KO mice vs 5.05±0.3 mmol/L in C57 wildtype). Both fasting serum insulin levels and HOMO-IR indexes were largely reduced in 24-week old Lcn2-KO mice, by 44.6% and 79.35% respectively comparing to those of the control mice. Similar phenomena were observed in DKO mice, which did not develop hyperinsulinemia as in the leptin receptor $(Lepr)^{-/-}$ (db/db) counterparts (516.778.421±73.225 μU/ml in wildtype db/db mice, 97.67±35.63 μU/ml in DKO mice, $p<0.05$). Lipocalin-2 deficiency attenuated peripheral insulin resistance and macrophage infiltration in adipose tissue. Further analysis revealed that obesity- and lipopolysaccharide-induced expressions of TNFα, a pro-inflammatory cytokine, were completed blocked by lipocalin-2 deficiency in epididymal adipose tissue as revealed by quantitative PCR analysis On the other hand, increased expression of IκB found in Lcn2-KO mice while incubation of lipocalin-2 in differentiated 3T3-L1 adipocytes lowered IκB expression and increased NFκB activity.
Conclusion: Lipocalin-2 plays critical roles in causing obesity-induced insulin resistance through regulating TNFα expressions, which might account for its systematic metabolic and pro-inflammatory activities.
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32

Chronic apelin treatment effects on lipid metabolism in wild type and insulin-resistant mice

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Background and aims: We have recently shown that apelin stimulates glucose utilization in skeletal muscle through an AMP-activated protein kinase (AMPK) dependent pathway. Since AMPK is also involved in lipid metabolism, we studied apelin chronic treatment effects on lipid plasma parameters, fatty acid oxidation and storage in the muscle of normal and insulin resistant mice.
Materials and methods: Chow fed or high fat fed C57bl6/J mice were ip injected with apelin (0,1μmol/kg/day) during 28 days. Total [1-^{14}C] palmitate oxidation was determined in soleus muscle as the sum of $^{14}CO_2$ release (corresponding to complete fatty acids oxidation) and acid-soluble metabolites (corresponding to the incomplete oxidation). Respiratory quotient (carbon dioxide production to oxygen consumption ratio) was measured by in vivo indirect calorimetry. Intramuscular triglycerides (IMTG) content was measured after extraction of muscle lipids. Plasma level of triglycerides (TG) and free fatty acids (FFA) were measured at the end of the treatment using commercial kits.
Results: In chow-fed mice, apelin treatment didn't change adipose tissue weight and body weight. TG plasma levels were significantly decreased in apelin treated mice and no change in plasma FFA concentration was observed. Palmitate oxidation was increased in soleus muscle (control 224 ± 42 vs apelin 456 ± 33 nmol/g of proteins, $p<0.001$) and IMTG content was decreased by 52% ($p<0.01$) in apelin-treated mice. In insulin resistant mice, apelin treatment didn't change neither adipose tissue and body weight or TG and FFA plasma levels. Moreover, total palmitate oxidation was increased (control 379 ± 40 vs apelin 642 ± 132 nmol/g of proteins, $p<0.05$). Of note, apelin increased complete fatty acid oxidation while no variation of incomplete fatty acids oxidation was observed. Apelin treatment decreased respiratory quotient which confirms in vivo a better fatty acid utilization in apelin treated mice.
Conclusion: Intramuscular triglycerides and incomplete fatty acid oxidation intermediates are known to be involved in insulin resistance development. Thus, by decreasing deleterious lipid accumulation and by stimulating fatty acid oxidation in muscle, apelin chronic treatment could improve insulin sensitivity.

33

Circulating visfatin levels reduced in patients with non-alcoholic fatty liver disease irrespective of type 2 diabetes mellitus status

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Background and aims: Visfatin/PBEF/Nampt, primarily produced by the liver, represents a novel adipokine with diabetogenic and immunomodulatory properties implicated in insulin resistance (IR) pathophysiology in patients with obesity and type 2 diabetes mellitus (T2DM). The influence of visfatin may be further exacerbated in non-alcoholic fatty liver disease (NAFLD), where several adipocytokines have been implicated in its pathogenesis. This study aimed to examine the influence of visfatin in NAFLD patients with/out T2DM; as such, we investigated (1) whether normal liver function impairment influenced visfatin production/expression, and (2) the relationship between serum visfatin levels and other adipocytokines, markers of IR and inflammation, in NAFLD patients with/out T2DM.
Materials and methods: Fasting human serum samples were collected from 104 patients (Age: 49.6±12.46years; BMI: 34.2±5.61kg/m^2; 69 males, 35 females) with biopsy confirmed NAFLD (71: Non-Diabetic (ND), 33: T2DM) and an additional 23 ND control patients without NAFLD (Age: 44.6±9.85years; BMI: 26.4±4.52kg/m^2; 8 males, 15 females). Serum visfatin, TNF-α, sCD14, leptin, adiponectin, resistin and insulin levels were assessed. Fasting glucose levels were analysed via a glucose oxidase method and IR calculated via HOMA. Inter-group comparisons were assessed via student's t-test. Correlations were calculated via a Pearsons Correlation Coefficient test. The significance threshold was $p<0.05$.
Results: Comparison between patients with NAFLD and ND controls without NAFLD revealed a significant increase in BMI ($p<0.001$), HOMA-IR ($p<0.05$) and circulating resistin ($p<0.01$) and insulin ($p<0.05$) concentrations, whilst circulating visfatin and TNF-α ($p<0.001$) levels were reduced with NAFLD. Visfatin concentrations remained similar following sub-division into level of NAFLD severity (FL, $p<0.001$; NASH, $p<0.001$; cirrhosis, $p<0.05$), although an increase was observed in serum visfatin levels from FL to NASH ($p<0.05$). Serum visfatin concentrations negatively correlated with BMI and insulin, dependent upon T2DM status. T2DM patients with NAFLD had more advanced levels of fibrosis (42%) than ND NAFLD subjects (11%). Further comparison between NAFLD patients with/out T2DM identified that serum visfatin was elevated in NAFLD T2DM patients however, these levels

were significantly lower than noted in the ND controls (ND, $p<0.001$; T2DM, $p<0.01$, respectively). Furthermore, a significant increase was exhibited in serum insulin ($p<0.05$), leptin (p<0.05), glucose ($p<0.001$), HOMA-IR ($p<0.01$) and sCD14 ($p<0.01$) levels in T2DM NAFLD vs. ND NAFLD patients.
Conclusion: The present study determined circulating visfatin concentration to be reduced in NAFLD, irrespective of T2DM status independent of severity of liver disease or fibrotic damage. In addition, this study illustrated a clear positive association between human NAFLD, increased IR, hyperresistinemia, hyperinsulinemia and adiposity. Taken together, these findings suggest that decreased circulating visfatin may be a potential biomarker of impaired liver function in NAFLD, whilst resistin shows a positive correlation with NAFLD pathogenesis.
Supported by: the DoH

34

Chemerin as an adipokine related to insulin resistance: chemerin decrease after bariatric surgery in morbidly obese patients
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Background and aims: Adipose tissue is a secretory and endocrine active organ producing a variety of bioactive proteins including the newly described adipokine and chemokine chemerin. We could recently demonstrate that chemerin induces insulin resistance in skeletal muscle cells in vitro. Conversely, a recent report suggests that there is a correlation of chemerin levels with BMI but not with insulin resistance in normal weight patients. The role of chemerin in obesity and type 2 diabetes remains unclear and has not been investigated after bariatric surgery.
Materials and methods: Chemerin plasma levels were measured in 60 morbidly obese female patients (BMI 50.0±1.0, age 43.1±1.5) before surgery as well as 3, 6 and 12 months after surgery. In a subgroup of 27 patients, chemerin levels were monitored for 2 years. At all time points blood levels of insulin, glucose, HbA1c, cholesterol, HDL, triglycerides, CRP, leptin, adiponectin and IL-6 were assessed in addition to weight and BMI.
Results: Chemerin concentrations are substantially elevated in obese patients compared to concentrations for normal weight persons (353.8±18.0 ng/ml compared to approximately 250 ng/ml described in the literature). Preoperatively, chemerin levels are correlated positively with body weight, BMI, CRP, IL-6 and insulin sensitivity (HOMA). Furthermore, chemerin is correlated negatively with HDL levels. After bariatric surgery, chemerin decreased significantly (275.6±17.9 ng/ml after 3 months, 255.9±12.0 ng/ml after 6 months and 253.0±14.9 ng/ml after 12 months) in parallel to leptin levels, but different from IL-6 that only displayed a marked reduction 12 months after surgery and adiponectin that was significantly increased after 6 and 12 months. In the subgroup of patients studied for 2 years, chemerin levels significantly decreased between 1 year and 2 years after surgery (preoperative 381.7±28.0 ng/ml, after 1year 290.7±26.9 ng/ml, after 2 years 215.1±14.7 ng/ml). 3 months after surgery chemerin levels only positively correlate with triglycerides, a correlation that was remaining apparent after 6 and 12 months. The prominent decrease of chemerin in the first 3 months after surgery was significantly associated with the increase in insulin sensitivity (HOMA) for this period. Analyzing diabetic patients (n=20) separately, a correlation of chemerin levels could be found with HOMA and HDL before surgery and with triglycerides 3 and 6 months after surgery. Differently in non-diabetic patients (n=37), chemerin correlates with BMI and IL-6 before surgery but not with HOMA or HDL.
Conclusion: This is the first study to demonstrate decreased chemerin plasma levels after bariatric surgery. The observed decrease in chemerin after bariatric surgery indicates together with our previous in vitro findings that chemerin might be a factor contributing to insulin resistance in obesity and the improvement of insulin sensitivity and other obesity-related morbidities after surgical intervention.

35

Adipokines induce degradation of IRS-1 in megakaryocytes resulting in insulin-resistant platelets
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Background and aims: Type 2 diabetes mellitus (T2DM) patients have a 2-8 fold higher risk for cardiovascular disease (CVD) and suffer from thrombotic complications. We demonstrated previously in normal platelets that insulin signals through IRS-1 and inhibits Ca^{2+} mobilization by interfering with Gi α-mediated suppression of cAMP, a platelet inhibitor. T2DM platelets are insulin-resistant and hyperactive which enhances the risk for thrombotic occlusion. In the present project we searched for the cause of insulin resistance in T2DM platelets. Since most of the properties of platelets are determined by the megakaryocyte and obese subjects are prone to develop insulin resistance, we investigated whether adipokines change the control of Ca^{2+} mobilization by insulin in the megakaryocyte.
Materials and methods: In megakaryocytic CHRF-288-11 cells incubated with adipokines and plasma from obese individuals and in T2DM platelets we measured (i) inhibition by insulin of thrombin-induced Ca^{2+} mobilization, (ii) phosphorylation of IRS-1 [Ser307] and protein kinase Bα [Ser473] as indicators of insulin signaling, (iii) expression of Suppressor Of Cytokine Signaling (SOCS) proteins which is under control of adipokines and, (iv) insulin signaling proteins.
Results: Leptin, resistin, PAI-1 and RBP-4 but not IL-6, TNFα and visfatin made cells resistant to Ca^{2+} suppression by insulin. Short contact with adipokines (2 h) aborted insulin signaling through IRS-1 illustrated by suppression of insulin-induced phosphorylation of IRS-1 and protein kinase Bα. The Ser/Thr phosphatase inhibitor cantharidin restored responses to those seen prior to adipokine addition demonstrating reversible insulin-resistance. Prolonged contact with adipokines (72 h) made cells unresponsive to phosphatase inhibition and revealed up-regulation of SOCS3 and IRS-1 degradation, demonstrating irreversible insulin resistance. Inhibition of adipokine signaling with the JAK2 inhibitor AG490 prevented cells from developing irreversible insulin-resistance. Poly-ubiquitination of IRS-1 by the proteosome inhibitor MG-132 was increased in leptin-treated cells (2 h). Indeed, incubation with leptin and resistin, but not IL-6 (24 h) resulted in decreased IRS-1 levels, whereas expression of insulin receptor, Giα2 and IRS-2 was unchanged. T2DM platelets but not platelets from healthy controls showed reduced levels of IRS-1. Plasma from metabolic syndrome subjects but not from matched controls mimicked the induction of insulin-resistance by adipokines in megakaryocytes. Insulin sensitivity was -13 ± 23% (2 hr) and -11.7 ± 20% (72 hr) compared with 61 ± 20% (2 hr) and 56 ± 16% (72 hr) in cells treated with obese and control plasma respectively (mean ± SEM, n=9). Leptin levels were increased in plasmas from the metabolic syndrome group compared to controls, whereas resistin levels were not different between groups.
Conclusion: This is a first report of insulin resistance induced by adipokines in the precursor of platelets, the megakaryocyte. The findings suggest that the increased CVD risk in T2DM patients is caused by synthesis of hyperactive platelets due to abnormal adipokine release.
Supported by: Dutch Diabetes Research Foundation

36

Acceleration of the interleukin-1 receptor antagonist (IL-1Ra) trajectory precedes the diagnosis of type 2 diabetes by 6 years: the Whitehall II prospective cohort study
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Background and aims: The proinflammatory cytokine interleukin-1ß (IL-1ß) inhibits beta-cell function and promotes beta-cell apoptosis, whereas IL-1 receptor antagonist (IL-1Ra), a naturally occurring inhibitor of IL-1ß, has been shown in a clinical trial to improve beta-cell function and glycaemic control in patients with type 2 diabetes (T2D). We recently reported high

levels of IL-1Ra to be associated with an increased risk of incident T2D, but this analysis was limited to a single serum sample from each individual. The present study exploits data from repeated measurements of IL-1Ra (i) to examine how serum IL-1Ra concentrations change during years preceding the manifestation of diabetes and (ii) to compare these trajectories with those from individuals who did not develop T2D.
Materials and methods: This is a case-cohort study within the Whitehall II cohort with a total of 2809 participants without T2D at baseline, 27.2% women, mean age at baseline 49.3 (SD 5.9) years and average BMI 25.3 (SD 3.5) kg/m^2. The study included 335 cases with incident T2D during the follow-up period and 2474 non-cases who remained T2D-free. Serum levels of IL-1Ra were measured at up to three time points for each participant (phase 3, the baseline: 1991-94; phase 5: 1997-99; and phase 7: 2003-04). Year 0 of the observation period was the year of diabetes diagnosis for cases and a randomly selected time point during follow-up for non-cases to approximate the follow-up time distribution of cases. IL-1Ra levels were traced backwards to participants' first participation in clinical screening. Multilevel models adjusted for age, sex and ethnicity were fitted to the data to assess changes in IL-1Ra during the preceding 13 years based on 755 serum samples in cases and 5095 serum samples in non-cases.
Results: Serum IL-1Ra levels were already higher in cases than non-cases 13 years before year 0. Mean (95% CI) levels were 404 (384-425) pg/ml in cases and 326 (314-339) pg/ml in non-cases. From 13 to 6 years before year 0, serum IL-1Ra levels increased only marginally in both cases and non-cases (1.5-2 pg/ml per year). From about 6 years before the manifestation of T2D, serum IL-1Ra concentrations of the cases showed a steep linear increase of about 20 pg/ml per year until the diagnosis, whereas the slope of the trajectory of the non-cases remained unaltered (P<0.0001 for the different slopes during the final 6 years). Adjustment for BMI and waist circumference as time-varying predictors had almost no impact on these trajectories.
Conclusion: IL-1Ra levels were already elevated more than 10 years before the manifestation of T2D and showed an accelerated increased during the last 6 years preceding the diagnosis (i.e., during a period also characterised by increases in plasma glucose levels and decreases in beta-cell function). These data provide support for the hypothesis of IL-1Ra upregulation to counterbalance metabolic and immunological disturbances preceding T2D.
IL-1Ra measurements were funded by a Medical Research Council (UK) New Investigator Award

OP 7 Beta cell regeneration and loss

37

Perinatal survivin expression is essential for the establishment of pancreatic beta cell mass
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Background and aims: The deficiency of functional β-cell mass is the common denominator in all forms of diabetes. Because new β-cells are primarily formed from the replication of mature pre-existing β-cells during adulthood, the establishment of adequate β-cell mass early in life is therefore critical in determining β-cell mass expansion in respond to metabolic alterations throughout the life of the organism. From late embryogenesis and neonatal periods, pancreatic β cells undergo dynamic remodeling to establish the normal β-cell mass with enhanced neogenesis, proliferation and apoptosis. The molecular mechanisms responsible for these processes are elusive. Survivin is an inhibitor of apoptosis, first described to be expressed exclusively in tumour and embryonic tissues with regulatory functions in mitosis and apoptosis. Our aim was to define the essential physiological role of survivin in the pancreas.
Materials and methods: Survivin expression profile was assessed in the mouse pancreas and we generated mice harboring a conditional deletion of survivin in pancreas using mice with a PDX-1 promoter-driven survivin knockout mouse by the Cre-loxP recombination system to determine the essential physiological function of survivin in the pancreas.
Results: Survivin was expressed within the pancreatic islets of mice from the second wave of pancreatic endocrine development at E15.5 to the postnatal 3 weeks. Targeted deletion of survivin in the pancreas resulted in a significant decline in β-cell mass throughout the perinatal period, leading to decreased insulin levels and glucose intolerance in adult. Survivin-deficient islets showed significantly decreased cell proliferation due to a delay in cell cycle progression with perturbations in cell cycle proteins such as phosphor RB, Cyclin-E, CDK2, p21, p27, Caspase-3 and Aurora B kinase. Islet development, islet architecture, microvasculature, and apoptosis were not affected by the absence of survivin in the pancreas.
Conclusion: Survivin expression in the pancreatic islets during the perinatal remodeling period is essential for the establishment of β-cell mass through cell cycle regulation. Survivin deficiency in the pancreas leads to defects in β-cell mass establishment early in life, resulting in a permanent decrease in β-cell expansion and glucose intolerance in adult.
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38

Regulation of beta cell mass during early life in somatostatin-deficient mice
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Background and aims: Early postnatal life represents a critical period when remodelling of the endocrine pancreas occurs, including proliferation of islet cells and their precursors, transient increases in apoptosis, and maturation of insulin secretory responses to glucose. Factors that regulate these processes are poorly understood. Somatostatin-secreting delta cells are abundant in the fetal and neonatal pancreas and since somatostatin acts to inhibit growth factor secretion and cell proliferation and promote apoptosis, the hormone may play a role in regulating islet expansion in early life. The aim of this project was to investigate the effect of somatostatin-deficiency on proliferation, apoptosis and beta cell expansion in the first three weeks of life in mice.
Materials and methods: Pancreases from wild-type or somatostatin-knockout (SST-/-) mice at 2, 7, 14, 21 days and 1 year of age were analysed for total pancreas, beta and alpha cell volumes by morphometry, and for cell proliferation and apoptosis by BrdU incorporation and TUNEL labelling, respectively. Activation of signalling pathways associated with proliferation and apoptosis was studied by Western blotting of tissue extracts.

Results: Significant decreases in pancreatic and beta cell volume were observed in pancreases from SST-/- mice in the first three weeks of life. Reduced beta cell volume (0.68±0.26 vs 2.95±0.7 mm^3, p<0.05) persisted to one year of age, whereas there were no significant differences in alpha cell or pancreatic volumes. BrdU incorporation into pancreatic cells was significantly reduced in SST-/- animals at 2 days (40.8±4.8 vs 117±19.5 BrdU-positive cells/mm^2, p<0.01) and TUNEL-positive cells increased at 14 days (235.7±58 vs 28.3±6.1 cells/mm^2, p<0.05). TUNEL positive nuclei were seen in all pancreatic cell types but were particularly abundant in pancreatic ducts. Decreased beta cell mass in SST-/- animals was not a consequence of impaired growth factor signalling through the Akt pathway, since Akt phosphorylation was increased 2.6-fold (p<0.01) and nuclear cyclin D2 increased 1.8-fold (p<0.05) in the SST-/- pancreas. However, levels of TGF-beta 1, a growth factor that stimulates apoptosis in epithelial cells and is implicated in tissue remodelling, were increased 2.5-fold (p<0.001) in the SST-/- pancreas. TGF-beta signalling is mediated by phosphorylation and nuclear translocation of SMAD proteins and SMAD phosphorylation was increased in the SST-/- pancreas.
Conclusion: Despite somatostatin being a negative regulator of growth, expansion of pancreatic beta cell mass was impaired in SST-/- mice as a consequence of reduced proliferation and increased apoptosis of beta cells and their precursors. Reduced expansion of beta cell mass was associated with increased TGF-beta 1 levels in the SST-/- pancreas and TGF-beta 1 may represent an important regulator of beta cell mass in early life.
Supported by: Diabetes UK

39

IGF-II reexpression in adult mice is essential for beta cell regeneration *in vivo*

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Background and aims: Identifying factors which can support beta cell regeneration and or prevent death is a key objective in diabetes research. The insulin-like growth factor (IGF) signaling system is well know in regulating growth and development of many tissues. Both IGF-1 and insulin have been shown to play important roles in adult adaptive increases in beta cell mass and in regeneration. Conversely, Insulin-like growth factor II (IGF-II), plays a critical role in fetal cell division and differentiation, but is generally believed to play no role in the adult pancreas under normal physiological conditions.
Materials and methods: We have employed the 'switchable' pInsMycERTAM mouse, which exhibits Myc-induced beta cell ablation, and is followed by complete beta cell regeneration over 3 months once Myc is deactivated. By crossing with IGF-II knockout mice we tested the hypothesis that IGF-II re-expression is important for beta cell regeneration in adult mice. Beta cell quantitative analyses were determined by immunohistochemistry and all results are expressed as means±SD and significance of differences are evaluated with Student's t test or ANOVA.
Results: Here we show for the first time that IGF-II is re-expressed early in the adult pancreas following beta cell ablation. Following subtotal beta cell ablation, subsequent regeneration was followed. By 4 days after Myc deactivation in pInsMycERTAM we already see some expansion of beta cell mass as determined by increasing cross-sectional area of insulin staining (0.00102±0.0002 to 0.00175±0.0003; p=0.0277). Whereas, in pInsMycERTAM /IGF-II KO mice this is completely prevented (0.00125±0.0011 to 0.00083±0.00013, p > 0.05). This was confirmed by cell counting- beta cell numbers increased by 1.5±0.3 folds, whereas in pInsMycERTAM /IGF-II KO mice cell numbers did not increase at all (0.8±0.2 fold change; p=0.0364). This early delay in beta cell regeneration is also seen at 3 months of recovery. At 96 days beta cell mass and numbers have returned to pre-ablation levels- in itself interesting given the fact that blood glucose levels were essentially normal from around 4 weeks and yet beta cell mass continues to increase until pre-ablation numbers are re-achieved. In pInsMycERTAM /IGF-II KO mice beta cell mass has also returned to similar levels to pre-ablation, but cell numbers remain substantially reduced suggesting that hyperplasia is compensating for impaired beta cell regeneration in IGF-II KO mice.
Conclusion: Our results demonstrate that early beta cell regeneration in adult mice depends on re-expression of IGF-II, suggesting the possibility that reactivation of an embryonic pathway contributes to this in adult mice. Moreover, full recovery of beta cell numbers is substantially impaired even after 3 months, though this is in part compensated by beta cell hypertrophy. This also suggests that IGF-II may be a novel potential target in treatment of diabetes.
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40

Islet cell proliferation is increased in human recent onset type 1 diabetes

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Background and aims: Type 1 diabetes (T1D) is a disease in which the β-cells of the islets of Langerhans are killed selectively by autoimmune-mediated attack. Very little information is available about the β-cell response to this immune assault in humans and, in particular, it is unclear whether endocrine cell proliferation can be re-initiated during this phase of the disease. Therefore, the aim of the present study was to assess the extent of islet endocrine cell proliferation in recent-onset T1D patients and to compare this with age-matched control patients.
Materials and methods: Paraffin-embedded sections from autopsy pancreases of 10 recent-onset T1D patients (mean age 14±3.8 years; range 1-42 years) and 12 non-diabetic controls (mean age 17.8±6 years; range 2-53 years) were employed. An antibody directed against Ki67, a protein expressed selectively during mitosis, was used to detect proliferating cells. Multiple antigen staining was performed with antibodies against insulin, glucagon and somatostatin to identify the islet cell subtypes which were Ki67-positive. Sections were examined using brightfield and fluorescence microscopy and islet morphometry was analysed using Adobe Photoshop CS4 software.
Results: The number of Ki67-positive ($Ki67^+$) endocrine cells within the islets of non-diabetic control patients was very low, such that only 1.1±0.3% of the islets contained any $Ki67^+$ cells in a given pancreatic section. By contrast, the percentage of islets containing $Ki67^+$ endocrine cells was increased markedly in recent-onset T1D patients (to 10.88±2.5%; p<0.005) The average number of $Ki67^+$ endocrine cells per islet section in those islets with $Ki67^+$ cells was also increased significantly, from a mean of 1.14±0.09 cells/islet section in controls to 1.91±0.12 cells/islet section in T1D patients (p<0.001). Dual-labelling studies revealed that the increase in $Ki67^+$ staining occurred in both α- and β-cells whereas the absolute numbers of $Ki67^+$ δ-cells were insufficient to allow firm conclusions to be drawn about their proliferative potential. In controls, a total of 3466 islets were studied (mean cell number = 124 cells/islet section) and these yielded labelling indices of 0.008% for β-cells and 0.006% for α-cells. In the T1D patients, the $Ki67^+$ labelling index (measured in a total of 1280 islets, of which approximately 75% were insulin positive) was raised to 0.070% for β-cells and to 0.066% for α-cells Thus, the $Ki67^+$ labelling index was increased by approximately 10-fold for both α- and β-cells in recent-onset T1D. $Ki67^+$ α-cells were present in both insulin-positive and insulin-negative islets.
Conclusion: This study reveals that both α- and β-cells cells undergo a marked increase in proliferation during the progression of T1D in humans, suggesting that endocrine cell proliferation is re-initiated in response to the autoimmune attack associated with T1D. Identification of the stimulus mediating this increase may provide a means for therapeutic intervention to restore β-cell numbers in patients who are newly diagnosed with T1D.
Supported by: JDRF

41

Human islets contain a population of mesenchymal stem cell capable of differentiation toward the endocrine lineage

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Background and aims: Islet transplantation is a promising treatment strategy in type-1 diabetes, but the scarcity of donors limits its widespread application. *In vivo* islet (re)generation would be the best therapeutic approach, although it is unclear whether this approach is possible. *In vitro* islet generation from adult stem cells represents an attractive alternative. So far, embryonic stem cells as well as mesenchymal stem cells from different origins such as bone marrow and adipose tissue have been evaluated for their ability to form beta cell-like cells. However, most of the studies showed an incomplete differentiation process leading to a low and often unregulated insulin production. In this study we describe the isolation and characterization of a mesenchymal stem cell (MSC) population from islets from human donors, here called islet-

derived cells (IDC). In contrast to bone marrow-derived MSC, human IDC have the capacity to aggregate, form clusters, and to induce the expression of insulin, glucagon and somatostatin. However, their origin still has to be clarified.
Materials and methods: Gene expression was analyzed and quantified by RT-PCR and Real-time PCR, respectively. Flow cytometry and immunofluorescence coupled to confocal microscopy were used to characterize and identify the cells of interest.
Results: After expansion, hIDC isolated from human donors express the mesenchymal stem cells markers CD105, CD90, CD73, CD44, CD29, and CD13 on their surface, synthesize nestin and vimentin, and can differentiate to adipocytes and osteocytes. Also, hIDC were found to express the pericyte markers CD146, NG2, αSMA and PDGF-Rβ. However, von Willebrand Factor, CD31, CD34 and CD45 are not expressed, demonstrating that hIDC are neither of hematopoietic nor of endothelial origin. Remarkably, and in contrast with other pancreatic progenitors, hIDC do not express the ductal markers CK19 and CA19.9. Using the combination of surface markers CD90 and CD105 for immunoflowcytometry we demonstrate that human islets contain 2.0±0.8 % CD105/CD90 double-positive cells. Confocal microscopy on freshly isolated islets positioned these cells within the islets.
Conclusion: From our data we conclude that human islets contain a population of mesenchymal stem cells with the unique capacity to differentiate toward the endocrine pathway. The identification of stem cells in human islets offers new avenues that can be explored for *ex vivo* and potentially *in vivo* islet regeneration.
Supported by: Dutch Diabetes Foundation

42

Islet autoantibodies in patients receiving pancreas transplantation alone
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Background and aims: Pancreas transplantation alone (PTA) in subjects with type 1 diabetes (T1D) and preserved kidney function is an established clinical procedure able to restore an endogenous and self-regulating insulin secretion and normalize blood glucose levels. In previous studies, post-transplant reappearance or titre increase of islet autoantibodies (Ab) was associated with transplant failure in a proportion of patients after islet or simultaneous pancreas and kidney transplantation. No data are available on PTA. Aim of this study was to investigate islet Ab in T1D patients undergoing PTA.
Materials and methods: Antibodies to the major autoantigens glutamic acid decarboxylase (GADA), protein tyrosine phosphatase IA-2 (IA-2A) and zinc transporter-8 (ZnT8A) were measured before and after PTA in 25 T1D patients (age: 36.9±7.8 yrs; males/females: 9/16; BMI: 23.7±2.7 kg/m^2; duration of T1D: 25.0±8.2 yrs; post-PTA follow-up: 38.8±14.4 mo). All patients underwent pancreas transplantation with portal-enteric drainage and received mycophenolate, tacrolimus and low-dose steroid as immunosuppressive treatment.
Results: Before transplantation, one or more Ab were detected in 12 (48%) patients: of them, 1 had all 3, 4 had 2 and 7 had 1 Ab only. After transplantation, all patients became insulin independent with normal blood glucose control. During follow-up, 5 patients lost pancreas function and resumed insulin therapy at 8, 15, 28, 55 and 77 months. The pre-transplant Ab status did not influence pancreas graft outcome. Out of patients with continuing graft function, only one showed Ab changes, defined as either appearance of previously undetectable Ab or doubling or more the titre of pre-existing Ab. Substantial changes in Ab levels were observed in 4 out of 5 patients whose transplant failed, and failure occurred after a mean of 18.5±22.0 mo from Ab changes detection. Pancreas biopsies were performed in 3 patients with failing pancreas graft and with Ab changes: immunohistochemistry showed CD3$^+$ and, to a less degree, CD20$^+$ infiltration in the exocrine tissue, with no pictures of selective insulitis or β-cell loss.
Conclusion: In conclusion, in T1D patients receiving PTA the pre-transplant islet Ab status is not predictive of graft outcome, while Ab reappearance or major titre increase is associated with transplant failure; the underlying immune pathogenetic processes remain to be clarified.

OP 8 Diabetic nephropathy and blood pressure

43

Higher blood pressure associated with lower mortality in elderly type 2 diabetic patients (ZODIAC-12)
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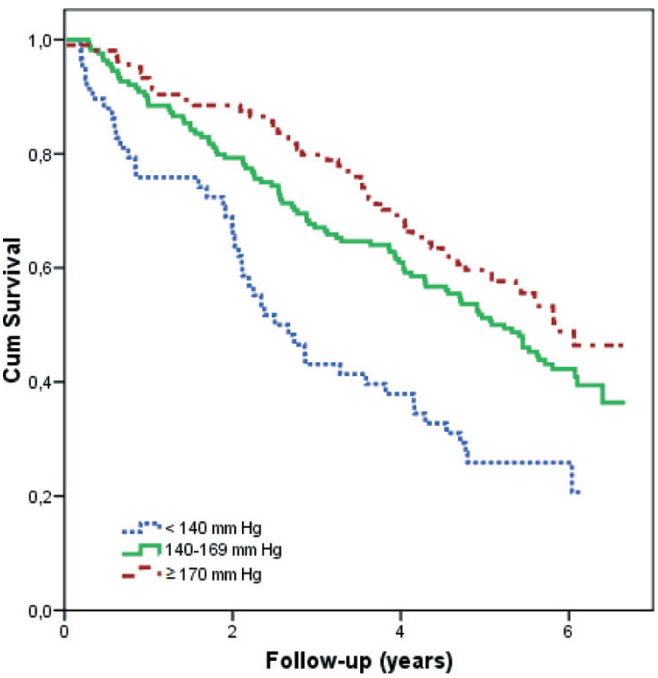
Background and aims: Hypertension is considered to increase the already high risk of cardiovascular disease in patients with type 2 diabetes mellitus (T2DM). However, the precise relationship between blood pressure and mortality in elderly T2DM patients remains unclear. The aim of this study was to investigate the relationship between blood pressure and pulse pressure over time and mortality in elderly T2DM patients.
Materials and methods: In 1998, 881 primary care patients with T2DM aged 60 years and older participated in the ZODIAC study. The cohort was divided into two age categories: 60-75 years and older than 75 years. After a median follow-up time of 5.7 years, updated means for systolic blood pressure, diastolic blood pressure and pulse pressure were calculated, and used, as time dependent covariates, in a Cox proportional hazard model. This analysis was used to evaluate the association between the different blood pressure measures over time and (cardiovascular and all-cause) mortality. The following variables were selected as possible confounders: gender, smoking (yes or no), body mass index, duration of diabetes, serum creatinine, cholesterol-HDL ratio, macrovascular complications (yes or no), albuminuria (yes or no), the use of lipid lowering and antihypertensive drugs (yes or no) and age.
Results: The mortality rate for the elderly patients (> 75 years) with a systolic blood pressure < 140 mm Hg was 76% compared to 50% in the group with a blood pressure ≥ 170 mm Hg (figure 1). All of the blood pressure measures were inversely related to all-cause mortality in this age group. An increase of 10 mm Hg in systolic blood pressure, diastolic blood pressure and pulse pressure led to a decrease of the hazard of mortality [95% confidence interval] of 19% [11%-27%], 27% [12%-43%] and 19% [9%-29%] respectively. For cardiovascular mortality the hazard ratios were 0,88 [0,77-1,00] for systolic blood pressure and 0,86 [0,72-1,00] for pulse pressure. In the low age group (60-75 years) the associations between blood pressure and mortality were not significant.
Conclusion: Blood pressure is a marker for mortality in elderly T2DM patients; however, the relationship is inverse. Known risk factors might have different consequences when assessed in different subgroups; in this case the elderly population.



44

Allelic variations in the SOD1 gene are associated with development and progression of nephropathy in type 1 diabetic subjects

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Background and aims: Oxidative stress is involved in the pathophysiology of diabetic nephropathy (DN), and the enzyme superoxide dismutase 1 (SOD1) is essential for reactive oxygen species detoxification. In this study, we tested the impact of SOD1 allelic variation in the development and progression of DN in subjects with Type 1 Diabetes Mellitus (T1DM).
Materials and methods: Seven SNPs (rs2173962, rs9974610, rs10432782, rs2070424, rs1041740, rs17880135 and rs202449), giving information on ~90% of the allelic variation of the haplotypic block containing SOD1 gene were analyzed in 1278 subjects from three independent T1DM cohorts: the SURGENE prospective study, GENEDIAB and GENESIS studies. Genotypes were determined by an Assay by Design kit from Applied Biosystems. Genotype associations with DN were assessed by logistic regression analyses. Associations with DN severity were assessed by ordinal logistic regression analyses, with stages of DN coded as ordinal polytomic dependent variables: absence (1), microalbuminuria (2), macroalbuminuria (3), reduced renal function (4) and end stage renal failure (5). A renal event was defined as progression to a further stage of nephropathy. Survival analyses without renal events were assessed by Cox's proportional hazards model.
Results: The rs1041740 SNP was associated with DN in the prospective SURGENE study both at baseline (odds ratio: 3.1, 95% CI: 1.3-7.9, p=0.01) and at 6 years follow-up (odds ratio: 5.22, 95% CI: 1.9-16.9, p=0.002). Associations were also observed with DN severity (p=0.009). Genotype tends to be associated with renal event incidence (Hazard ratio: 1.33, 95% CI: 0.97 - 1.84, p = 0.08). The rs17880135 variant was associated with DN in GENEDIAB (odds ratio: 2.5, 95 % CI: 1.1-6.0, p=0.03) and GENESIS (odds ratio: 2.0, 95% CI: 1.04 - 3.8, p=0.03) cohorts. Significant association with DN severity was also observed in both cohorts (p=0.05 and p=0.04, respectively). Associations of both SNPs with albuminuria were observed in the 3 cohorts. Associations with arterial hypertension were also observed.
Conclusion: We have observed associations of SOD1 allelic variations with nephropathy, its severity and intermediate phenotypes in subjects with T1DM. We are currently performing haplotypic studies in the 3 cohorts and analysis of 10 year follow-up data of SURGENE.

45

Different sets of risk factors for the development of albuminuria and renal impairment in type 2 diabetes - the Swedish National Diabetes register (NDR)

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Background and aims: Type 2 diabetes (T2D) is one of the leading causes of end stage renal disease (ESRD) but not all patients with T2D develop renal dysfunction (albuminuria and/or renal impairment). The aim of this study was to identify clinical risk factors associated with development of renal dysfunction in T2D patients.
Materials and methods: 3677 patients with type 2 diabetes with age 30-74 years with no signs of renal dysfunction at baseline (no micro- or macroalbuminuria and eGFR >60 ml/min//1.73m^2 according to MDRD (Modified Diet in Renal Disease) were followed for 5 years (2002 to 2007). Clinical and biochemical variables were reported at baseline and renal outcomes, development of albuminuria and/or renal impairment (eGFR <60 was ml/min/1.73m^2 MDRD or eCrCl <60 ml/min according to Cockcroft-Gault; C-G) were assessed at follow-up. Univariate logistic regression analyses for baseline variables with renal outcomes as dependent variables and then adjusted odds ratios, 95% confidence intervals, Wald X^2 and p-values in stepwise logistic regression analyses were calculated. In addition, two significant models for adjusting the odds ratios for BMI as a predictor of development of albuminuria or renal impairment were created.
Results: 20 % of patients developed albuminuria (16 % micro- and 4 % macroalbuminuria) and 11 % (MDRD) or 7 % (C-G) developed renal impairment. Among patients who developed albuminuria 16 % (MDRD) or 10 % (C-G) developed renal impairment. 29% (MDRD) or 32% (C-G) of patients who developed renal impairment also developed albuminuria. Development of albuminuria or renal impairment was independently associated with high age (all p<0.001), high systolic BP (all p<0.02), and elevated triglycerides (all p<0.02). Additional independent risk factors for albuminuria were high BMI (p<0.01), high HbA1C (p<0.001), smoking p<0.001), HDL (p<0.05) and male sex (p<0.001), and for renal impairment elevated plasma creatinine at baseline and female sex (both p<0.001). High BMI was an independent risk factor for renal impairment when defined by MDRD (p<0.01), but low BMI was when defined by C-G (p<0.001). Adverse effects of BMI on HbA1C, blood pressure and lipids accounted for approx 50% of the increase risk for albuminuria, and for 41% of the increased risk for renal impairment (MDRD).
Conclusion: Distinct sets of risk factors were associated with the development of albuminuria and renal impairment consistent with the concept that they are not entirely linked in type 2 diabetes. Interestingly effects of BMI accounted for a substantial proportion of the increased risk for both development of albuminuria and renal impairment. The equations used to define renal function (MDRD vs C-G) have an impact on interpretation of data.

46

Age at onset and sex influence the risk of developing end-stage renal disease in young patients with type 1 diabetes

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Introduction: Young age at onset of type 1 diabetes (T1D) can postpone the development of diabetic nephropathy (DN) and end-stage renal disease (ESRD). It has been suggested that puberty could promote the development of diabetic complications. If puberty is associated with increased risk of ESRD, the cumulative incidence of DN and ESRD for subjects with diabetes onset after puberty should be similar to that of pre-pubertal T1D cases. The aims of this study were to assess the cumulative risk of ESRD due to DN in a large population-based prospective T1D cohort and to study the effects of sex and age at onset of T1D on this risk.
Study population: Since 1977 all incident cases of T1D in the ages 0-14 years are recorded in the Swedish Childhood Diabetes Register (SCDR) and since 1983 all incident cases in the age-group 15-34 years are recorded in the Diabetes Incidence Study in Sweden (DISS). The Swedish Renal Registry (SRR) started in 1991 and collects data on all patients with active uraemia treatment, ESRD. Patients with >13 years duration of T1D were entered in the analyses since these patients would have equal chance of entering the SRR. In total, 6788 patients from the SCDR and 4847 patients from the DISS were included.
Results: The median time of follow up was 21.2 years for SCDR and 18.9 years for DISS (range 13-30 years), 125 patients had developed ESRD due to DN. At 25 years of T1D duration the cumulative incidence was 3.0% in males and 1.9% in females. Among patients developing T1D before the age of 15 there was no difference between males and females. Female patients developing T1D at 15-34 years had a similar risk of ESRD as the 0-9 year age-group and lower risk than the 10-14 years age-group (Table 1). In contrast, developing diabetes after 15 years of age doubled the risk for males as compared with females, hazard ratio (HR) 2.4, 95% CI=1.2-4.8. Among male patients with T1D development at 15-34 years the ESRD risk was four times higher than the 0-9 years age-group but not significantly different from the 10-14 years age-group.

Table 1: Hazard ratios and 95% CI comparing different age at onset of T1D.

Age at onset (years)	**Males**	**Females**
15-34 vs. 0-9	4.0 (2.0-8.0)	1.8 (0.7-4.7)
15-34 vs. 10-14	1.4 (0.8-2.4)	0.5 (0.2-0.99)

Conclusion: The cumulative risk of ESRD due to DN in Sweden at 25 years of diabetes duration is low. In females, pubertal age at onset was associated with an increase in risk that disappeared in post-pubertal onset patients but males had a continuing risk increase with older age at onset. The findings suggest a role for age at onset/puberty and sex hormones in the development of diabetic renal complications.

47

Efficacy and safety of long-acting nifedipine (GITS) in patients with diabetes and symptomatic stable angina pectoris: insights from the ACTION trial

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Background and aims: In the ACTION (A Coronary disease Trial Investigating Outcome with Nifedipine GITS) trial, the benefits of adding nifedipine GITS to the treatment of patients with stable symptomatic coronary artery disease were particularly apparent in those with concomitant hypertension. This further analysis has assessed whether or not the addition of nifedipine GITS is particularly beneficial in the treatment of patients with the combination of diabetes mellitus and chronic stable angina.
Materials and methods: Of the 7665 randomised patients in the ACTION study, 1113 (14.5%) had diabetes. In addition to their existing "best practice" cardiovascular (CV) treatment, all patients were randomised to receive either nifedipine GITS 60 mg once daily or placebo. The primary endpoint for efficacy was the combined rate of death of any cause, myocardial infarction, refractory angina, new overt heart failure, debilitating stroke or peripheral revascularisation.
Results: There were significant treatment-related differences between diabetic and non-diabetic patients: for example, diabetics were more likely than non-diabetics to receive drugs blocking the renin-angiotensin system (RAS blockade) both at baseline (36 v 20%) and during the study (68 v 43%). Despite the use of RAS blockade, 60% of the diabetic patients were uncontrolled with BP>140/90mmHg and 15% with BP > 130/80 mmHg. After 4 years treatment, 56% of diabetics treated with nifedipine were controlled at < 140/90 compared to 45% in the placebo group and at <130/80 mmHg the respective figures were 25 and 18%. With respect to endpoint analysis, there was an overall consistency whereby nifedipine treatment tended to be superior to placebo across all the parameters. Furthermore there was a trend towards a greater magnitude of benefit in the diabetic versus non-diabetic patients. Although there was no difference in the occurrence of the primary end-point, nifedipine treatment resulted in a statistically significant reduction (p=0.001) in the combined endpoint of death, any cardiovascular event or cardiac procedure by 21% in the diabetics compared to 9.8% in the non-diabetic patients.
Evolution of blood glucose and serum creatinine measurements was not affected by nifedipine treatment: fewer diabetics assigned nifedipine required increased anti-diabetic medication.
Conclusion: Despite the well-recognised high level of CV risk in patients with established coronary artery disease and diabetes mellitus, BP control was poor and well short of recommended target levels, even although the majority of patients were receiving some form of RAS blockade. The addition of nifedipine to "best practice" CV treatment was not only safe in diabetic patients with stable symptomatic coronary artery disease but it led to improved BP control, reduced CV risk and a reduction in "hard" CV end-points (cardiovascular events and interventions.)
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48

Impact of baseline renal function on the efficacy and safety of aliskiren added to losartan in patients with type 2 diabetes and nephropathy

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Background and aims: Patients with low GFR are at high risk of renal disease progression and treatment-related adverse events (AEs). This *post hoc* analysis assessed the efficacy and safety of aliskiren (ALI) or placebo (PBO) added to losartan (LOS) according to baseline renal function (estimated GFR [MDRD equation] <60, ≥60-<90 and ≥90 mL/min/1.73 m^2) in the Aliskiren in the EValuation of PrOteinuria In Diabetes (AVOID) study.
Materials and methods: In AVOID, 599 patients aged 18-85 years with hypertension and diabetic nephropathy received 6 months' ALI (150 mg force titrated to 300 mg after 3 months) or PBO added to LOS 100 mg and optimal antihypertensive therapy. Exclusion criteria included eGFR <30 mL/min/1.73 m^2 and serum potassium >5.1 mmol/L.
Results: Baseline characteristics were similar between treatment arms in all eGFR subgroups. In the overall population, adding ALI to LOS reduced urinary albumin:creatinine ratio (UACR) by 20% (p<0.001). The antialbuminuric effects of ALI were consistent across eGFR subgroups (19%, 22% and 18% reduction, respectively). Overall, eGFR declined by 2.4 mL/min/1.73m^2 with ALI but by 3.8 mL/min/1.73 m^2 with PBO (p=0.07 vs ALI); numerically smaller eGFR reductions were also observed with ALI vs PBO in all eGFR subgroups. In the eGFR <60 subgroup, baseline serum creatinine levels were equal (ALI 129.3 μmol/L vs. PBO 133.9 μmol/L) but renal dysfunction prespecified as a post randomization serum creatinine level >176.8. μmol/L (2.0 mg/dL) occurred more frequently in PBO vs. ALI (30.1% vs. 16.4%, p=0.02). Serum potassium elevations >5.5 mmol/L on a single measurement were more frequent with ALI (22.5% vs 13.6%), while the occurrence was equal in the two other groups. AE rates were highest in the eGFR <60 subgroup (68.1-72.9%) and lowest in the eGFR ≥90 subgroup (59.4-60.8%); there were no differences between ALI and PBO in each subgroup.
Conclusion: ALI added to LOS preserved renal function, reduced prespecified renal dysfunction and UACR vs. add-on PBO and was well tolerated, except for hyperkalemia, independent of baseline renal function in patients with type 2 diabetes, hypertension and nephropathy.
Supported by: Novartis Pharma AG

OP 9 Diabetes in childhood

49

Defects in the dynamic control of beta cell function and in glucose effectiveness underly the initial decline in intravenous glucose tolerance of obese European children

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Background and aims: Europe Europeans are thought to be less vulnerable to obesity induced type 2 diabetes. Detailed study of the main components of the glucose (G)-insulin (I) system were never performed in Europe European obese children. We aimed at assessing the main determinants of G levels in Italian obese children with normal G regulation (NGR).
Materials and methods: Sixty-five Italian obese children (age: 10.3±1.8 yrs, mean±SD; BMI: 28.1±3.2 Kg/m^2) with NGR underwent a frequently sampled intravenous G tolerance test (IVGTT) [12 g/m^2 of body surface area] lasting 200'. G/C-peptide curves and I/G curves were analyzed with state-of-art mathematical models to quantify the sensitivity of beta-cell to both the rate of increase of G (DC: dynamic or derivative control; units: [pmol·m^{-2}] per [mM·min^{-1}]) and to G concentration (PC: static or proportional; analyzed as I secretion rate at G=4.0,5.5, 8.0, 11.0 mmol/L; units:pmol·min^{-1}·m^{-2}) and the routes of G utilization [global, i.e. liver+periphery, insulin sensitivity, SI, presented as G utilization rate at G=5 mM and I=420 pM; units: mmol·min^{-1}·m^{-2}; G effectiveness, SG units: ml·min^{-1}·m^{-2}]. Acute I response (AIR) was calculated as the AUC under I concentration between 0' and 10' of the IVGTT. Median FPG (4.6 mM) and median G level at 60' during the IVGTT (1h-PG: 5.3 mM) were used to classify children in one of 4 groups: low (below median) FPG and low 1h-PG (LowLow, n=20, FPG:4.4±0.16 mM, 1h-PG: 4.4±0.47 mM), high (above median) FPG and low 1h-PG (HighLow, n=13, FPG: 4.9±0.23 mM, 1h-PG: 4.4±0.58 mM), low FPG and high 1h-PG (HighHigh n=13, FPG: 4.3±0.25 mM, 1h-PG: 7.1±0.10 mM), and high FPG and high 1h-PG (HighHigh, n=19, FPG: 5.0±0.22 mM, 1h-PG: 6.5±0.7 mM).
Results: Worsening of G regulation was associated with increasing age (from 9.3±1.7 to 10.5±1.8 yrs, p<0.05) and BMI (from 26.6±2.4 to 28.8±3.2 kg/m^2, p<0.05). AIR was similar across the 4 groups (4.9± 2.9, 4.6± 1.6, 3.4± 2.1 and 5.2± 5.2 in the LowLow, HighLow, LowHigh and HighHigh group, respectively, p=0.31). Both SI and SG were lower (p<0.05-0.01) in the LowHigh and HighHigh groups (SI: 0.95± 0.27 and 0.96± 0.31; SG: 102± 45 and 99± 23) than in the 2 Low 1h-PG groups (SI: 1.5± 0.4 and 1.3± 0.4; SG 137±33 and 152±50). SI, but not SG, was inversely related to both DC and PC of beta-cell function (r=-0.44 and -0.43, respectively, p<0.01 for both). PC, but not DC, of beta-cell function, was higher in the LowHigh (p=0.02) and HighHigh (p=0.03) groups than in the LowLow group, but after adjusting for SI these differences disappared. After adjusting for both SG and SI, the DC of beta-cell function was lower by~47% (p<0.01) in the LowHigh and HighHigh groups than in the other two groups.
Conclusion: In Europe European (Italian) obese children with NGR: 1. elevated 1h-PG is associated to decline in DC of beta-cell function and in both SI and SG. 2. SI, but not SG, is accompanied by an apparently compensatory increase in PC of beta-cell function, 3. hence, the fall in DC of beta-cell function and the uncompensated decline in SG play primary roles in determining the initial decline in i.v. G tolerance.
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50

Tracking insulin secretion and sensitivity in children who subsequently develop diabetes: a seven year study

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Background and aims: Blood glucose is controlled by the interaction between insulin secretion and insulin action, which constantly adjust to maintain optimal glucose levels, and whose vector can be plotted over time. We tracked the vector plots of three children who developed diabetes in the context of age-matched children who did not.
Materials and methods: Participants: 258 healthy children from the Early-Bird cohort (recruited from randomly-selected schools in 2000/1), studied annually from 5-12y. Girls A and B were diagnosed diabetic at 12.2y and 11.9y, boy C at 8.1y. Measures: BMI (sds), glucose, insulin-sensitivity (HOMA-%S), insulin-secretion (HOMA-%B).
Results: The table shows trends in cohort and diabetic children over time. The path traced by the mean vector was biphasic in the cohort as a whole, and in children A, B and C. While glucose rose throughout (change 5-12y p<0.001), HOMA-B first fell and HOMA-S increased in compensation (changes 5-7y both p<0.001). At 7-8y, however, the vector switched direction towards lower HOMA-S and higher HOMA-B (changes 8-12y both p<0.001). The inflexion occurred at 7y in Girl-A, 8y in Girl-B, and 6y in Boy-C. In contrast to that of the remaining cohort, their insulin secretion never recovered.
Conclusion: There is limited data on pre-diabetes in children. The inflexion we describe is novel, and may reflect early strain on beta-cell reserve in contemporary children. The children who developed diabetes were distinguishable from their peers, not by higher BMI or insulin resistance, but by early beta-cell failure which was apparent up to 12 months pre-diagnosis. Vector plotting may help better understand which children are at risk of beta cell failure, and why.

Trends in cohort and children A,B,C 5-12y

	Cohort girls n=114 Mean (SD)	**Girl A**	**Girl B**	**Cohort boys** n=144 Mean (SD)	**Boy C**
BMI (sds)	0.54 (0.9)	1.09	1.60	0.19 (1.1)	-1.28
5y	0.50 (1.0)	1.12	1.50	0.14 (1.1)	-0.47
6y	0.56 (1.0)	0.69	1.31	0.18 (1.1)	-0.37
7y	0.54 (1.0)	0.63	1.60	0.28 (1.1)	-0.96
8y	0.64 (1.1)	1.06	1.30	0.34 (1.1)	-
9y	0.65 (1.2)	1.05	1.77	0.42 (1.1)	-
10y	0.59 (1.3)	1.53	1.40	0.33 (1.2)	-
11y	0.68 (1.2)	1.45	-	0.42 (1.2)	-
12					
Glucose	4.25 (0.5)	4.3	4.1	4.31 (0.4)	3.8
(mmol/l)	4.36 (0.3)	4.5	4.1	4.49 (0.4)	3.5
5y	4.56 (0.3)	4.5	4.3	4.62 (0.5)	4.4
6y	4.61 (0.3)	4.6	4.4	4.78 (0.5)	6.2
7y	4.74 (0.3)	5.0	4.7	4.80 (0.4)	-
8y	4.74 (0.3)	4.9	4.5	4.88 (0.3)	-
9y	4.77 (0.3)	4.8	4.9	4.82 (0.3)	-
10y	4.88 (0.4)	6.1	-	4.92 (0.3)	-
11y					
12y					
HOMA-S (%)	180 (126)	132	70	252 (150)	204
5y	234 (145)	278	89	318 (149)	500
6y	274 (129)	270	476	332 (136)	476
7y	155 (143)	278	75	307 (148)	417
8y	165 (109)	109	202	228 (133)	-
9y	138 (110)	108	61	152 (83)	-
10y	116 (87)	91	120	173 (113)	-
11y	89 (67)	69	-	146 (105)	-
12y					
HOMA-B(%)	112 (47)	109	187	87 (39)	107
5y	91 (41)	60	159	67 (32)	71
6y	68 (24)	61	46	57 (21)	45
7y	71 (26)	57	85	59 (30)	23
8y	91 (30)	89	68	71 (25)	-
9y	107 (41)	84	43	87 (29)	-
10y	114 (41)	111	87	87 (36)	-
11y	131 (47)	80	-	92 (34)	-
12y					

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51

Paediatric ONSET-Study: impaired QoL in children and depressed mood in mothers at onset of diabetes mellitus type 1 in children

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Background and aims: The diagnosis of diabetes mellitus type 1 (DMT1) is a significant psychological burden for a child affected and the future of the whole family. The acute psychological implications of the diagnosis, as well as their determinants in children and their mothers are investigated during the first year after the diagnosis. This is a part of the pediatric ONSET-Study, a study comparing sensor augmented pump therapy with pump therapy without continuous subcutaneously glucose monitoring in children. Data on mother's well-being and children's quality of life following the diagnosis of DMT1 will be presented.
Materials and methods: This is a prospective study with children (aged 1-16 years), randomised within 4 weeks of DMT1-diagnosis. 160 children (age: 8,7 ± 4,4 J.; 47,5% girls) were included. The psychological wellbeing of the mothers or other primary caregivers of the child was assessed a few days after the diagnosis with a questionnaire (WHO-5, a well-being index). The QoL was evaluated in children (>/= 8yrs) via KIDSCREEN-27- and in parents via KIDSCREEN-27-proxy-questionnaires.
Results: The mean sum-score of mothers in the WHO-5 questionnaire (negative - positive: 0-100) was 46,3 ± 22,8, with 55,3% of mothers with a sum-score ≤ 48 indicating poor well-being / depressed mood. The mothers of the children below 6 years were significantly more affected than those of children aged 6 to 11 yrs. (52,1 ± 21) or 12 - 16 yrs. (46,0 ± 21) (p< .05). 69% of mothers with children < 6 yrs. showed symptoms of a depressed mood. The T-Values of the children's Quality of life scores (parents' perspective) were 39.4 ± 9,8 for physical wellbeing, 40,1 ± 10,7 for psychological wellbeing, 49,9 ± 9,5 for parents and autonomy, 44,7 ± 13,9 for social integration and 46,3 ± 12,7 for school. Compared with the representative European norm data of the Kidscreen the quality of life was significantly lower in all dimensions except "parents and autonomy"(each p < .01). The children (>/=8yr.) rated their quality of life according their parents' assessments (T-values on the five dimensions: 41,5 ± 8,9; 44,4 ± 10,7; 49,7 ± 9,1; 45,3 ± 11; 46,1 ± 11). Mainly their physiological and psychological wellbeing were significantly impaired compared to European norm data of the Kidscreen. After adjusting the data according to age of the children there were no significant differences between the five diabetes centres.
Conclusion: The high psychological burden of mothers, especially of those with young children and the impaired wellbeing of children diagnosed with diabetes underline the importance of an integrated medical and psychosocial care in the first phase of coping with the chronically illness. Psychotherapeutic interventions for mothers to cope better with affective disorders should become a standard of care.
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52

A new model of therapeutic education in pre-school and school-aged children with type 1 diabetes

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Background and aims: Therapeutic Diabetes Education is a key aspect of diabetes care, underlining the importance of psycho educational programs in type 1 diabetic child, adolescents and their family. For effective results, such programs need to focus on physical and psychological outcomes of every single phase of the child developmental stage. There are a number of studies on educational programs for school aged children and adolescence but very few of them concern the psychological adjustment and the Diabetes Education of very young children affected by diabetes mellitus type 1 (DM1).
The aim of this project is to implement a new education curriculum for children with DM1 (4-12 yrs old) and their families and to assess its effectiveness to obtain a good diabetes control, to increase knowledge about diabetes and moreover to help them developing the skills necessary to identify, confront and resolve problems with confidence.
It will use a progressive modular-based structure to improve self-management in children with DM1, following the criteria of EASD and IDF and the methodology of the Service of Children Hospitals Bambino Gesù (Rome, Italy) .
Materials and methods: A randomized control study (N=83) was designed to assess the effect of psycho-education and its relationship with HbA1c. A knowledge test and some psychometric (projective personality tests) and and pediatric quality of life measures were administered using a pre and post test design. A special psycho educational program was developed using the methodology of Childen Hospital Bambino Gesù, adapted for age (4-7 and 8-12 yrs old). A group of 43 children (standard diabetes education- Group B) matched by age and gender was considered as control.
Results: The study is still ongoing but preliminary findings in Group A, when compared to the control group, at week IV, XII and week XXIV showed: 1) a significant improvement in the overall scoring of PedsQL and in the mean scores of diabetes self efficacy scale; 2) an increase in dietary and diabetes knowledge; 3) at school, the need to speak of their condition to the classmates, instead of hide it. Morevoer a preliminary analysis of children's draws evidenced a typical structure in the family of a child with diabetes type 1; 4) HbA1c values were significant lower at 6 months (p<0.005).
Conclusion: we observed a better comprehension and acceptance of DM1 in the intervention group (especially for preschoolers) and a significant difference in HbA1c. Furthermore data obtained from Personality Projective tests offer important cues for the study of familiar functioning in diabetes.

HbA1c during the study

patients	Age 6.7±1.6	HbA1c at DM1 onset	3 months	6 months
Group A n.40	6.7±1.6	12.8±1.4	7.5±0.4	7.2±0.3
Group B n.43	7.0±1.8	13.1±1.1	7.6±0.3	7.7±0.5

53

Diabetes-related quality of life and glycaemic control among youth with type 1 diabetes

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Background and aims: Glycemic control is a key measure of diabetes (DM) management and risk for long term DM complications. We hypothesized that poor glycemic control would be associated with poorer DM-related quality of life (QoL) among youth with type 1 diabetes (T1DM).
Materials and methods: These cross-sectional analyses included 2,601 youth ages 5-23 years (mean 13.6 yrs.) with T1DM duration of ≥ 12 months who completed a child self-report pediatric quality of life-diabetes (PedsQL-DM) module and had glycated hemoglobin (A1c) measured by SEARCH, a population-based study of youth with DM onset <20 yrs. Glycemic control was categorized using age-specific cut-points based on American Diabetes Association target values for good and intermediate control. Poor control was defined as A1C ≥9.5% regardless of age. Demographic and DM treatment variables were self-reported.
Results: Of these youth, 75% were non-Hispanic white; 80% were privately insured; 50% were female; mean T1DM duration was 5.2 years; 29% received insulin < 3 times/day, 49% ≥ 3 times/day, 22% were on a pump. Mean A1c was 8.0% for youth aged <6 years (n=167), 8.3% for 6-12 yrs. (n=1011), 8.7% for 13-18 yrs. (n=1134), and 8.7% for ≥ 19 yrs (n=289). Glycemic control was poor in 20.1% and intermediate in 47.6% of participants. In unadjusted linear regression models, PedsQL-DM total score and all five subscale scores were associated with glycemic control (p<0.0001) (Table). After adjustment for age, race/ethnicity, sex, duration, insulin regimen, and insurance status, these associations remained (p<.0001). The strongest association with glycemic control for both the unadjusted and adjusted models was for the treatment barriers subscale. In the adjusted model, youth with good control had a mean score that was 9.95 points higher (fewer barriers) than those in poor control; those with intermediate control were 6.24 points higher on the scale,

while the weakest association was for the worry subscale. Additionally, girls and those without private insurance had significantly lower (worse) PedsQL-DM scores in all models.

	Outcomes for the Six Unadjusted and Adjusted Linear Regression Models					
	PedsQL-DM Total score	**DM Symptoms Subscale**	**Treatment Barriers Subscale**	**Treatment Adherence Subscale**	**Worry Subscale**	**Communication Subscale**
Cronbach's alpha	0.85	0.78	0.56	0.59	0.71	0.90
Mean ± 1 SD score	74.1 ± 13.4	65.7 ± 15.9	79.8 ± 17.9	81.9 ± 16.1	74.0 ± 22.5	79.5 ± 22.3
Unadjusted Model						
Good Control β coefficient*	8.37	6.08	12.64	8.66	10.93	8.14
Intermediate Control β coefficient*	4.86	3.28	8.27	4.49	5.35	6.44
Adjusted Model**						
Good control β coefficient*	6.91	6.06	9.95	6.31	5.77	8.47
Intermediate control β coefficient*	3.78	3.12	6.24	3.15	2.31	5.57

*Reference = Poor Glycemic Control, A1C ≥9.5% ** Linear regression models are adjusted for age category, race/ethnicity, sex, DM duration, insulin regimen, and insurance status.

Conclusion: In this large racially/ethnically diverse sample of youth with T1DM, our analysis showed that youth in good or intermediate glycemic control have significantly better diabetes-related QoL than those in poor control. While the causal direction of the association cannot be established from cross-sectional data, these findings suggest that poor glycemic control, in addition of being a strong risk factor for long-term DM complications, is negatively associated with QoL for young people with T1DM.

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54

Tracking of metabolic control from pediatric onset until adulthood: metabolic control during the first year of type 1 diabetes predicts HbA_{1c} during adulthood: DPV-analysis on 6578 patients from 300 centers

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Background and aims: The importance of metabolic control is well established in type-1-diabetes. However, most studies address patients in a cross-sectional manner, rather than following patients longitudinally over prolonged periods of time. In this analysis we tried to answer the question, whether tracking of metabolic control is present in patients with T1DM of pediatric onset, followed continuously over the pubertal period until adulthood under routine care.

Materials and methods: The DPV initiative is based on a multicenter, standardized electronic documentation of patients with diabetes in Germany and Austria. Data are recorded at the participating institutions, anonymized and transmitted for central analysis. Inconsistent data are reported back to the centers for confirmation or correction. Until March 2009, a total of 41797 patients with T1DM of pediatric onset (prior to age 15) are included in the database. 300 institutions (294 from Germany and 6 from Austria) participate. 6578 patients from 185 centers were followed longitudinally from the first year of diabetes until adulthood (age > 16 years). The mean time of follow-up was 6.0 ± 0.4 years. Preschool onset (below 6th birthday) of T1DM was present in 505 patients (mean follow-up 12.8 ± 0.07 years), 2210 patients showed an elementary school onset (6.1 - 11.0 years; mean follow-up 7.9 ± 0.03 years) and 3863 patients had a high school diabetes-onset (11.1 - 15.0 years; mean follow-up 4.0 ± 0.02). Data were analyzed using SQL and SAS 9.13. A mixed hierarchical model with treatment center as random variable was applied.

Results: HbA1c during the first year of diabetes (3 - 12 months after onset) averaged 6.99 ± 0.02 %, compared to 8.35 ± 0.02 % during adulthood. In the whole group, after adjustment for age at onset, diabetes duration, insulin therapy and BMI during adulthood, gender, migration background, treatment center and center characteristic, HbA1c during the first year of diabetes was a strong, significant predictor of HbA1c during adulthood: ß = +0.3418, $p < 0.001$. This relationship was already significant in patients with a preschool onset of diabetes (ß= +0.12, $p<0.05$), as well as in patients with elementary school onset (ß=+0.28, $p < 0.0001$) and with high school onset of diabetes (ß = +0.41, $p < 0.0001$).

Conclusion: This study on a large, multicenter group of patients with T1DM of pediatric onset provides convincing evidence for longterm tracking of metabolic control. This result is in agreement with the importance of an early focus on metabolic control, even during the first months of diabetes (remission phase). Alternatively, disease heterogeneity or different personal traits in diabetes management might be responsible for the longterm stability of HbA1c levels in individual patients with pediatric diabetes onset, despite the considerable variability observed during puberty.

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OP 10 Retinopathy

55

HbA_{1c} and fasting plasma glucose are predictors of retinopathy at ten years: the French desir study

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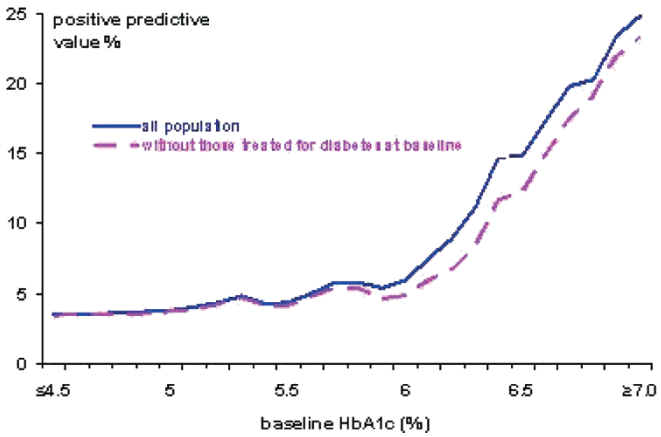
Aims: To evaluate the predictive values of HbA_{1c} and fasting plasma glucose (FPG) for retinopathy ten years after baseline examinations.
Methods: 700 men and women from the D.E.S.I.R. study were evaluated for retinopathy using a non-mydriatic digital camera: 235 had diabetes (treated, FPG≥7.0mmol/l at least once over preceding nine years), 227 had an impaired fasting glucose (IFG:6.1-6.9 mmol/l) at least once, 238 always had normal glucose (<6.1mmol/l).
Results: The 44 participants with retinopathy at ten years had higher baseline mean (SD) FPG and HbA_{1c} than those without retinopathy, 7.24 mmol/l (2.72) vs 5.90 mmol/l (1.22) mmol/l and 6.4% (1.6) vs 5.7% (0.7) (both p<0.0001). The frequency of retinopathy at ten-years, standardized over the entire D.E.S.I.R. population was 3.6%, and over 16% for FPG≥6.5 mmol/l and HbA_{1c}≥6.5%. The positive predictive value for retinopathy increased more rapidly for higher values of HbA_{1c} than for FPG. In our population, FPG of 6.0 mmol/l and 6.5 mmol/l had positive predictive values of 8.6% and 17.4% respectively for retinopathy at ten years, while HbA_{1c} of 6.0% and 6.5% had positive predictive values of 6.8% and 15.9%. After ten years of follow up, retinopathy was equally frequent (95%CI) in diabetes 7.9% (4.4-11.3) and in IFG 8.6% (5.0-12.2).
Conclusion/interpretation: In this population, baseline HbA_{1c} had better profile of positive predictive values for retinopathy than baseline FPG ten years later. The ten-year frequency of retinopathy in individuals with IFG (at least one value in the abnormal range during follow-up) - was as high as for those with diabetes.



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56

Retinal microaneurysm count predicts progression and regression of diabetic retinopathy

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Background and aims: To examine whether the number of microaneurysms (MAs) predicts progression and regression of retinopathy in type 1 and type 2 diabetes.
Materials and methods: 893 type 1 and 526 type 2 diabetic patients with microaneurysms only (ETDRS levels 20/20 or 20/10) were subjected to yearly 7-field stereo retinal photographs for a mean of 4.6 years as part of the Diabetic Retinopathy Candesartan Trials (DIRECT) Programme. Retinopathy progression was defined as a 2 step increase on the ETDRS scale for the patient, regression as a 2 step decrease. For the latter, analysis was restricted to those 441 type 1 and 217 type 2 patients with sufficient potential to regress, i.e. ETDRS level 20 in both eyes. MAs were counted and summed for both eyes. Mean age (+/-SD) and duration (+/-SD) of diabetes was 31.2 (8.4) and 9.8 (4.3) years in type 1, 56.2 (8.0) and 7.2 (4.5) in type 2 patients, respectively.
Results: In type 1 diabetes, we found a significantly increased risk of 2-step progression of retinopathy with increasing MA count at baseline (Hazard Ratio (HR) 1.08 per MA, 95% CI 1.05-1.12, p< 0.0001), adjusted for diabetes duration, systolic blood pressure and HbA_{1c}. Conversely, people with higher MA counts at baseline were less likely to regress (HR 0.79, 95% CI 0.73-0.86, p<0.0001). Similarly, in type 2 diabetes there was a significantly increased risk of progression of retinopathy (HR 1.07, 95% CI 1.01-1.13, p< 0.02), adjusted for diabetes duration, systolic pressure, $HbA_{1c,}$ urinary albumin excretion rate and use of antihypertensive therapy with increasing MA count, and a lesser likelihood of regression (HR 0.85, 95% CI 0.77-0.93, p<0.0009,).
Conclusion: In both type 1 and type 2 diabetic patients a greater MA count significantly predicted a greater risk of retinopathy progression, and a lesser likelihood of retinopathy regression. As clinical trials need to focus on earlier stages of disease, our findings support the use of MA counts as a possible surrogate clinical endpoint for progression and regression of retinopathy.
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57

WITHDRAWN

58

Effectiveness of diabetic retinopathy screening in primary health care. Agreement with the ophthalmological service

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Background and aims: To assess the effectiveness of diabetic retinopathy screening using the non-mydriatic camera and to estimate the specificity and sensitivity of referrals from general to specialist services.
Scope: A Basic Care Unit of 15885 inhabitants
Materials and methods: Observational study
A non-mydriatic retinography was performed on patients with type 1 and 2 Diabetes Mellitus (except for those already suffering from retinopathy) visited in the Primary Health Care Office in the period November 2007 to December 2008.
329 patients were selected to undergo this exploration. 46 retinographies were considered pathological in the Primary Health Care Offices. They were sent to be revalued by the ophthalmological service in the reference hospital where the diagnosis was communicated to the family doctors.

Before the study was carried out, the general practitioners were intensively trained on funduscopic examination.
In parallel with this study a statistical analysis was carried out in a sample of 879 cases, including other Basic Care Units in our territory as well as the 329 cases of our center. This analysis tried to determine the reliability (sensitivity and specificity) in detecting retinal pathology in the Primary Health Care.
Results: Number of retinographies: 329
Number of non-pathological retinographies: 283 (86,02%)
Retinographies assessed by the ophthalmologist: 46 (13,98%)
Pathological retinographies: 15 (4,55%)
Slight Diabetic retinopathy (DR): 3 (0,91%)
Moderate DR: 3 (0,91%)
Severe DR: 1 (0,30%)
Age-related *macular degeneration:* 1 (0,30%)
Others: 7 (2,13%)
Non-assessable: 3 (0,91%)
Conclusion: 4,55% of the screened showed pathological retinography , of which 46,67% due to DM. Using non-mydriatic retinography in diabetic patients offers the possibility of an early diagnosis in primary care, with a sensitivity and specificity of over 90%. Its easy access and application allow the quality improvement of the diabetic patient assistance and the rationalization of the referral system to the hospital.
Supported by: Department of Ophthalmology, Hospital Sant Joan, Reus, Department of Epidemiology, University Rovira i Virgili, Tarragona

59

Thiamine and benfotiamine counter apoptosis induced by intermittent high glucose exposure in human retinal pericytes
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Background and aims: Human retinal pericytes are more vulnerable to fluctuating than stable high glucose exposure, showing great differences with bovine pericytes, used so far as models for diabetic retinopathy. For this reason, we created an immortalized human retinal pericyte line (Bmi-HRP), with the same morphology and metabolic characteristics of the wild-type cells from which it was derived. Thiamine and benfotiamine are well-known protective agents against the damaging effects of high glucose in vascular cells. Our aim was to verify if thiamine and benfotiamine are able to counter intermittent high glucose-induced damage in human retinal pericytes.
Materials and methods: Wild-type and immortalized pericytes were kept intermittently at 48h intervals in high (28 mmol/l) or physiological (5.6 mmol/l) glucose for 8 days, with or without the addition of 50 or 100 μmol/l thiamine or benfotiamine. Control cells were cultured in stable physiological or high glucose for the whole period. DNA fragmentation (ELISA and TUNEL), Bcl-2 and Bax mRNA expression and protein concentration, as markers of glucose-induced apoptosis, were determined.
Results: DNA fragmentation measured through ELISA showed a dramatic increase in apoptosis in intermittent HG only ($p<0.001$ vs NG in both cases), which was completely prevented in both wild type and Bmi-HRP by the addition of thiamine and benfotiamine at both concentrations throughout the whole period. Results obtained with the ELISA were confirmed by *in situ* TUNEL staining. Intermittent exposure to high glucose resulted in both cell types in a 50-60% decrease in Bcl-2 to Bax ratio, for both expression and concentration ($p<0.005$). Addition of thiamine and benfotiamine at both concentrations to intermittent high glucose completely reverted this damaging effect.
Conclusion: The hypothesis that daily blood glucose fluctuations play a major role in the development of diabetic retinopathy is reinforced by the confirmation that apoptosis in human pericytes is increased following intermittent high glucose exposure only. Bmi-HRP show the same behaviour of wild-type pericytes when exposed to fluctuating glucose levels, making this cell line a promising candidate model to study pathogenic mechanisms and therapeutic applications in diabetic retinopathy. Thiamine and benfotiamine are able to prevent pericyte apoptosis, suggesting once again that this vitamin could be an inexpensive approach to the prevention and/or treatment of diabetic vascular complications.
Supported by: an EFSD/Lilly fellowship

60

Safety and efficacy of ranibizumab treatment in patients with diabetic macular oedema: 12-months results of the RESOLVE study
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Background and aims: Evaluate safety and explore effect of ranibizumab in diabetic macular edema (DME).
Materials and methods: Multi-center, double-masked study involving 151 DME patients treated over 12 months with 6 mg/ml ranibizumab (n=51), 10 mg/ml ranibizumab (n=51), or sham injection (n=49). Three initial monthly injections followed by re-treatment based on success, futility, or safety criteria. Injection volume doubled as of Month 1 if central retinal thickness (CRT) was >300 μm or >225 μm and reduction from prior assessment <50 μm. Laser photocoagulation as rescue medication allowed from Month 3 onwards. Best-corrected visual acuity (BCVA; Early Treatment for Diabetic Retinopathy Study charts) and CRT (optical coherence tomography) were assessed monthly.
Results: Injection volume doubled in 92% sham and 73/65% ranibizumab patients (6/10 mg/ml). Rescue treatment in 33% sham and 9% ranibizumab patients.Over 12-months, most frequent ocular adverse events (AEs): conjunctival hemorrhage (14% sham, 23% ranibizumab); eye pain (20% sham, 18% ranibizumab). Serious ocular AEs: 1 sham patient (retinal detachment), 4 ranibizumab patients (endophthalmitis, transient retinal artery occlusion, peripheral retinal ischemia). Non-ocular arterial thromboembolic events: 2 sham and 3 ranibizumab (10 mg/ml) patients. Mean BCVA increased and mean CRT decreased continuously over time in ranibizumab patients. Mean change in BCVA from baseline to Month 12 was -1.4 letters (sham) +11.8 letters (6 mg/ml), +8.8 letters (10 mg/ml), and +10.3 letters (pooled ranibizumab groups).
Conclusion: Safety of ranibizumab in DME was comparable to previous studies of neovascular age-related macular degeneration. Ranibizumab treatment demonstrated superior efficacy to sham treatment in DME and was associated with continuous improvement in BCVA and CRT throughout 12 months.
Supported by: Novartis Pharma

OP 11 Fatty acids and lipids

61

Serum and adipose tissue adipokine concentrations are affected by non-esterified fatty-acids in healthy individuals

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Background and aims: Non-esterified fatty acids (NEFA) are implicated both in the development of insulin resistance and initiation of inflammatory pathways in different tissues. To the moment, the hypothesis of an interaction between NEFA and adipose tissue-derived cytokines in the genesis of insulin resistance was not tested *in vivo*. The aim of the present study was to investigate the effect of experimentally elevated NEFA concentrations on cytokines measured in circulation and in interstitial fluid effluent from subcutaneous tissue of healthy volunteers.
Materials and methods: Ten healthy volunteers were randomly assigned to two subsequent experiments, either receiving continuous venous lipid-heparin infusion or saline during 28 hours. Two open-flow microperfusion (OFM) catheters were inserted in the subcutaneous adipose tissue (SAT) of each volunteer for continuous sampling of interstitial fluid effluent. Blood samples were withdrawn concordantly. Effluent, plasma and serum samples were analyzed using a multiplexed ELISA system to measure the concentrations of interleukins (IL) 6, 8, 10, tumor-necrosis factor alpha (TNF-alpha), adiponectin, C-reactive protein (CRP), plasminogen activator inhibitor-1 (PAI-1) and macrophage chemoattractant protein-1 (MCP-1). NEFA, triglycerides, glucose and insulin concentrations in blood were measured by validated methods in parallel.
Results: Plasma concentrations of triglycerides and NEFA were higher in the lipid-heparin infusion group. High levels of insulin (saline: 2.69±1.30μU/mL vs. lipid-heparin: 4.78±3.2μU/mL; p<0.05), glucose (saline: 86±8mg/dL vs. lipid heparin: 94±8mg/dL; p<0.05) and the calculated HOMA-R index (saline 0.40±0.06 vs. lipid-heparin 0.57±0.09; p=0.03) indicated lower insulin sensitivity during lipid infusion. Distinct time-profiles were observed for cytokines measured in SAT effluent samples. IL-10 concentrations were higher in the control intervention in comparison to lipid infusion during the last 4 hours (saline: 449.2±105.9 vs. lipid-heparin: 65.4±15.4pg/ml; p=0.02). PAI-1 (saline: 67.8±15.6ng/mL vs. lipid-heparin: 77.2±15.1ng/mL; p<0.01) and MCP-1 (saline: 160.1±40.2pg/mL vs. lipid-heparin: 211.7±48.8; p<0.001) were substantially higher during the lipid experiment in serum. Adiponectin also presented a slight increase during lipid-heparin infusion (treatment effect: p=0.03; saline: 7.9±0.8 vs. lipid-heparin: 8.5±1.0mg/l). No additional differences in terms of cytokine concentrations were observed.
Conclusion: Elevated concentrations of NEFA promote a systemic pro-inflammatory response and a depressed anti-inflammatory response in SAT. These effects might contribute for the development of insulin resistance in conditions where NEFA is elevated in plasma, such as in obese and type-2-diabetic patients. The increase of adiponectin in serum might be interpreted as a counter-regulatory response.
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62

Skeletal muscle fatty acid handling in the pre-diabetic state

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Background and aims: Already in the pre-diabetic state, fat storage in non-adipose tissues, such as skeletal muscle, is increased and associated with insulin resistance ("lipid overflow" hypothesis). When the uptake of free fatty acids (FFA) or triacylglycerol (TAG) exceeds the capacity or the need to oxidize fat in skeletal muscle the fatty acids are stored, leading to ectopic fat accumulation. Therefore, the aim of the present study was to examine fasting and postprandial skeletal muscle fatty acid handling in subjects with impaired fasting glucose (IFG), impaired glucose tolerance (IGT) (combined or not combined with IFG), compared to normal glucose tolerance (NGT).
Materials and methods: 6 NGT (age 55.0±3.0yrs, BMI 32.3±0.9 kg/m^2), 14 IFG (age 60.3±1.5yrs, BMI 29.5±1.0 kg/m^2), 12 IGT (age 58.0±1.9yrs, BMI 32.0±1.2 kg/m^2) male and female subjects were included. During fasting and after ingestion of a standardized high fat mixed meal (2.6 MJ,61% of total energy content of fat), skeletal muscle fatty acid handling was examined by measuring arterio-venous differences across forearm muscle in combination with measurements of forearm blood flow using venous occlusion plethysmography. Insulin sensitivity (IS) was determined using the hyperinsulinemic-euglycemic clamp.
Results: IS was lower in IFG and IGT compared to NGT (4.3±0.5, 4.2±0.6 and 10.7±0.9 mg·kg^{-1}·min^{-1} respectively, ANOVA p<0.001; Bonferroni p<0.001 for IFG and IGT vs NGT). Arterial TAG concentrations increased more in the early postprandial state (0-2h) in IFG (iAUC=265.5±32.3 mmol·L^{-1}·min^{-1}) and IGT (iAUC=294.1±58.9 mmol·L^{-1}·min^{-1}) compared to NGT (iAUC=82.6±13.8 mmol·L^{-1}·min^{-1}), (ANOVA p=0.02; Bonferroni p=0.06 and p=0.03 respectively). Furthermore, despite the consistently higher insulin concentrations in IFG and IGT compared to NGT (ANOVA p=0.03; Bonferroni p=0.03 and p=0.08 respectively), net forearm muscle glucose uptake was not significantly different between groups, suggesting skeletal muscle insulin resistance in pre-diabetic subjects. In addition, postprandial FFA uptake across the forearm muscle was less suppressed in IFG compared to IGT (ANOVA p=0.04; Bonferroni p=0.04) in the mid postprandial state (2-4h). TAG extraction across the forearm muscle in the early postprandial state (0-2h) was increased in IFG (AUC=57.7±11.2 nmol·100ml^{-1}·min^{-1}) and IGT (AUC=70.7±20.0 nmol·100ml^{-1}·min^{-1}) compared to NGT (AUC=3.8±15.4 nmol·100ml^{-1}·min^{-1}), (ANOVA p=0.06; Bonferroni p=0.04 and 0.07 respectively).
Conclusion: No differences between IFG and IGT were found in whole body and skeletal muscle IS (when compared to NGT). Postprandial TAG extraction across the forearm muscle was higher in subjects with IFG and IGT compared to NGT subjects, which may lead to fat accumulation in skeletal muscle and consequently, decreased insulin sensitivity in the pre-diabetic state.

63

Non-esterified fatty acids dynamics during an oral glucose tolerance test in women with a history of gestational diabetes

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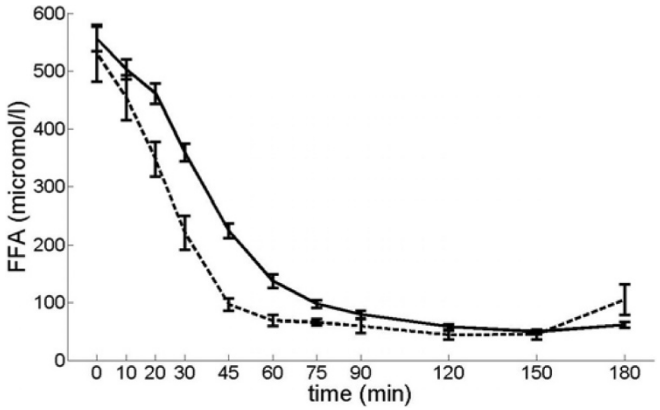
Background and aims: Elevation in non-esterified fatty acids (NEFA) has been shown to modulate insulin secretion and to be a risk factor for the development of type 2 diabetes. Since women with former gestational diabetes (fGDM) are at increased risk of diabetes, in this population we studied NEFA kinetics and its relationships with insulin during an oral glucose tolerance tests (OGTT).
Materials and methods: We analyzed 122 3h-OGTT of non-diabetic fGDM women (age=34.9±0.4 years (mean±SE); body mass index, BMI=26.6±0.5 kg/m^2, fasting glucose, G_b=5.13±0.09 mmol/l), compared to those of 19 healthy women after normal pregnancy, CNT (age=28.8±1.1, BMI=22.7±0.8, G_b=4.55±0.07). Glucose, insulin and NEFA were frequently measured during OGTT. We computed the OGTT area under the curve of insulin (AUC_{INS}) and NEFA (AUC_{NEFA}). Through mathematical modeling, we assessed parameters of NEFA kinetics: the sensitivity of lipolysis inhibition to suprabasal insulin variations, S_{NEFA}, and the fractional NEFA turnover rate, K_{NEFA}. Univariate regression analysis was performed between some parameters. ANOVA on log-transformed parameter values was used to assess differences in the two groups (P<0.05 statistically significant).
Results: fGDM women were slightly older and had higher BMI than CNT women. Glycemic levels were higher in fGDM, both at basal and at 120 min (not shown). NEFA at basal, $NEFA_b$, was not different in the two groups (538±19 μmol/l fGDM, 508±44 CNT), while basal insulin was higher in fGDM (I_b=62.4±2.7 pmol/l *vs.* 45.2±4.1 of CNT, P<0.008). After the glucose load, AUC_{NEFA} was higher in fGDM (29.3±1.4 mmol/l min *vs.* 22.0±1.6 of CNT, P<0.04). NEFA curves during the OGTT are reported in the Figure (solid line: fGDM; dashed line: CNT). Also insulin levels were higher in fGDM (AUC_{INS}=57.5±3.1 nmol/l min *vs.* 31.6±3.4 of CNT, P<0.0001). NEFA kinetic parameters were lower in fGDM: S_{NEFA}=2.3±0.3·10^{-2} ml/μU *vs.* 2.9±0.6 of CNT, P<0.04; K_{NEFA}=4.9±0.2·10^{-2} min^{-1} *vs.* 7.6±1.3 of CNT, P<0.005. In fGDM women regression analysis showed a significant but very weak relationship between $NEFA_b$ and I_b (R=0.25, P<0.005), as well as between AUC_{NEFA} and AUC_{INS} (R=0.19, P<0.03).

Conclusion: A limited number of investigations assessed NEFA in fGDM women and no study performed detailed analysis of NEFA kinetics during an OGTT based on sophisticated modeling. NEFA levels were found higher in fGDM than in CNT, despite the fact that insulin, which has an inhibitory effect on NEFA production, was higher in fGDM: in fact, these women exhibited lower sensitivity to insulin of lipolysis inhibition (S_{NEFA}) and lower NEFA turnover (K_{NEFA}), which can be further signs of early metabolic derangements in a population at risk for diabetes.



Supported by: Austrian Nationalbank Jubiläumsfonds

64

Ursodesoxycholic acid (UDCA) promotes the hepatic oxidation of free-fatty acids in Zucker fatty rats

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Background and aims: Ursodesoxycholic Acid (UDCA) is a natural bile acid commonly used to improve liver function in diseased livers by protecting mitochondrial membranes and avoiding their functional disruption. Its beneficial effects include decrease of hepatic triglyceride (HTG) levels hence it may have potential for treatment of non alcoholic fatty liver disease (NAFLD). On the basis that UDCA improves several aspects of mitochondrial function, we hypothesized that it reduces HTG accumulation by promoting the mitochondrial oxidation of non-esterified fatty acids (NEFA).
To test this hypothesis, we quantified mitochondrial oxidation of NEFA relative to non-fat substrates (pyruvate/lactate) and endogenous sources in Zucker Fatty Rats - a model of NAFL. UDCA action was studied either in Zucker and normal Wistar Rats.
Materials and methods: Male Zucker rats were maintained on a standard diet with (ZF+UDCA) or without (ZF) the UDCA (15mg/Kg) daily treatment for 4 weeks. Control groups were also maintained under the same conditions with (Ctrl+UDCA) or without the UDCA treatment. After 24h of fasting, livers were isolated and perfused with a Krebs-Henseleit buffer containing 0.2mM of [U-13C]long-chain fatty-acids bound to albumin, 0.1 mM [3-13C]pyruvate and 1mM [3-13C]lactate for 30 minutes. Oxidation of these exogenous 13C-labeled substrates and unlabeled endogenous sources were assessed by determining their fractional contributions to the mitochondrial acetyl-CoA pool. For this, livers were freeze clamped and soluble metabolites extracted with perchloric acid. 13C-enrichment of the hepatic Krebs cycle and acetyl-CoA pools were determined by 13C NMR analysis of hepatic glutamate 13C isotopomers and the fractional contributions of exogenous and endogenous substrates to the mitochondrial acetyl-CoA pool was determined by a metabolic model relating 13C-isotopomer distributions to acetyl-CoA sources. With this model, acetyl-CoA sources are resolved as follows: [1,2-13C]acetyl-CoA, reflecting the oxidation of exogenous [U-13C]fatty acids; [2-13C]acetyl CoA, reflecting oxidation of exogenous pyruvate/lactate, and unlabeled acetyl-CoA, reflecting the oxidation of unlabeled endogenous substrates.
Results: ZF rats exhibited a weaker capacity for NEFA oxidation, with only 23 ± 5% of acetyl-CoA derived from NEFA compared to Ctrl rats with 44 ± 3%. The contribution of exogenous pyruvate/lactate was residual in both control and ZF (3 ± 1% and 4 ± 1%, respectively) reflecting low PDH activity of the fasted liver. For the ZF rats, UDCA treatment caused a significant increase in the fraction of acetyl-CoA derived from oxidation of exogenous NEFA (46 ± 6% in UDCA-treated rats vs 23 ± 5% for untreated rats, $p < 0.05$). UDCA also increased acetyl-CoA recruitment from NEFA in Ctrl rats (53 ± 6% UDCA vs 44 ± 3% untreated, p=0.06). These increases were not accompanied by significant changes in exogenous pyruvate/lactate contributions but were related to significant reduction in the contribution of endogenous sources to acetyl-CoA (45± 8% ZF+UDCA vs 71 ± 4% in ZF; 40± 5% Ctrl+UDCA vs 53 ± 3% in Ctrl).
Conclusion: UDCA promoted the recruitment of NEFA for mitochondrial β-oxidation in a rat model of NAFLD. UDCA increased the recruitment of acetyl-CoA from NEFA over endogenous triglycerides suggesting that its site of action is downstream of lipolysis at the level of mitochondrial fatty acid oxidation.
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65

Osteopontin deficiency prevents obesity-associated hepatic steatosis and insulin resistance

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Background and aims: Obesity is a major risk factor for the development of hepatic steatosis and insulin resistance. The chronic low grade inflammation associated with obesity as evidenced by increased systemic concentrations of inflammatory markers probably represents a crucial link between obesity and insulin resistance. Osteopontin (OPN) is an inflammatory cytokine, the expression of which is strongly upregulated in adipose tissue and liver upon obesity. OPN has recently been linked to diet-induced adipose tissue inflammation but the role of OPN in hepatic inflammation and insulin sensitivity is still elusive. This study aimed at investigating the impact of OPN in obesity-associated alterations of the liver.
Materials and methods: OPN deficient mice (OPN-/-) and wild-type (WT) littermates were fed a high-fat diet (HF) for six months to induce obesity followed by euglycemic-hyperinsulinemic clamp testing. Hepatic triglyceride content, alanine aminotransferase and gene expression were measured by enzymatic tests and quantitative real-time PCR, respectively.
Results: Obese OPN-/- mice displayed markedly improved insulin sensitivity and suppression of hepatic glucose production compared with WT controls. Hepatic steatosis was significantly reduced in obese OPN-/- compared to WT mice as assessed by histological analysis and liver triglyceride content. Moreover, plasma alanine aminotransferase concentrations were lower in mice lacking OPN compared to WT animals. Hepatic gene expression of the lipogenic transcription factor sterol regulatory element-binding protein-1c, of the inflammatory cytokines TNF-α and TGF-β and of the gluconeogenic enzyme glucose-6-phophatase was significantly downregulated by OPN deficiency.
Conclusion: These findings indicate a critical role of OPN in the pathogenesis of obesity-induced hepatic steatosis and insulin resistance. Hence, targeting OPN could provide a novel therapeutic approach to prevent obesity-associated metabolic disorders including hepatic steatosis and type 2 diabetes.
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66

Contribution of intra-abdominal and liver fat to components of the metabolic syndrome

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Background and aims: The contribution of liver vs. intra-abdominal (IA) fat to components of the metabolic syndrome cannot be studied invasively in humans. IA fat could be important independent of liver fat. Second, it might be merely an innocent bystander playing little or no role, while liver fat would

be the critical factor. Third, IA and liver fat could contribute to variation in components of the metabolic syndrome independent of each other. We explored these three possibilities in a large group of subjects in which IA and liver fat were determined by state-of-the-art techniques.
Materials and methods: We studied 356 subjects (mean age 42 yrs, mean BMI 29.7 kg/m2) in whom liver fat (proton magnetic resonance spectroscopy), IA fat (MRI), and features of insulin resistance were measured.
Results: IA and liver fat contents were closely correlated (r=0.65, p<0.0001). In multivariate linear regression analyses, liver fat explained variation in fasting serum (fS) triglyceride, fS-HDL cholesterol, fasting plasma (fP) glucose, fS-insulin, and liver enzyme concentrations (alanine and aspartate aminotransferaces) independent of age, gender, subcutaneous fat, and/or lean body mass (LBM). The same was true when liver fat was replaced by IA fat. In multivariate linear regression analyses including both liver and IA fat, liver and IA fat independent of each other explained variation in fS-triglyceride, fS-HDL cholesterol, and fS-insulin concentrations independent of age, gender, subcutaneous fat and LBM. In similar models, only liver fat was an independent predictor of fP-glucose and liver enzymes. Subcutaneous fat, i.e. overall obesity, and age explained variation in blood pressure.
Conclusion: Both IA and liver fat independently contribute to variation in serum triglyceride, HDL cholesterol, and insulin concentrations, thus supporting the idea that both fat depots independently contribute to components of the metabolic syndrome. Liver, but not IA fat, also independently explained variation in fasting glucose and liver enzyme concentrations. Age and overall obesity but neither liver not IA fat explained variation in blood pressure.
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OP 12 Protecting beta cell mass and function

67

Evaluation of the effect of GLP-1 receptor activation on ER stress mediated beta cell damage in Akita mice
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Background and aims: Recent studies have revealed that the ER stress is one of the causes of β-cell damage in diabetes. On the other hand, several researchers have reported the cytoprotective effect of exendin-4(Ex-4) on β-cell. Last year, we have shown in vivo effects of Ex-4 on pancreatic β-cell in Akita mice, an animal model of ER stress-mediated diabetes. In this report, we further investigated the underlying mechanism by which Ex-4 treatment can attenuate ER stress-mediated β-cell damage.
Materials and methods: To exclude the possibility that the effects of Ex-4 on β-cell is through the decreased blood glucose level, we made three groups of animals treated with Ex-4, PBS, and phlorizin, a sodium-coupled glucose transporter inhibitor which decreases blood glucose levels without increasing insulin. The mice were given twice daily intraperitoneal injections of Ex-4 (24nmol/kg), phlorizin (0.3g/kg), or PBS for 2 weeks (from 3 to 5 weeks of age). Plasma glucose levels were measured every third day and pancreas samples from each animal groups were then obtained at 5 weeks of age for the histological evaluation, and islets were also isolated for RNA extraction. The differences of insulin-immunopositive areas and the numbers of cells exhibiting both nuclear CHOP and cytoplasmic insulin immunopositivity between the groups were determined by the staining with anti-CHOP and with anti-insulin antibodies,respectively. We also evaluated the effect of Ex-4 treatment on β-cell replication and apoptosis histologically by Proliferating cell nuclear antigen (PCNA) staining and TdT-mediated dUTP-biotin nick-end labeling (TUNEL) staining, respectively. Additionally, mRNA expression level of the CHOP was assessed by real-time quantitative RT-PCR.
Results: Ex-4 treatment significantly reduced blood glucose levels, but there was no significant difference in body weight between Ex-4-treated group and PBS-treated group. Immunohistochemical examination indicated an increase in insulin-positive areas in the islets in Ex-4-treated group compared with PBS-treated group. In contrast, we could not find any difference in insulin-positive areas between PBS-treated group and phlorizin-treated group whose glucose levels were identical to those of Ex-4-treated group. Furthermore, treatment of Akita mice with Ex-4 resulted in significant decrease in the number of CHOP-positive β-cells and TUNEL-positive β-cells adjusted by β-cell area,while there was no significant difference in the number of CHOP-positive or TUNEL-positive β-cells between PBS-treated group and phlorizin -treated group. In PCNA staining, we could not find significant difference in proliferation of β-cells between the three groups but Ex-4 treatment considerably increased the number of PCNA-positive β-cells compared to the other two groups. Ex-4 treatment significantly lowered the mRNA expression levels of CHOP, whereas there was no significant difference in the mRNA expression levels of CHOP between phlorizin-treated group and PBS-treated group.
Conclusion: Our data suggest that Ex-4 treatment can attenuate ER stress-mediated β-cell damage through the reduction of apoptotic cell death, partially through the promotion of β-cell replication, and the in vivo effect of Ex-4 on pancreatic β-cell is independent of the decreased blood glucose levels.

68

The once-daily human GLP-1 analogue liraglutide increases the beta cell mass in normoglycaemic mice by directly accelerating cell differentiation and proliferation
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Background and aims: GLP-1 increases pancreatic β-cell mass by stimulating cell proliferation and inihibiting apoptosis. Previously, we demonstrated that liraglutide (LIRA) increases pancreatic β-cell mass in diabetic *db/db* mice through not only regulating cell kinetics as an acute effect but also suppressing oxidative stress and endoplasmic reticulum (ER) stress by ameliorating glucolipotoxicity as a chronic effect. In this study, to further clarify the

proliferative effect of GLP-1 on pancreatic β-cell by eliminating the influence of dysmetabolism, we assessed the mode of action of LIRA on β-cell function and cell mass in the normoglycemic *m/m* mice model.
Materials and methods: Ten week-old male BKS.Cg-m +/m +/Jcl (*m/m*) mice recieved LIRA (0.2mg/kg twice daily *s.c.*) or vehicle (control) for 2 weeks. Body weight (BW), fasted blood glucose (FBG), insulin (FIRI), and triglyceride (TG) were measured before and after LIRA intervention. The β-cell mass and cell proliferation/apoptosis was assessed by histological analysis including proliferating cell number antigen (PCNA), 4-hydroxynonenal modified protein (4-HNE) immunostaining, and TUNEL assay of the islet tissue. Gene expressions specific for the core area of the pancreatic islets were analyzed by Laser Capture Microdissection method and real time RT-PCR. Primer pairs encoding genes associated with pancreatic hormones, cell proliferation, apoptosis, cell cycle, ER and oxidative stress were prepared, and real-time PCR with Sybr Green was applied. Gene expression was quantified by the comparative Ct method with each result corrected by 18SrRNA quantity. Glucose stimulated insulin secretion (GSIS) and the insulin content in islets were assessed after 2 weeks of intervention.
Results: Although food intake and BW were significantly lower in the LIRA group than in the control (food intake: 21.1±0.7 vs. 27.0±0.8 g, n=6 for each, $p < 0.05$, BW: 21.1±0.2 vs. 23.2±0.5 g, $p < 0.01$) after 2 weeks intervention, FBG, FIRI, and TG levels were similar between LIRA group and control group (glucose: 48±1.6 vs. 51±2.6 mg/dl, insulin: 0.4±0.1 vs. 0.3±0.1 ng/ml, TG: 142±5.2 vs. 156±10.9 mg/dl). The pancreatic β-cell mass was greater in LIRA treated mice than in the control (1.0±0.1 vs. 0.7±0.1 mg , $p < 0.05$, n=3 for each). The PCNA-positive cells were increased in number by LIRA treatment, but, there was no difference in 4-HNE immunostaining and TUNEL assay between two groups. The mRNA levels of Hlxb-9, NeuroD and ERK-1 associated with cell differentiation/proliferation were significantly higher in LIRA treated mice than in controls. Gene expression of Hes-1 related to anti-cell differentiation was down-regulated in LIRA-treated mice compared with controls. Gene expression of ER stress related XBP-1, anti-oxidative stress related catalase and GSHPx, and lipid synthesis associated SREBP-1c and FAS were not affected by 2 weeks of treatment with LIRA. The islet insulin content was greater in LIRA group than in controls (59.0±3.7 vs. 44.4±3.9 ng/ islet, $p < 0.05$). Furthermore, GSIS with 16.7mM glucose was greater in LIRA group than in controls (1.7±0.1 vs. 1.2±0.03 ng/ml/ islet, $p < 0.01$).
Conclusion: Liraglutide increases pancreatic β-cell mass through directly regulating cell differentiation and cell proliferation, and promotes β-cell responsiveness to glucose stimulation in normoglycemic model *m/m* mice.

69

Gene profiles of islets after combined treatment with sitagliptin and metformin in Zucker diabetic fatty rats
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Background and aims: The combination of dipeptidyl peptidase IV (DPP-IV) inhibitor and metformin has been shown to be an efficient, safe and tolerable therapy for type 2 diabetes. However, the detailed mechanism of this combination therapy compared with mono therapy on beta cell was less known. The aim of this study was to investigate genes regulated after treatment with one of DPP-IV inhibitors, sitagliptin and its combination with metformin by using c-DNA microarrary, when treatment begins before the development of overt diabetes.
Materials and methods: Nine-week-old ZDF rats which were randomly chosen were divided into 4 groups and treated; control group, sitagliptin group (450mg/kg), metformin group (520mg/kg), and sitagliptin plus metformin group (300mg/kg+450mg/kg). After 5 weeks of treatment, oral glucose tolerance test and insulin tolerance test were performed; plasma active GLP-1 level and c-DNA microarrary were assessed.
Results: Sitagliptin plus metformin group showed significant improvement in glucose tolerance test compared to sitagliptin group. As for insulin sensitivity, there was no difference between combination group and either metformin group or sitagliptin group. Plasma active GLP-1 level was increased in sitagliptin plus metformin group compared to sitagliptin group. Sitagliptin plus metformin group upregulated genes involved in anti-apoptosis and proliferation compared with sitagliptin or metformin group (Table 1).
Conclusion: We identified upregulated genes after combined treatment with sitagliptin and metformin compared with either metformin or sitagliptin treatment. These genes may be associated with additive effect of glucose control of combined treatment with sitagliptin and metformin.

Table 1.

Genebank Accession No.	Gene	Fold change		
		Sitagliptin	Metformin	Sitagliptin+ Metformin
NM_031535	Bcl2-like 1	1.044	1.905	3.111
NM_178866	Insulin-like growth factor 1	1.648	2.048	2.774
BM390560	Defender against cell death	3.250	2.945	4.277
NM_022274	Baculoviral IAP repeat-containing 5(survivin)	2.423	1.785	2.914
BM986266	Clusterin	2.017	2.487	3.386
AF235993	Bcl2-associated X protein	2.339	2.272	2.344
NM_019305	Fibroblast growth factor 2	1.814	4.359	5.058
NM_020087	Notch gene homolog 3(Drosophila)	2.716	3.151	3.587
NM_013109	Orthodenticle homolog 1 (Drosophila)	2.473	2.797	4.604
BG377323	ELK1, member of ETS oncogene family	2.487	2.541	4.010
AI071874	Pleiotrophin	2.613	3.332	4.109
NM_031577	Growth hormone releasing hormone	4.677	2.330	7.471
NM_032075	Growth hormone secretagogue receptor	7.183	4.434	9.349

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70

The direct effects of DPP-4 inhibition on isolated human islets include protection from glucotoxicity
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Background and aims: Glucagon-like peptide-1 (GLP-1) is an important physiological incretin secreted from the intestinal L-cells after pro-glucagon cleavage by pro-convertase (PC)-1/3 and rapidly degraded by DPP-IV. We have recently observed that GLP-1 is present in human pancreatic islets, with differences in the case of type 2 diabetes. The aim of the present study was to evaluate the direct effects of DPP-IV inhibition on several features of isolated human islets.
Materials and methods: Islets were prepared from the pancreas of 6 multiorgan donors (4 females/2 males; age, 56±20 yrs; body mass index, BMI, 25.6±3.3 kg/m2) and then incubated for 24h in M199 culture medium, in the presence of 5.5 (g) or 22.2 (G) mmol/l glucose, without (g- and G-) or with (g+ and G+) the addition of 0.1 nmol/l DPP-IV inhibitor (ILE-PRO-ILE, replaced after 12 h). Then, GLP-1 active form was measured in islets incubation medium by radioimmunoassay, insulin release in response to acute glucose stimulation was assessed by IRMA and expressed as stimulation index (SI), and gene expression studies were performed by quantitative RT-PCR.
Results: Active GLP-1 peptide was detected in islets incubation medium, and co-culture with the DPP-IV inhibitor significantly (p2-fold), in particular at G+ culture condition. SI was 3.2±0.35 at g- and decreased to 1.6±0.07 at G- ($p<0.01$). The presence of the inhibitor ameliorated SI in G+ (2.15±0.06, $p<0.01$ vs G-). Gene expression studies showed that G- caused a marked reduction of insulin, GLUT2 and glucokinase transcription and no major change of GLP-1 receptor. At G+, and in comparison with G-, DPP-IV inhibition significantly ($p<0.01$) increased insulin (+356±76%), GLUT2 (+334±86%), glucokinase (+342±45%) and GLP-1 receptor (+423±82%).

Conclusion: In conclusion, these results show that DPP-IV inhibition exerts a protective effect directly on islets exposed to high glucose, possibly mediated by an increased availability of intact GLP-1.

71

Prevention of islet of Langerhans degeneration with rosiglitazone: demonstration of a novel automated image analysis approach with dual insulin and collagen immunohistochemistry

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Background and aims: The search for small molecule therapies of diabetes often involves an understanding of their potential for reducing the islet of Langerhans degeneration rate. Following Rosiglitazone administration to male obese ZDF rats, islet degeneration is substantially reduced. We used this model to validate a technique of dual insulin/ collagen immunohistochemistry (IHC) with automated image analysis to assess drug-induced islet alterations.
Materials and methods: Obese ZDF rats were randomised prior to either daily gavage dosing with 3 mg/kg/day Rosiglitazone for 24 days or given vehicle only. Lean ZDF rats received vehicle only. Animals were terminated, the pancreas fixed in formalin, processed into paraffin wax and sections stained with either H&E for histopathological assessment or dual IHC stained with insulin or collagens1 and 3 antibodies using peroxidase and alkaline phosphatase techniques respectively and imaged on the Aperio scanscope. Stored images were automatically analysed by an in-house designed rule set using Definiens Developer software. The programme created subsets of islets from a reduced resolution image of the pancreatic sample, followed by high resolution analysis of islet subsets to assess relative numerical data representative of morphological changes observed in size and shape of islets and associated distribution of beta cell insulin and fibrosis. Parameters measured included: area of insulin, islet-associated collagen and islet area, the number of beta cell and islet cell nuclei, intensity of insulin and an islet shape factor.
Results: Lean ZDF islets were comparable to those of non-diabetic rat strains. Obese ZDF rat islets showed β cell vacuolation, degeneration and apoptosis with associated mononuclear inflammatory cell infiltration and fibrosis. Dual insulin/collagen IHC revealed brown-stained β cells in centrally located clusters within islets and red-stained collagen adjacent to blood vessels, exocrine ducts and within and around islets. Insulin staining was heterogeneous and pale compared with lean ZDFs. Islets of obese ZDF Rosiglitazone treated rats resembled more those of lean ZDFs and showed reductions in both β cell degenerative changes and inflammation/fibrosis. When compared to obese control rats, reductions in collagen (506±991 vs 769±1588 pixels^2 p<0.0008) and islet cell areas (42790±30570 vs 71800±75130 pixels^2 p<0.003) were seen in Rosiglitazone treated rats. Beta cell numbers remained unchanged in the **3** groups. The islet cell population was reduced in Rosiglitazone treated rats compared to obese controls (total islet nuclei, 303±191 vs 480±437 p<0.002) due to reduced interstitial tissue. The degree of insulin and collagen staining varied between groups and individual islets depending on animal strain and drug treatment.
Conclusion: Islets of lean and obese ZDF rats were characterised using combined β cell and collagen IHC. Lean ZDF islets showed intense insulin staining and minimal collagen whilst obese ZDFs had increased β cell separation, heterogeneous insulin staining and increased fibrosis. Islets of obese ZDFs given Rosiglitazone for 24 days resembled more those of lean ZDFs. Future applications will focus on reparative effects of novel anti-diabetic therapeutic agents.

72

The SGLT2 inhibitor dapagliflozin prevents the disruption of pancreatic islet morphology in the high fat-fed-female ZDF rat

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Background and aims: Dapagliflozin is a potent, selective inhibitor of the renal sodium-glucose co-transporter 2. Previous studies have demonstrated dapagliflozin reduces blood glucose in rodent models of hyperglycaemia in association with urinary glucose excretion. We have investigated whether improved glycaemic control with dapagliflozin can prevent the disruption of pancreatic islet morphology observed in obese female Zucker diabetic fatty (ZDF) rats following high fat feeding.
Materials and methods: Following randomisation on body weight and blood glucose, ZDF rats (7-8 weeks of age) were placed on a high-fat diet (48% kcal), and dosed with either dapagliflozin (1mg/kg/day, p.o, n=14) or vehicle (n=14) for up to 34 days. Lean, chow fed ZDF rats (n=14) were vehicle dosed. On day 34, 48 hours after the final dose of dapagliflozin, paraffin embedded formalin fixed pancreatic sections were fluorescently labeled from a cohort of animals (n=7 / group). Automated imaging systems and Definiens analysis software were used to image and measure β-cell mass and islet morphological changes. Islet morphological changes were measured by calculating the degree of scattering of β-cells within islets and normalising this to the total area of insulin staining within the image.
Results: On day 24, blood glucose (BG) and glycated haemoglobin (GHb) levels in the dapagliflozin treated group were significantly reduced compared with obese vehicle controls (BG, 8.7±0.46mM vs 13.4± 1.34mM p<0.005; GHb, 3.4±0.17% vs 4.8±0.4% p<0.01). This is consistent with the improvement in glucose homeostasis seen in previous studies. By day 34 fasting plasma insulin (I) and triglyceride (TG) concentrations were also reduced in obese dapagliflozin treated animals compared to obese vehicle controls (I, 483±81pM vs1101±134pM, p≤0.001; TG, 7.1±0.2mM vs 10.8±1.1mM,p≤0.01 respectively). The β-cell mass in the lean, obese vehicle and dapagliflozin obese groups were 0.68+/-0.11%, 1.15+/-0.33% (ns vs lean) and 1.49+/-0.16% (P<0.002 vs lean) of total cell count, respectively. Dapagliflozin resulted in marked, quantifiable improvement of islet morphology assessment by 2.3 fold+/-0.45 (p<0.05) compared with the obese vehicle control group. The insulin staining intensity of islets post dapagliflozin treatment was also elevated (2.2 fold+/-0.2 p<0.05) relative to obese vehicle animals to the extent that there is no observable difference between these and the lean animal group. Histopathological assessment of pancreatic sections also showed islet characteristics in obese dapagliflozin groups similar to those seen in lean vehicle controls ie. substantial reductions in β-cell degeneration, vacuolation and islet inflammation/fibrosis.
Conclusion: These data suggest that, by inducing renal glucose excretion, dapagliflozin treatment not only improved glycaemic control, it also prevented the marked disruption of pancreatic islet morphology affecting β-cells, which is a characteristic of ZDF rats.

OP 13 Type 2 diabetes mellitus genetics and genomics

73

Genome-wide association meta-analysis and replication involving 141,547 individuals of European descent identifies further loci influencing type 2 diabetes risk

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Background and aims: Genome-wide association (GWA) studies have provided novel insights into the biology of T2D and glucose homeostasis, with close to 20 confirmed T2D-susceptibility loci to date. The DIAGRAM consortium has extended previous GWA meta-analyses and replication efforts to identify additional loci influencing T2D susceptibility in European-descent samples.
Materials and methods: We combined high-density GWA data from 8 studies: DECODE (Iceland), DGDG (French), DGI (Sweden/Finland), ERGO (Netherlands), EUROSPAN (isolates from UK; Croatia; Netherlands; Italy); FUSION (Finland), KORA (Germany) and the WTCCC (UK) using standard meta-analysis approaches. These stage 1 samples included 8,130 cases and 38,987 controls (effective sample size 22,570). After setting aside 17 previously-proven associations, lead SNPs representing the 25 next strongest associations (all $p<10^{-5}$ on meta-analysis) were followed-up through de novo genotyping in 13 studies (Steno [Denmark], DGDG2 [French], DGI_GCIP [Poland], DGI_GCIUS [US], DGI_MALMO [Sweden], DUNDEE [UK], ERGO_DIAGENE [Netherlands], KORA [Germany], ULM [Germany], W2C_UKBS [UK], HUNT [Norway], METSIM [Finland] and FUSION2 [Finland]) and in silico analyses in ARIC and NHS [both US]. The maximum effective sample size of the stage 2 samples was 82,364.
Results: Of the 25 signals for which replication was attempted, 23 (binomial $p=1.6x10^{-6}$) showed consistent direction of effect in the follow-up samples, and 18 stage 2 association p values were nominally-significant as well as directionally-consistent ($p<0.05$). When stage 1 and stage 2 data were combined (fixed-effects meta-analysis), 9 SNPs generated association P-values consistent with genome-wide significance (p-values from $1.9x10^{-8}$ to $6.7x10^{-17}$). Three of these represent signals (near *MTNR1B*, *IRS1* and *KCNQ1*) for which there is already overwhelming evidence of T2D-susceptibility effects. One (rs243021 in *BCL11A*) has been the subject of previous reports of association but now reaches genome-wide significance for the first time ($1.1x10^{-13}$). The remaining five signals (which map to chromosomes 5, 7, 8, 11, and 12) represent entirely novel T2D-susceptibility loci (point estimates of effect size ranging from 1.07-1.13). The recombination intervals within which these 5 novel signals lie encompass the coding regions of at least 25 genes (range 1-9) encoding multiple members of the phosphodiesterase and carboxypeptidase gene families and other proteins of potential relevance to diabetes pathogenesis.
Conclusion: Our findings provide compelling evidence for six novel T2D-susceptibility loci and bring the number of regions known to confer risk for T2D in European populations to 26. The ongoing efforts of the DIAGRAM consortium are directed towards systematic follow-up of these data, including deeper follow-up, fine-mapping to identify the causal variants and functional studies to develop insights into the pathophysiology of diabetes. We also plan to extend these meta-analyses and replication efforts to non-European-descent populations.
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74

Genome wide analysis of African American type 2 diabetes

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Over 3.2 million African Americans have type 2 diabetes (T2DM). This represents approximately 13.3% of the African American population and a significant proportion of the 20.8 million Americans believed to be living with diabetes. On average, an African American is twice as likely to have T2DM as a European American peer. We have carried out both gene-targeted and Genome Wide Association Studies (GWAS) of African American T2DM genetics in samples of T2DM-affected cases and non-diabetic controls recruited from the southeastern portion of the United States. African Americans are an admixed population and admixture in these studies was estimated based on 70 Ancestry Informative Markers genotyped on all DNA samples. Taking advantage of the LD structure of African-derived chromosomes, we have localized TCF7L2 association with T2DM to a 4kb LD block. With resequencing of this block in 96 African American DNAs we identified 35 novel and 14 known SNPs which were genotyped in 1033 cases and 1106 controls along with the T2DM-associated microsatellite DG10S478. The SNP rs7903146 was by far the most strongly associated (admixture adjusted, additive $P=1.59x10^{-6}$; OR 1.39, CI 1.21-1.60) suggesting this is a functional polymorphism. In contrast to TCF7L2, other SNPs associated with T2DM in European-derived samples showed little or no evidence of association with T2DM even in an enlarged sample of 2841 cases and 1964 controls. Only SNPs in CDKAL1, rs10946398 and rs7754840, showed even nominal evidence of association (P=0.06). Evaluation of these SNPs in the Human Genome Diversity Panel, however, suggests some of these SNPs may be fixed in the African population since Yoruba are monomorphic for the risk allele observed in Europeans. We have carried out a GWAS analysis on 966 cases and 1033 controls using with the Affymetrix 6.0 chip. After extensive quality control 835,042 SNPs were evaluated in admixture-adjusted association analysis focusing on the additive model. Approximately 40 SNPs had P-values $< 10^{-6}$. In addition, SNPs in two previously identified T2DM genes from studies of European-derived populations were among the top hits. SNPs rs17703228 ($P=7.42x10^{-5}$) in "juxtaposed with another zinc finger gene 1" (*JAZF1*) and rs10906180 ($P=6.38x10^{-5}$) in the calcium calmodulin-dependent protein kinase 1D (*CAMK1D*) were associated with T2DM in African Americans. These SNPs are different from SNPs previously observed to be associated with T2DM in Europeans. Replication studies of high scoring African American SNPs are underway in a two stage replication analysis totaling over 4,000 cases and 4,000 controls, and evaluation of imputed SNPs and copy number variation is also in progress.
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75

Genome-wide meta-analysis identifies novel genetic loci associated with OGTT-induced post-challenge glucose

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Background and aims: Two-hour post-load glucose measured during the oral glucose tolerance test (OGTT) defines glucose tolerance, reflects insulin resistance and influences cardiovascular risk - yet common genetic determinants of two-hour glucose are largely unknown.
Materials and methods: To identify genetic factors influencing 2h-glucose levels, we combined results from nine genome-wide association studies (GWAS) involving 15,234 non-diabetic individuals of European descent through collaborative MAGIC (Meta-Analyses of Glucose and Insulin-related traits Consortium) efforts and followed up 10-29 signals in up to 27,922 replication samples from 15 studies.
Results: Compelling genome-wide significant evidence of association with 2h-glucose was observed for SNPs at the *ADCY5* ($P = 8.6 \times 10^{-14}$), *TCF7L2* ($P = 4.7 \times 10^{-10}$), *GCKR* ($P = 4.0 \times 10^{-10}$), *GIPR* ($P = 1.5 \times 10^{-13}$) and *VPS13C* ($P = 4.3 \times 10^{-08}$) loci. These associations were independent of body mass index and fasting glucose levels, indicating a specific role of these loci for the post-challenge rise in glucose levels. Detailed physiological investigation showed that individuals carrying the GIPR risk allele displayed lower glucose stimulated insulin secretion early, at 2h and during the course of the OGTT (insulinogenic index ($P = 2.8 \times 10^{-10}$), 2h-insulin adjusted for 2h-glucose ($P = 8.1 \times 10^{-10}$), and AUC insulin to glucose ratio ($P = 6.9 \times 10^{-12}$) as well as exhibiting a lower incretin effect ($P = 0.007$).

Conclusion: The discovery of common genetic variants that are specifically and robustly associated with 2-hour glucose levels in diabetes free individuals provides novel insight into the pathogenesis of hyperglycaemia, type 2 diabetes and related metabolic disorders.

76

Novel genetic loci implicated in fasting glucose homeostasis and their impact on related metabolic traits

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Background and aims: Genetic analyses of continuous glycaemic traits can offer insights into the regulation of physiological processes, and have previously led to the discovery of *MTNR1B*, a novel locus influencing type 2 diabetes (T2D) risk.
Materials and methods: To identify additional glycaemic trait loci and investigate their metabolic impact, we performed meta-analyses of 21 genome-wide associations studies informative for fasting glucose (FG, N=46,263), fasting insulin, and indices of β-cell function (HOMA-B) and insulin resistance (HOMA-IR) (N=38,413). Follow-up genotyping of 25 loci was carried out in 61,219 independent samples.
Results: We discovered nine new loci for FG (in or near *ADCY5*, *MADD*, *ADRA2A*, *CRY2*, *FADS1*, *GLIS3*, *SLC2A2*, *PROX1* and *FAM148B*) and a novel genome-wide significant ($P<5\times10^{-8}$) association with fasting insulin and HOMA-IR (*IGF1*). Also associated with FG were T2D-associated loci *TCF7L2* and *SLC30A8,* and previously reported loci *GCK*, *GCKR*, *G6PC2*, *MTNR1B* and *DGKB/TMEM195*; *GCKR* also achieved genome-wide significant association with fasting insulin and HOMA-IR. The impact of FG loci on T2D and related metabolic traits suggests shared genetic determinants: *DGKB/TMEM195*, *ADCY5* and *PROX1* with T2D and *FADS1/FADS2* and *MADD* with lipid levels.
Conclusion: Within the associated loci the most likely biological candidate genes influence signal transduction, development, glucose-sensing and circadian regulation. The wealth of novel FG loci contrasts with the sole fasting insulin/HOMA-IR novel finding and suggests a different genetic architecture for β-cell function and insulin resistance. Our findings demonstrate considerable divergence between the genetic variants influencing glucose levels in healthy populations, those altering T2D-predisposition and other influencing related metabolic traits highlighting the complex relationships that may exist between continuous endophenotypes and the disease states to which they relate.
Replication genotyping was supported in part by Diabetes UK

77

Decreased TCF7L2 protein levels in type 2 diabetes mellitus correlated with downregulation of GIP and GLP-1 receptors and impaired beta cell function

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Background and aims: Recent human genetics studies have revealed that common variants of the TCF7L2 gene are strongly associated with type 2 diabetes mellitus (T2DM). We have shown that TCF7L2 expression in the β-cells is correlated with function and survival of the insulin producing pancreatic β-cell. In order to understand how variations in TCF7L2 influence diabetes progression, we investigated its mechanism of action in the β-cell.
Materials and methods: RNA and protein were extracted from isolated islets from three diabetic animal models, the *db/db* mouse, the high fat diet fed mouse and the VDF Zucker rat, and RT-PCR and western blot analysis for TCF7L2 were performed subsequently. TCF7L2 expression in pancreatic sections from 8 healthy controls and from 8 patients with T2DM were analyzed by immunofluorescence staining. For transfection experiments, isolated human islets were exposed to TCF7L2 small interfering RNA or pCMV-TCF7L2 plasmids and cultured with or without GLP-1/GIP. Beta cell function was assessed by glucose-, GLP-1-/GIP-, KCl- or cAMP- stimulated insulin secretion assays in static incubations and islet perifusions.
Results: We show robust differences in TCF7L2 expression between healthy controls and models of T2DM. While mRNA levels were ~2-fold increased in isolated islets from the diabetic *db/db* mouse, the VDF rat and the high fat/ high sucrose diet treated mouse compared to the non-diabetic controls, protein levels were decreased. A similar decrease was observed in pancreatic sections from patients with T2DM. In parallel, expression of GLP-1 and GIP receptors was decreased in islets from humans with T2DM as well as in isolated human islets treated with siRNA to TCF7L2 (siTCF7L2). Also, insulin secretion stimulated by glucose, GLP-1 and GIP, but not KCl or cAMP was impaired in siTCF7L2 treated isolated human islets. Loss of TCF7L2 resulted in decreased GLP-1 and GIP stimulated AKT phosphorylation, and AKT mediated Foxo-1 phosphorylation and nuclear exclusion.
Conclusion: Our findings suggest that β-cell function and survival are regulated through an interplay between TCF7L2 and GLP-1R/ GIP-R expression and signaling in T2DM.
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78

The impact of differential splicing of TCF7L2 on target gene expression in human islet of Langerhans

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Background and aims: Variants in *TCF7L2* have been shown to be highly associated with type 2 diabetes. We have previously reported that the risk allele rs7903146 does not significantly influence the overall amount or splicing pattern in human islets of Langerhans, however, the expression level of the exon4-containing-TCF7L2 transcript correlates positively with plasma HbA1c levels (r = 0.758; p = 0.018). The aims of this study are identify *TCF7L2* target genes and to investigate the effect of TCF7L2 exon 4 splicing on these genes in human islets of Langerhans.
Materials and methods: All samples were from non-diabetic individuals (6 female, 11 male, aged 26-73 years, BMI 17.7-29 kg/m^2). Total RNA was isolated using the AllPrep DNA/RNA Mini Kit (Qiagen) and analyzed using Gene 1.0 ST whole transcript based assays (Affymetrix). The percentage of the exon4-containing-TCF7L2 transcripts was measured with QPCR using absolute quantification. Statistical analyses were performed using non-parametric Spearsman correlation (SPSS 16.0). Candidate genes were analysed using Gene Set Analysis Tool kit (http://bioinfo.vanderbilt.edu/).
Results: The TCF7L2 binding motif (A-C/G-A/T-T-C-A-A-A-G) was investigated in the promoter region (5 kb upstream and 1 kb downstream of transcriptional start site) of all annotated genes and regions in the human genome using a bio-informatics approach. All together, 2759 annotated genes were found to contain the motif. Genes not represented on the array and genes with expression below the detection level were excluded. 205 genes correlated significantly (p = 0.05), with exon4-containing-TCF7L2 transcript expression. Using pathway analysis, several pathways were found to be significantly enriched, including protein transport (p = 8.69 x 10e-4) and intracellular transport (p = 1.15 x 10e-2). In order to select a suitable model system to replicate these findings, the splicing pattern in the INS-1 cell line was established and was found to resemble the human pattern. Currently, the functional consequences of reduced expression of a number of the correlated genes are being investigated using siRNA.
Conclusion: Based on our results, intronic variation in *TCF7L2* could potentially perturb the binding of splice factors and thereby influence the splicing pattern. Differential splicing, e.g. exon 4 inclusion/exclusion, could then affect target gene expression and propagate the increased risk of type 2 diabetes. Future studies are obviously necessary to fully elucidate the mechanism/s whereby genetic variation in *TCF7L2* leads to increased risk of the disease and the role of differential splicing.
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OP 14 Endothelium and coagulation

79

***MiR-217* regulates aging and angiogenic functions in human endothelium via SirT1**

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Background and aims: Increasing evidence indicates that premature senescence contributes to endothelial dysfunction, a pivotal step in development of diabetes and metabolic syndrome. Molecular targets relevant for this process remains to be clarified. To this aim we have performed a microarray analysis of MicroRNAs (miRNAs), crucial post transcriptional modulators of gene expression, in an endothelial aging model in vitro, comparing young (Population doubling levels, PDLs8) to old (PDLs44) HUVEC.
Materials and methods: To this aim we have performed a microarray analysis of MicroRNAs (miRNAs), crucial post transcriptional modulators of gene expression, in an endothelial aging model in vitro, comparing young (Population doubling levels, PDLs8) to old (PDLs44) HUVEC.
Results: We found a progressive induction of *miR-217* during aging. Bioinformatic analysis and a promoter reporter assay revealed that SirT1, a NAD^+-dependent hystone deacetylase playing a critical role in stress resistance, longevity and metabolism, is a potential target of *miR-217*. Transfection of young HUVEC with an oligonucleotide that decreases *miR-217* (Anti-miR-217) increased SirT1 expression. Conversely, overexpression of the *miR-217* precursor decreases SirT1 expression in young HUVEC. In young HUVECs overexpressing *miR-217,* senescent cells were increased up to 30% of the control ($p< 0.0005$) in a Senescence Associated-β-gal activity assay. On the contrary, in old HUVEC transfection with Anti-miR-217 decreased the percentage of senescent cells approximately to 30% compared with the control ($p< 0.005$), indicating that *miR-217* plays a role in endothelial premature senescence in vitro. SirT1 controls endothelial angiogenic functions via deacetylation of forkhead transcription factor FoxO1. In young endothelial cells, *miR-217* overexpression significantly impaired tube forming activity ($p< 0.0005$) and induced a marked FoxO1 acetylation. Conversely, Anti-miR-217 in old HUVECs significalntly increased tube formation ($p< 0.005$). Next, we compared, by real time PCR, the mRNA expression of targets coordinately regulated by Sirt1 and of Foxo1, such as GADD45, Flt1 and eNOS, in young and old HUVEC transfected with miR-217 or Anti-miR-217, respectively. Our results confirmed that *miR-217* is involved in FoxO1 targets modulation. Functional changes in senescent endothelial cells in vivo may play an important role in the pathophysiology of age associated vascular disorders. Therefore we performed real time PCR experiments in plaques from patients who underwent carotid endarterectomy for symptomatic disease and we confirmed in vivo a negative correlation between *miR-217* and Sirt1 expression and FoxO1 acetylation state; similarly, we observed a positive correlation between Sirt1 and FoxO1 targets GADD45, Flt1 and eNOS.
Conclusion: These data suggest that miR-217, via regulation of SirT1/FoxO1, can regulate senescence and angiogenesis in endothelial cells thus providing a molecular explanation to age-related endothelial dysfunction.
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80

Endothelial and endocrine alterations in diabetic pancreas. Role of angiopoietin-2 and thrombospondin-1

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Background and aims: In the events leading to type 2 diabetes, inadequate compensatory increase of the beta cells mass could be a direct consequence of vascular disease, either by endothelial dysfunction or secondary to defective secretion of angiogenic peptides by beta cells. To dissect the role of angiogenic process in the maintenance and growth of islets, we studied pancreatic chimeras, consisting of endocrine chick cells and murine endothelial cells, obtained by grafting embryonic chick pancreas in SCID mice. We analyzed angiogenesis during development of the pancreatic chimera and examined the impact of hyperglycemia on the vasculature of the pancreas. We then used an avian-specific retrovirus, the RCAS system, to over-express VEGF during pancreatic development.
Materials and methods: Seven-week-old female SCID mice were made diabetic by a single intra-peritoneal injection of streptozotocin (160mg/kg). Embryonic chick pancreases (E14) were grafted under the kidney capsule of SCID mice and collected 14 days later. Pancreatic vasculature was analyzed by immunohistochemistry using nestin, a mouse-specific endothelial marker, and in situ hybridization with both mouse- and chick-specific VEGFR-2 probes. Expression of angiopoïetin-2 (Ang2), thrombospondin-1 (Tsp1) and VEGF was analyzed by in situ hybridization. To over-express VEGF, an avian-specific retrovirus RCAS carrying the gene encoding the VEGF (RCAS-VEGF) was prepared. Chick pancreases were infected in vitro with the RCAS-VEGF for 1 hour before graft. Mammalian cells were not susceptible to the RCAS infection. All analyzes were performed on n=5 animals. This study was carried out along the principles of laboratory animal care.
Results: As compared to normoglycemia, hyperglycemia increased beta-cell density ($p<0.05$). Pancreatic vasculature was disorganized with large blood-filled spaces and vessels discontinuities. Vessel density was decreased as evidenced by quantification of nestin- and VEGFR-2-positive cells ($p< 0.05$). Expression of two anti-angiogenic factors, Tsp1 and Ang2 was increased in grafts ($p<0.05$), whereas no clear difference was observed for VEGF.
In order to correct the vascular defects, we over-expressed VEGF in pancreas. In normoglycemic condition, number of both beta- and endothelial-cells was not altered by VEGF over-expression. In hyperglycemic conditions, number of both beta- and endothelial-cells was increased by VEGF over-expression ($p<0.05$). Ang2 and Tsp1 determinations are pending.
Conclusion: Hyperglycemia induced major defects of the pancreatic vasculature associated with Ang2 and Tsp1 over-expression. These two anti-angiogenic growth factors have been associated with vasculopathy in diabetes. We demonstrated for the first time up-regulation of Ang2 expression in the pancreas in hyperglycemic conditions. In normoglycemic condition, there was no change after VEGF over-expression, whereas in hyperglycemic condition, VEGF over-expression increased both endocrine and endothelial cells.
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81

Tissue Factor pre-mRNA splicing in platelets from healthy subjects and type 2 diabetes mellitus patients

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Background and aims: Type 2 diabetes mellitus (T2DM) patients have a 2-8-fold higher risk of cardiovascular disease than healthy individuals and eighty percent will die of thrombosis-related disorders. T2DM induces a prothrombotic state caused by imbalance of the hemostatic mechanism characterized by hypercoagulation and platelet hyperaggregability. Patients with T2DM have increased plasma Tissue Factor (TF), the starter of coagulation. Recent findings suggest a new source for TF expression. Platelets contain Splicing Factor 2 (SF2), a regulator of constitutive and alternative splicing, together with pre-mRNA with message for TF. Splicing of TF pre-mRNA results in TF protein and the capacity to initiate coagulation. We investigated whether abnormal platelet TF production was the cause of the hypercoagulant state in T2DM patients.
Materials and methods: Platelets form T2DM patients and matched controls were depleted from leukocytes by a double incubation with anti-CD14/CD45 antibody-coupled beads and magnetic separation. RT-PCR for CD14 was negative in 30 cycles RT-PCR. Platelets were adhered to different surface-coated proteins (0.5 - 4 hrs, 37°C) and phosphorylation of SF2, TF-pre-mRNA splicing, TF protein and TF procoagulant activity (Xa assay) were measured. Numbers of adhered platelets were based on alkaline phosphatase content.
Results: Adhered platelets showed adhesion-induced Ser phosphorylation of SF2 and TF pre-mRNA splicing (blocked by the splicing inhibitor Tg003) and translation-dependent TF activity (blocked by the protein synthesis inhibitors puromycin and cycloheximide). Collagen induced TF-pre-mRNA splicing started at 30 min. and was optimal after 4 hrs. Collagen-adhered platelets produced 600 fold more TF than suspensions (0.17 ± 0.09 and 0.0003 ± 0.0001 ng TF/ $2.0 \cdot 10^8$ platelets, mean +/- SEM, n=3). Compared with fibrinogen (100%), TF production on horm collagen, collagen I, thrombin, collagen III and von Willebrand Factor was 143 ± 42, 132 ± 29, 126 ± 20, 71 ± 10 and 38 ± 14%. Incubation with AR-C699331 MX and indomethacin, which mimic the action of clopidogrel and aspirin respectively, inhibited collagen-induced TF activity, which was almost zero when co-incubated with both inhibitors. Pharmacologic blockade showed signaling through cAMP (increased by iloprost, forskolin) and PI3-kinase/protein kinase B (inhibited by wortmannin,

LY and ML9). In normal platelets, insulin (1-100 nM, 5 min) interfered with cAMP regulation, inhibited collagen-induced TF-pre-mRNA splicing and reduced TF procoagulant activity by 63 ± 10 % at 100 nM (n=5). The inhibition was two-fold lower in T2DM platelets and reached 30 ± 7% (n=4).
Conclusion: These results demonstrate that in normal platelets TF synthesis is inhibited by insulin through interference with cAMP. T2DM platelets have become partially resistant to this inhibition leading to upregulation of TF synthesis. We conclude that the hypercoagulant state in type 2 diabetics might be caused by increased platelet TF production.
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82

Absence of fibronectin EDA exon increases endothelial dysfunction and oxidative stress in diabetic mice aortas
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Background and aims: Fibronectin (FN) pre-mRNA undergoes alternative splicing at three positions, in a tissue and develomental-specific manner generating up to 20 different protein isoforms. One of the alternatively spliced exons is the Extra Domain A (EDA). EDA exon inclusion is normaly low in adult tissues but it is upregulated in several injure-repair processes. Hyperglycemia induces overexpression of selected vascular extracellular matrix proteins, including FN. In addition, FN is known to modulate endothelial nitric oxide synthase (eNOS) activation and the absence of regulated splicing of EDA reduces atherosclerosis in mice. The precise role of the EDA segment in vascular oxidative stress and endothelial dysfunction in diabetes needs further clarification. The aim of this study was, therefore, to assess endothelial function and oxidative stress in diabetic mice constitutively expressing ($EDA^{+/+}$) or lacking ($EDA^{-/-}$) the EDA exon of the FN gene.
Materials and methods: Three months old $EDA^{+/+}$ (n=18), $EDA^{-/-}$ (n=16) and C57/BL6 (wild type, WT: n=14) mice were treated by multiple streptozotocin injections to induce diabetes. After 20±2 weeks aortas were harvested and endothelial function immediately assessed in organ chamber vasorelaxation experiments. The remaining aortic rings were frozen for RNA extraction. eNOS, Nox4 and p22phox gene expression analysis was performed by labelled-probe real-time RT-PCR. Normalisation was performed by ratio to 28S rRNA expression. Statistical analysis was performed by one-way ANOVA or Student's t-test as appropriate.
Results: Streptozotocin resulted in diabetes induction as demonstrated by increased ($p<0.01$) blood glucose levels in all genotypes (pre- vs. post treatment: $EDA^{+/+}$: 163.3±5.9 vs. 411.3±12.1 mg/dl; $EDA^{-/-}$: 168.1±3.7 vs. 474.7±8.5 mg/dl; WT: 141.9±5.0 vs. 429.3±21.0 mg/dl). Acetylcoline (10^{-6} mol/l) mediated endothelium-dependent vasorelaxation was lower in $EDA^{-/-}$ mice compared to control aortas (36.5±5.7% vs. 52.4±4.4%, $p<0.05$) and to $EDA^{+/+}$ (52.7±2.9%, $p<0.05$). Endothelium-independent vasorelaxation to DEA-NONOate was not different among groups. To investigate the mechanisms of impaired endothelial-dependent vasorelaxation in $EDA^{-/-}$ mice, we analysed eNOS, Nox4 and p22phox gene expression. eNOS mRNA was significantly reduced in $EDA^{-/-}$ samples compared to control ones (0.145±0.105 vs. 0.456±0.094 a.u., $p<0.05$). In contrast, both Nox4 and p22phox mRNA levels were significantly higher in $EDA^{-/-}$ aortas than in control vessels (Nox4: 1.167±0.320 vs. 0.226±0.038 a.u., $p<0.01$; p22phox: 0.444±0.118 vs. 0.099±0.004 a.u., $p<0.01$). Nox4 mRNA was also significantly higher in $EDA^{-/-}$ than in $EDA^{+/+}$ (1.167±0.320 vs. 0.340±0.052 a.u., $p<0.01$).
Conclusion: These data demonstrate that constitutive absence of fibronectin EDA exon increases endothelial dysfunction in diabetes and suggest an important role for this exon as a modulator of eNOS and oxidative-stress related gene expression.
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83

Intracellular methylglyoxal levels explain hyperglycaemia-induced impaired endothelium-dependent vasorelaxation in an oxidative stress-dependent pathway
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Background and aims: In hypergglycaemic conditions, increased formation of the glycolysis-derived reactive dicarbonyl compound methylglyoxal (MGO) and MGO-derived advanced glycation endproducts (AGEs) are thought to be implicated in endothelial dysfunction and, subsequently, in the development of diabetic vascular complications. A common feature of endothelial dysfunction is an impaired nitric-oxide (NO)-dependent vasodilatation. We now investigated the effect of hyperglycaemia on impaired vasoreactivity and a putative role of MGO and glyoxalase-I (GLO-I), i.e. the enzyme detoxifying MGO, therein.
Materials and methods: Isolated mesenteric arteries from wild type and transgenic GLO-I rats were mounted in a myograph. The effects of hyperglycaemia and MGO on NO-dependent vasodilatation were tested with acetylcholine (ACh; 0.01 - 10μM) in the presence of indomethacin (10μM) during contraction with potassium (65mM). AGE formation was determined by immunohistochemistry with an antibody against the major MGO-adduct 5-hydro-5-methylimidazolone (MG-H1). Reactive oxygen species (ROS) formation was measured with a DCF-probe and an antibody against nitrotyrosine.
Results: Treatment of wild type mesenteric arteries with high glucose (30mM for 2-hours) resulted in reduced acetylcholine-induced vasorelaxation. Impairment of dilatation by hyperglycaemia was not observed in mesenteric arteries of GLO-I transgenic rats compared with their wild type littermates, indicating a specific intracellular MGO effect. Direct exposure of mesenteric arteries to 0.1, 0.33 and 1.0mM MGO (for 1-hour) significantly reduced the acetylcholine-induced vasorelaxation, as indicated by decreased potency (log EC50 was -6.33, -6.21, -6.17 and -5.94 M, respectively) and efficacy (Emax was 71; 58; 51 and 49 % dilatation, respectively). This effect was endothelium-dependent as indicated by unaltered relaxing responses to the NO donor Na-nitroprusside. Impairment of dilatation by MGO was not observed in mesenteric arteries of GLO-I transgenic rats indicating a specific intracellular MGO effect. Pre-incubation of healthy mesenteric arteries with MGO increased staining of MG-H1 in endothelial cells and adventitia accompanied by an increase in the oxidative stress marker nitro-tyrosine. The MGO-induced impaired vasoreactivity was normalized by preincubation of the vessels with the antioxidants N-Acetyl Cysteine, EUK-134 and Mn(III)TMP.
Conclusion: We found that hyperglycaemia-induced impairment of endothelium-dependent NO-mediated vasodilatation could be prevented by GLO-I overexpression. In addition, exposure to MGO decreased NO-mediated dilatation due to an increase in oxidative stress. These data implicate that elevated MGO leads to impaired endothelial function in diabetes and that GLO-I may prevent this.

84

Relations between endothelial function and peripheral microcirculation and cardiac autonomic neuropathy in type 2 diabetic patients
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Background and aims: Peripheral endothelial function is impaired in patients with coronary disease. Endothelial dysfunction and autonomic neuropathy may alter peripheral microcirculation. The aim of this study was to examine in type 2 diabetic patients whether endothelial dysfunction or a reduction of peripheral microcirculation might be markers of coronary disease and the role of autonomic dysfunction in these disorders
Materials and methods: We included 116 patients without any cardiac history or symptom. Silent myocardial ischemia (SMI) was assessed using an ECG stress test and if SMI was found, coronary stenoses were assessed by coronary angiography. Endothelial function and peripheral microcirculation were examined by measuring forearm post-ischemic hyperemia (echo-dop-

pler), through the changes in humeral artery diameter (D2/D1) and blood flow (F2/F1), respectively. CAN was assessed using Ewing' tests which mainly estimate cardiac parasympathetic activity.
Results: SMI was found in 34 patients and was associated with significant coronary stenoses (SCS) (>70%) in 18 of them, and CAN was present in 82 patients. D2/D1 and F2/F1 did not correlate with age, BMI and HbA1c, and did not differ in the patients with or without hypertension. D2/D1 did not differ in the patients with or without SMI or CAN, but was significantly lower in patients with SCS (p=0.04). F2/F1 was significantly lower in patients with CAN (p= 0.002) and decreased with CAN severity (p = 0.02), and was lower in patients with SCS (p=0.01). In multivariate analyses F2/F1 levels were associated independently with CAN and its severity (p< 0.001 and < 0.005).
Conclusion: These data suggest that neither endothelial function nor peripheral microcirculation may be considered as markers of silent coronary disease but that a defect in vagal activity and a relative excess of sympathetic activity may play a role in the alterations of microcirculation in type 2 diabetic patients
Supported by: ANDRS

OP 15 Glucose monitoring

85

Effect of self-monitoring of blood glucose in patients with type 2 diabetes treated with oral medication on quality of life parameters: a 1-year randomised controlled trial
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Background and aims: Self-monitoring of blood glucose (SMBG) improves glycemic control in patients using insulin. Apparently, in patients with type 2 diabetes mellitus (T2DM) not using insulin SMBG does not result in improvement of glycemic control. In previous studies, either no effect on health related quality of life (HRQoL) was found, or, like in the ESMON trial, SMBG resulted in more depressive symptoms. Our aim was to investigate the effect of SMBG (postprandial values) on HRQoL and treatment satisfaction in patients with T2DM not using insulin who are in persistent moderate glycemic control.
Materials and methods: Patients were eligible when between 18 and 70 years of age, with an HbA1c between 7 and 8.5% at the start of the study (at annual check-up) and at the previous annual check-up, using 1 or 2 different oral blood glucose lowering drugs without regimen change during the preceding 3 months, and without use of SMBG in the preceding 6 months. Forty patients were randomly assigned to receive either SMBG added to usual care or to continue with usual care during 1 year. SMBG was performed using the Accu-check Aviva blood glucose meter. Twice a week (including either Saturday or Sunday), a fasting glucose value and 3 postprandial glucose values were measured. All participants were instructed about acceptable glucose values (fasting: 4-8 mmol/L, postprandial: 4-10 mmol/L) and no specific (dietary) advice was given. HbA1c was measured every 3 months. Blood glucose lowering therapy was intensified when HbA1c was ≥ 8.5%. At baseline, after 6 and after 12 months, HRQoL and treatment satisfaction were assessed using the short form 36 questionnaire (SF-36), the Type 2 Diabetes Symptom Checklist (DSC-type 2), the Diabetes Treatment Satisfaction Questionnaire (DTSQ) and the WHO-wellbeing index (WHO-5).
Results: Recruitment took place between March 2006 and October 2007. At baseline, mean age was 59 years, median diabetes duration was 6.0 years, mean HbA1c was 7.6%, and mean BMI was 31 kg/m^2. At the end of the study, there were no significant changes between groups on the DTSQ, the DSC-type 2, the WHO-5 or on the physical and mental component summary scores of the SF-36. The SF-36 dimension 'health change' was lower in the SBMG-group (mean difference: -12 (95%CI: -20.9, -3.1; p=0.008)).
Conclusion: Tablet-treated T2DM patients rating their health over a 1 year period experienced a worsening on the dimension 'health change' when performing SMBG. On top of the absence of a clinical benefit, we conclude that there is no evidence for a positive impact of SMBG on HRQoL or treatment satisfaction in T2DM patients not on insulin. We argue therefore, that the use of SMBG in this patient group should be reconsidered.
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86

A randomized, controlled trial of blood-glucose self-monitoring in type 2 diabetic patients receiving conventional insulin treatment
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Background and aims: Blood-glucose self monitoring (SMBG) is necessary and cost-effective within intensified insulin regimens. Whether SMBG helps obtaining better glycaemic control in type 2-diabetic patients on conventional insulin regimens (premixed insulin twice daily or basal insulin once daily, with or without OAD) is not known. Our randomized, controlled, multicentric study aimed at clarifying potential improvement in glycaemic control with a once-weekly glucose profile (SMBG) in such patients.

Materials and methods: 244 patients participated (300 patients randomized, 56 early drop-outs). All patients were instructed to use urinary glucose monitoring once daily (after breakfast). 125 were randomly selected not to perform SMBG (- SMBG), 119 were instructed to use once-weekly 4-point glucose profiles (+ SMBG). Baseline age was 66 ± 8 vs. 65 ± 8 years, 64.0 vs. 56.3 % were male, premixed insulin was used in 52.8 vs. 58.0 %, HbA_{1c} was 7.3 ± 1.2 vs. 7.3 ± 1.3 %, BMI was 30.8 ± 5.4 vs. 30.0 ± 4.6 kg/m^2, and diabetes duration was 12 ± 9 vs. 12 ± 8 years for the - SMBG and + SMBG groups (mean ± SD, all not significant). Patients were seen (at least) at 3-monthly intervals to evaluate any necessity for therapy intensification (adding antidiabetic agents or increasing their doses) and for the determination of HbA_{1c} (HPLC, normal values 4.0-6.2 %). Primary endpoint was HbA_{1c} after 12 months (adjusted ANCOVA with baseline HbA_{1c} as covariate). Secondary analyses determined the probability of therapy intensification with increasing percentage of glucose-positive urine tests (all patients), with increasing HbA_{1c} values, and with increasing glucose concentrations as determined by SMBG (only those assigned to this arm), estimated by logistic regression (generalized linear mixed models) using corresponding quintiles as independent class variables, adjusted for visit, treatment group (fixed effects) and repeated measurement.
Results: Adherence to urinary glucose control was 90.7-98.4 % in both groups. 95.7-98.3 % of those assigned to + SMBG performed glucose profiles vs. 6.5-8.1 % in the - SMBG group. HbA_{1c} was reduced in both groups, to 7.0 ± 1.0 (- SMBG) vs. 7.0 ± 1.2 % (+ SMBG; $p = 0.93$ for a difference between groups). The frequency of therapy intensification was similar in both groups. The odds ratios for therapy intensification rose with increasing proportions of urines testing positive for glucose ($p = 0.0007$; $n = 235$). The odds ratios for therapy intensification, likewise, rose with increasing HbA_{1c} values ($p < 0.0001$; $n = 134$), increasing fasting glucose (as determined by SMBG; $p = 0.0007$; $n = 117$), and increasing postprandial glucose ($p = 0.0004$; $n = 116$).
Conclusion: On a background of urinary glucose testing, additional 4-point glucose profiles once weekly did not further improve glycaemic control in type 2-diabetic patients receiving conventional insulin treatment. However, in those patients performing SMBG, therapy intensification was significantly more likely with higher values measured, indicating a clear association to SMBG. The magnitude of therapy intensification introduced may have been too small to finally affect overall glycaemic control.
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87

Exploring patient beliefs about blood glucose monitoring
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Background and aims: Self-monitoring of blood glucose (SMBG) is a cornerstone of personal diabetes management. However, recent evidence suggests that many people with diabetes do not check blood glucoses regularly and/or do not use SMBG data to adjust their own care. A variety of psychosocial factors may contribute to low SMBG, but few studies have explored these potential relationships. We examined the impact of affective factors (depressed mood, diabetes-related distress) and specific attitudinal factors (personal beliefs about the value of SMBG) on SMBG frequency (number of SMBG tests/week) and personal use of SMBG results (number of SMBG tests/week that were then used to adjust therapy) in type 2 diabetes (T2DM).
Materials and methods: Four hundred and three poorly-controlled ($HbA_{1c} \geq 7.5\%$), insulin-naive T2DM subjects were drawn from a large, multicenter study in the United States. Subjects completed a questionnaire that included five sets of variables: demographic information; diabetes status (e.g., HbA_{1c}, BMI); diabetes self-care behaviors; affective status (e.g., depressed mood, diabetes-related distress); and SMBG-related attitudes (7 newly created items; e.g., "I just find the SMBG results to be discouraging", "there is no rhyme or reason to the results I see"). The variables were included in multiple regression equations predicting frequency of SMBG and use of SMBG results.
Results: Sixty-four percent of subjects were white and they were evenly divided by gender (53.0% male). Mean age, HbA_{1c}, BMI, and duration of diabetes were 56.0 years (± 10.7), 8.9% (± 1.2), 34.5 (± 7.2) and 7.6 years (± 5.9), respectively. Many subjects evidenced negative SMBG attitudes; e.g., approximately one-quarter or more agreed that their own SMBG results were typically discouraging (31.7%), confusing (19.5%), and/or that they could do nothing to influence the results (20.6%). The seven SMBG items were submitted to a Principal Factor Analysis with Promax rotation, yielding a single coherent factor accounting for 52% of the total variance (alpha=0.84). We therefore computed a total SMBG beliefs score, with higher numbers indicating a more negative attitude towards SMBG. Negative SMBG attitude was associated with depression ($r = 0.15$; $p < 0.05$) and diabetes-related distress ($r = 0.33$; $p < 0.05$). Multiple regression analysis indicated that, adjusted for demographic variables, negative SMBG attitude, but not depressed mood or diabetes-related distress, was independently associated with lower SMBG frequency ($p < .02$) as well as less personal use of SMBG results ($p < .05$).
Conclusion: Beliefs about SMBG are linked to actual SMBG frequency and use of test results, independent of all other study variables. We found that negative beliefs about SMBG are common and are associated with depressed mood and diabetes-related distress, but neither depressed mood nor distress are associated with SMBG use. This suggests that patients may not follow recommendations for regular SMBG use because they do not understand how to interpret and/or make use of their results. This underscores the importance of HCPs and patients working collaboratively to make effective use of SMBG data.
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88

Glucose concentrations at the site of subcutaneous insulin delivery in patients with type 1 diabetes
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Background and aims: Type 1 diabetes is presently treated by self-administration of insulin, either by subcutaneous bolus injection or by continuous subcutaneous infusion. Type 1 diabetic patients furthermore separately self-monitor glucose levels in blood obtained by finger-pricking to guide the adjustment of insulin dosage. In order to simplify and improve the treatment of type 1 diabetes, we propose to use the site of subcutaneous insulin administration for the measurement of glucose. The aim of the present study was to test the feasibility of estimating blood glucose concentrations from the glucose levels directly measured at the site of subcutaneous insulin delivery in diabetic subjects.
Materials and methods: Three 24-gauge microperfusion (MP) catheters were inserted into the subcutaneous adipose tissue of ten type 1 diabetic subjects (2 females, 8 males; age 39.8 ± 2.9 years; BMI 25.3 ± 1.0 kg/m^2; HbA_{1C} 7.6 ± 0.3 %, all C-peptide negative). One MP catheter was perfused with rapid-acting insulin (100 U/ml, Aspart), and used for insulin delivery and simultaneous glucose sampling during an overnight fast and after ingestion of a standard glucose load (75 g). As controls, the other two MP catheters were perfused with an insulin-free perfusate (5 % mannitol), and used for glucose sampling only. Blood glucose was measured frequently at the bedside. Tissue glucose concentrations were calculated from glucose and conductivity levels determined in the effluent samples of the MP catheters.
Results: Insulin delivery with the MP catheter was adequate to achieve normoglycemia during fasting and to restore normoglycemia by ~5 hours after glucose ingestion. Furthermore, tissue glucose concentrations derived with the insulin-perfused probe agreed well with glucose levels measured in blood plasma. Bland & Altman analysis revealed a median residual 2SD value of 31.6 % (interquartile range: 26.4 - 34.6 %), and error grid analysis indicated that the percentage number of the tissue values falling in the clinically acceptable range is 99.6 %. Comparable analysis results were obtained for the two mannitol-perfused MP catheters.
Conclusion: Our data suggest that estimation of plasma glucose concentrations from the glucose levels directly observed at the adipose tissue site of insulin delivery is feasible and its quality is comparable to that of estimating plasma glucose concentrations from the glucose levels measured in insulin-unexposed adipose tissue.

89

The RealTrend study: effect of continuous glucose monitoring on metabolic control in addition to pump therapy in poorly controlled type 1 diabetic patients

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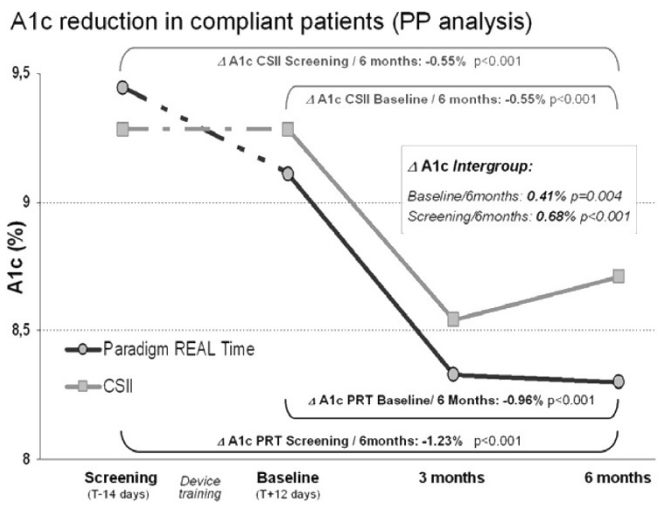
Background and aims: Efficacy of insulin pump augmented with continuous glucose monitoring (CGM) versus Continuous Subcutaneous Insulin Infusion with standard self-monitoring of blood glucose has not yet been determined.
Materials and methods: In this randomized, controlled, multi-center trial, 132 adults and children with type 1 diabetes, insufficiently treated with multiple daily insulin injections (A1c≥ 8%) were assigned to a 6 months treatment in one of 2 study arms: PRT arm, fitted with the Paradigm REAL Time System (Medtronic insulin pump with integrated CGM), or CSII arm, fitted with an insulin pump and conventional blood glucose self-monitoring. In the PRT arm, patients wore glucose sensors for 9 training days prior to baseline HbA1c. HbA1c change between baseline and study end served as a primary endpoint in the 2 study arms. Secondary endpoints included hyper- and hypoglycemia parameters measured by CGM: average glucose, time spent above and below hyper- and hypoglycemia limits, and respective area under the curve.
Results: HbA1c was analyzable for 115 patients (46 children, 69 adults) of the full analysis population (FAS) and improved between baseline and study end in the two groups (PRT, n=55, -0.81%±1.09; CSII, n=60, -0.57%±0.94; p=0.087). A per protocol (PP) analysis of 91 patients (35 children, 56 adults) who wore sensors over 70 % of the time (as required by the inclusion criteria) showed a significant difference in A1c reduction between groups (PRT; n=32; -0.96%±0.93, CSII, n=59; -0.55%±0.93, p=0.004). Ancillary analyses revealed a significant decrease in HbA1c levels between the screening visit and the end of the study (PRT -1.14±1.21, p<0.001; CSII group -0.57±0.91, p<0.001), as well as a significant difference in favor of the PRT group (p=0.006) for the entire study population as well for the per protocol population (PRT -1.23±1.08, p<0.001; CSII -0.55±0.90, p<0.001; inter-group comparison: p<0.001). In PRT group, CGM hyperglycaemia parameters decreased in line with HbA1c, without increased hypoglycaemia.
Conclusion: In both FAS and PP populations HbA1c decreased in both study arms after treatment was changed from MDI to CSII or PRT, but improved significantly more in the PRT group when patients wore the CGM more than 70% of the time versus CSII.



90

Sensor augmented pump therapy substantially lowers HbA_{1c}; a randomized controlled trial

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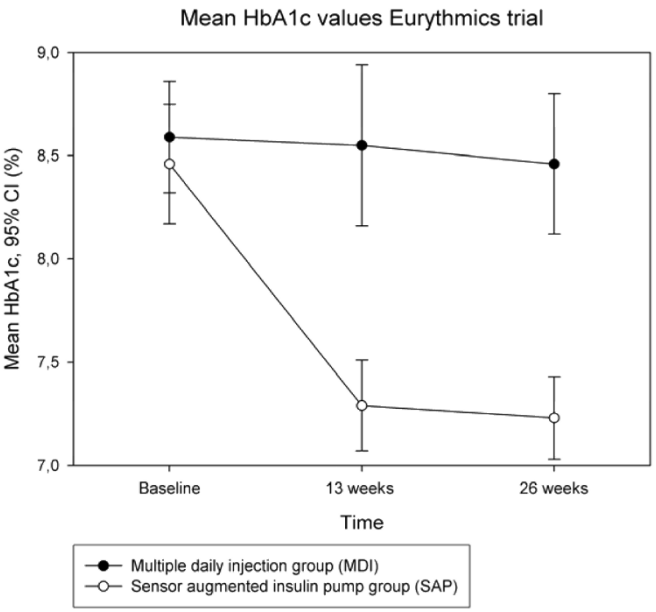
Background and aims: An insulin pump augmented with a continuous glucose monitor (CGM) and mealtime insulin dose advisor is the first step towards a closed-loop system. This is the first randomized trial to investigate efficacy of this integrated system as compared to multiple daily injection therapy with SMBG.
Materials and methods: In an investigator-initiated multinational multicenter controlled trial (Eurythmics trial ISRCTN22472013), we randomized 83 diabetes type 1 patients, age 18-65 years and HbA1c ≥ 8.2%, to receive either 26 weeks of continuous treatment with a sensor augmented insulin pump (SAP) (Paradigm® REAL-Time) or standard care with multiple daily injection therapy (MDI). In both arms, treatment was preceded by 6 days of retrospective CGM use and treatment was optimized based on this information. A blinded six day CGM recording was taken in the MDI group at 13 and 26 weeks. HbA1c at 13 and 26 weeks was measured and the occurrence of severe hypoglycaemia (requiring third party assistance) was scored. Analyses were performed using ANCOVA models, adjusting for baseline values and centre.
Results: Baseline characteristics in the SAP group (n=44) and MDI group (n=39) were: mean age (± SD) 39 ± 12 and 37 ± 11 years; diabetes duration 17 ± 11 and 21 ± 9 years and 50% and 46% female, respectively, while 43/44 (98%) and 35/39 (90%) patients completed the trial. Mean HbA1c (± SD) in the SAP group decreased from 8.46 ± 0.95% to 7.23 ± 0.65% after 26 weeks. In the MDI group mean HbA1c was 8.59 ± 0.82% at baseline and 8.46 ± 1.04% after 26 weeks.
The mean difference in HbA1c change after 26 weeks was -1.10% (95% CI -1.41 to -0.71, *P*<0.001) in favour of the SAP group (Figure). The proportion of patients reaching EASD/ADA HbA1c target of <7.0% was 34.1% in the SAP group and 0.0% in the MDI group, *P*<0.001. Neither the difference in number of severe hypoglycemia episodes (4 in the SAP group versus 1 in the MDI group, *P*=0.22) nor the difference in change in sensor mean area under the curve (AUC) for hypoglycaemia (<72 mg/dl) (-0.20 mg/dl*min 95% CI-0.52 to 0.11, *P*=0.20) was statistically significant.
Conclusion: Sensor augmented pump therapy effectively lowers HbA1c in poorly regulated type 1 diabetes patients without increase in hypoglycaemia. The magnitude of the difference in HbA1c change of -1.10% suggests an additive and possibly synergistic effect of the insulin pump, mealtime insulin dose advisor and CGM.



Supported by: Medtronic MiniMed, Inc.

OP 16 Metabolic effects of bariatric surgery

91

Effect of laparoscopic ileal interposition on beta cell function and insulin sensitivity in non-obese patients with type 2 diabetes mellitus

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Background and aims: Bariatric surgery is very effective in achieving weight loss and improving type 2 diabetes mellitus (T2DM) in obese patients. Our aim was to evaluate T2DM in patients with a BMI below 30 kg/m2 the impact of two versions of laparoscopic ileal interposition and sleeve gastrectomy: one in which the duodenum is kept in continuity with the stomach (II-SG) and another in which a diversion of the second portion of the duodenum is applied (II-DSG).:
Subjects and methods: Twenty-two (13M/9F; 58±2 years; BMI=26.0±0.7 kg/m2) and 20 (10M/10F; 60±2 years; BMI=27.3±0.3 kg/m2) T2DM patients underwent II-SG and II-DSG, respectively. A standard OGTT (75 g) was performed before and 8-15 months after surgery; C-peptide deconvolution and mathematical modelling were used to reconstruct insulin secretion rates and beta cell function. In particular, beta cell glucose sensitivity (b-GS, slope of the insulin secretion/glucose concentration dose-response curve) and total insulin output in response to oral glucose (TIR) were calculated. Insulin sensitivity was estimated from the plasma glucose and insulin responses by the OGIS (Oral Glucose Insulin Sensitivity) index.
Results: Mean A1c decreased from 8.15 +- 2.1% to 6.06 +- 1.19 %. With II-SG, BMI fell to 21.3±0.3 kg/m2 ($p<0.05$). Fasting plasma glucose (9.4±0.7 vs 6.6±0.3 mmol/l, $p<0.001$), 2-hour glucose (20.0±1.1 vs 9.3±1.0 mmol/l, $p<0.001$) and incremental glucose area-under-curve (AUGC) (902±59 vs 643±76 mmol.1-1.h-1, $p<0.05$) were lower after surgery. Similar effects were seen after II-DSG (BMI to 22.3±0.6, $p<0.0001$, fasting plasma glucose, 11.3±1.1 vs 6.3±0.5mmol/l, $p<0.001$; 2-hour glucose, 19.8±1.2 vs 10.1±1.1 mmol/l, $p<0.001$). TIR increased from 36.8±4.3 to 55.0±6.5 nmol/m2 ($p<0.05$) with II-SG and from 41.3±3.4 to 53.6±3.4nmol/m2 ($p<0.05$) with II-DSG, while b-GS rose from 18±4 to 47±7 pmol.min-1.m-2.mM-1 ($p<0.001$) with II-SG and from 24±5 to 42±6 pmol.min-1.m-2.mM-1 with II-DSG ($p<0.05$). Insulin sensitivity rose from 265±37 to 410±20 ml.min-1.m-2 ($p<0.001$) with II-SG and from 218±23 to 428±19 ml.min-1.m-2 ($p<0.001$) with II-DSG. In the pooled data from both groups, the changes in 2-hour-glucose concentration were independently related to the changes in BMI (standardised r=-0.20), b-GS (standardised r=-0.36) and OGIS (standardised r=-0.59), with a total explained variance of 72%, $p<0.0001$.
Conclusion: In non-obese (BMI<30) T2DM, laparoscopic ileal interposition/sleeve gastrectomy, with or without diversion, effectively improves glucose tolerance by augmenting both beta cell function and insulin sensitivity. These findings lend support to the concept that the terminal ileum releases neuroendocrine signals in response to feeding which favourably impact on glucose tolerance (hindgut hypothesis).
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92

Early effects of biliopancreatic diversion on beta cell function in morbidly obese subjects with type 2 diabetes mellitus

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Biliopancreatic diversion (BPD) normalizes glucose tolerance in morbid obese subjects with type 2 diabetes (T2DM) improving insulin sensitivity and restoring acute insulin response to glucose (AIRg). Since other secretagogues, such as arginine, stimulate insulin secretion acting on cellular pathways different from those activated by glucose, we investigated whether arginine stimulated insulin secretion was affected by BPD. We studied 13 morbid obese subjects (5 male), 6 with and 7 without T2DM, before and 1 month after BPD. We measured body weight (BW), BMI, plasma fasting glucose, HOMA-IR, insulin secretion after arginine test (5 gr of arginine were infused IV in 45 sec at basal glucose concentrations and after infusion of 20% glucose solution to reach a glucose concentration of 14 mmol/L). The acute insulin response to arginine was calculated as the mean insulin concentration in the first 5 min of test minus the value at fasting glucose (AIRa1) and at 14mmol/L glucose concentration (AIRa2). The Slope of the AIRa [(AIRa2-AIRa1)/Δplasma glucose] was calculated as a measure of glucose potentiation of β cell insulin secretion. Preoperatively T2DM were different from controls (NFG) for higher fasting glucose (9.8±0.9 vs. 4.9±0.2mmol/L,$p<0.001$), lower AIRa1 (243±111 vs. 502±86pmol/L,$p<0.01$), AIRa2 (420±179vs.1395±149 pmol/L,$p<0.001$), slope of AIRa (18.5±18.6 vs. 181.9±41.6,$p<0.001$). Insulin sensitivity, measured as HOMA-IR, was not different between T2DM and NFG. One month after BPD, in both groups, BW was reduced by 13% but all subjects were still morbid obese (mean BMI 40.8). In T2DM fasting glucose returned to normal values and HOMA-IR was improved in both groups (2.0±0.3 in T2DM,2.1±0.5 in NFG,$p<0.01$ vs.pre BPD). AIRa1 was reduced in T2DM (163±45 pmol/L) and NFG (502±86 pmol/L), probably in relation to the reduction of insulin resistance and fasting glucose. Post BPD any significant difference in AIRa2 and slope AIR disappeared due to an increase in T2DM (AIRa2 668±338 pmol/L, slope AIR 110.4±18.0) and a reduction in NFG (AIRa2 1032±123 pmol/L, slope AIR 88.8±45.8,p=ns vs.T2DM). These findings were not correlated with BW changes. The present data show that BPD induces an improvement of β function at least partially independent by weight loss, acting both on glucose- and not-glucose stimulated insulin secretion. The mechanisms underlying the improvement of β cell function after BPD are still unknown.
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93

Cholesterol absorption decreases after Roux-en-Y gastric bypass but not after gastric banding

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Background and aims: Cholesterol synthesis is increased and cholesterol absorption is decreased in obese and insulin resistant humans. Dietary weight loss changes this balance towards decreased synthesis and increased absorption. Of the different forms of obesity surgery Roux-en-Y gastric bypass (RYGB) is superior to gastric banding (GB) in the treatment of diabetes. Our aim was to compare effects of RYGB and GB on cholesterol metabolism in morbidly obese subjects.
Materials and methods: Fifty-five obesity surgery patients, 29 RYGB and 26 GB, were examined at baseline and 1 year after surgery. Levels of serum cholesterol precursors cholestenol, desmosterol and lathosterol (markers of whole-body cholesterol synthesis), and serum plant sterols campesterol, sitosterol and avenasterol (markers of cholesterol absorption) were measured preoperatively and at 1 year follow-up.
Results: Weight decreased by 25% and 17% in RYGB and GB groups ($p<0.001$ in both groups), respectively. As expected both operations decreased serum levels of cholesterol synthesis markers by 10-25% (all $p<0.001$), without a significant difference between the groups. However, the expected increase in cholesterol absorption markers in response to weight loss was only observed after GB and not after RYGB. The ratio of sitosterol to cholesterol, reflecting fractional cholesterol absorption, increased by 18% in GB group and decreased by 22% in RYGB group ($p=2x10^{-6}$ for difference between the groups). Although fasting insulin levels improved significantly more after RYGB than GB (-59% vs -29%, $p<0.001$), reflecting more improvement in insulin sensitivity after RYGB, the difference in sitosterol ratio between the groups remained significant after adjustment for BMI and fasting insulin levels ($p=2x10^{-4}$), indicating a specific effect of RYGB on cholesterol absorption. In both groups the change in serum sitosterol levels correlated positively with the change in serum cholesterol levels ($r=0.464$ and 0.537, $p<0.05$ in both groups), suggesting that cholesterol absorption has a role in the regulation of serum cholesterol levels after obesity surgery. However, serum total cholesterol decreased significantly only after RYGB (-9%, $p=2x10^{-3}$).
Conclusion: Decrease in cholesterol absorption post RYGB, in addition to rapid improvement in blood glucose, is a novel beneficial effect of RYGB in comparison to dietary weight loss or GB. More detailed analysis of lipid metabolism in response to different forms of bariatric surgery is required.
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94

Mechanisms of improvement of glucose tolerance in type 2 diabetes mellitus after bariatric surgery: gastric bypass vs sleeve gastrectomy

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Background and aims: In morbidly obese patients with T2DM, gastric bypass (GBP) restores euglycaemia early after surgery, but data on the effectiveness of sleeve gastrectomy in resolving or improving type 2 diabetes mellitus are scarce. Aims of this study were to investigate extent and mechanisms of recovery of ß-cell function and insulin sensitivity in severely obese patients with type 2 diabetes mellitus (T2DM) undergoing Roux-en-Y gastric by-pass (RYGB, predominantly restrictive) or sleeve gastrectomy (SLV, restrictive).
Materials and methods: 14 obese T2DM subjects (8 subjects treated with RYGB and 6 with SLV) were studied before, 15 and, 45 days after surgery. The early effects of surgery were assessed by comparing the response to a Mixed Meal Test (MMT) (before and 15 days after surgery, in both cases following a week of constant, low caloric intake). Insulin sensititvity was assessed by the MMT-derived OGIS index and ß-cell function by modelling analysis of the C-peptide response to MMT. An OGTT (75 g) was also performed before and 45 days after surgery (under conditions of free caloric intake).
Results: At 15 days post-surgery, BMI decreased to the same extent in RYGB and SLV subjects (from 46.6±7.8 to 44.3±7.5 kg.m-2 in RYGB and from 46.4±5.2 to 43.9±5.6 in SLV, mean±SD, p<0.0001). Glucose tolerance improved in both groups 15 days after surgery (mean glucose during the MMT: from 8.2±1.9 to 7.4±2.1 in RYGB and from 8.0±1.0 to 6.4±0.4 mmol/l in SLV, p=0.02). Mean insulin during MMT decreased in both groups (from 166±67 to 111±46 in RYGB and from 346±115 to 233±55 pmol/l in SLV, p=0.001). ß-cell glucose sensitivity (i.e., the slope of the dose-response curve of insulin secretion rates and plasma glucose concentrations) improved after surgery in both groups (from 23.2±23.7 to 46.7±38.8 in RYGB and from 44.8±45.3 to 77.2±83.0 pmol.min-1.m-2.mM-1 in SLV subjects, p=0.05). At baseline, insulin sensitivity was similar in RYGB and SLV (299±54 vs 287±16 ml.min-1.m-2, p=ns). Following surgery, insulin sensitivity tended to improve in all subjects (to 314±51 in RYGB and 358±44 ml.min-1.m-2 in SLV, p=0.05). Forty-five days after surgery, BMI decreased similarly in both groups and insulin sensitivity improved in both groups (from 302±67 to 330±44 in RYGB and from 263±20 to 320±49 ml.min-1.m-2, p=0.015).
Conclusion: Glucose tolerance, ß-cell function and insulin sensitivity are all improved within 2 weeks of bariatric surgery under conditions of constant calorie intake. These effects, which are stabilised 45 days after surgery, are equivalent with gastric bypass and sleeve gastrectomy.
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95

Early metabolic effect of Roux-en-Y gastric bypass on insulin-resistance in morbidly obese type 2 diabetic subjects

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Background and aims: Weight loss after bariatric surgery improves insulin resistance (IR). Recent evidence has shown that biliopancreatic diversion, a predominantly malabsorptive procedure, restores peripheral insulin sensitivity early after surgery before important weight loss. Roux-en-Y gastric bypass (RYGB), a predominantly restrictive surgery, also has been associated with hormonal changes and improved HOMA-IR prior to substantial weight loss. However, there are no data regarding the early effect of RYGB on directly measured insulin sensitivity, endogenous glucose production (EGP) and acute insulin response to intravenous glucose (AIR).
Materials and methods: We studied eleven (3m/8f) morbidly obese type 2 diabetic (T2DM) subjects (age 49±2 years, BMI=49±2 $kg \cdot m^{-2}$) before and 19±1 days after RYGB with the use of the euglycaemic hyperinsulinaemic (240 $pmol \cdot min^{-1} \cdot m^{-2}$) clamp technique in combination with tracer infusion (6,6-$^2H_2$-glucose and 1-^{2}H-glycerol) to measure EGP and whole-body lipolysis, respectively. AIR was measured following an intravenous glucose bolus with the use of C-peptide deconvolution.
Results: After surgery, body weight had decreased by 7% (133±9 *vs* 123±8 kg, p=0.003). At this time, fasting plasma glucose (7.9±0.6 *vs* 6.8±0.4 mmol/l, p=0.01) and insulin levels (162±26 *vs* 92±13 pmol/l, p=0.003) were significantly reduced. On the clamp, peripheral insulin sensitivity was increased (M/I=46±6 *vs* 33±7 $\mu mol \cdot min^{-1} kg_{FFM}^{-1} pM^{-1}$, p=0.03). EGP was not significantly modified (HGP=12.6±0.7 *vs* 11.4±0.7 $\mu mol \cdot min^{-1} \cdot kg_{FFM}^{-1}$, p=ns) but the hepatic insulin resistance index (EGP x basal plasma Insulin) was significantly reduced (from 2.26±0.36 to 1.25±0.12 $mmol \cdot min^{-1} \cdot kg_{FFM}^{-1} \cdot pM$, p=0.04) as was whole-body lipolysis (as indexed by the rate of systemic glycerol appearance), from 494±51 to 267±43 $\mu mol \cdot min^{-1}$, p=0.02. AIR, on the other hand, did not change significantly (65±19 *vs* 67±20 ($pmol \cdot min^{-1} m^{-2} \cdot mM^{-1}$, p=ns).
Conclusion: We conclude that in morbidly obese T2DM subjects, a mostly restrictive bariatric operation such as RYGB is associated with an early improvement in glycaemia due to increased peripheral, hepatic and adipose tissue insulin sensitivity, while beta cell function is unchanged. As weight loss at this time postsurgery is minimal, these metabolic changes are likely to be the result of calorie restriction.

96

Variation in M1/M2 macrophage balance and HDL-C phenotype in obese subjects before and after gastric bypass induced weight loss

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Background and aims: Macrophages accumulate in adipose tissue in obesity and most likely participate to inflammatory related metabolic complications. Macrophage amounts vary with adipose tissue amount, site and weight modification but it is unknown whether this is accompanied by phenotypic changes in humans. Furthermore macrophages infiltration in visceral adipose tissue is negatively associated with HDL-Cholesterol concentrations. HDL-C subspecies profiles and functions have not yet been precisely characterized in obesity and after weight loss. Our aim was to characterize the activation state of adipose tissue macrophages in obese subjects, before and after gastric bypass surgery and establish a link with HDL-C phenotype variation.
Materials and methods: We performed a single center prospective study. 36 premenopausal obese women (Body mass index = 44 ± 6 kg/m^2) were followed before (T0) and three months (T3M) after a laparoscopic gastric by-pass. Quantitative HDL subspecies were evaluated at T0 and T3M as well as their qualitative features on cellular free cholesterol efflux through SR-BI and ABCG1 pathways. Paired surgical biopsies of subcutaneous and visceral adipose tissue were obtained during gastric surgery in a subgroup of 16 women. A surgical subcutaneous adipose tissue biopsy was obtained in the same obese individuals at T3M, and in 10 normal weight individuals during programmed surgery. The number of CD40 (M1 cell marker) and CD163 or CD206 (M2 markers) positive cells was determined by immunochemistry. Differences between before and after surgical weight loss were tested with a Student t test.
Results: Gastric by-pass induced a 15% weight loss and a significant improvement in insulin resistance, triglyceride and total cholesterol levels. We observed a significant 18% increase in large HDL-C subspecies concentrations (p=0.004) and a significant 25% enhancement in large HDL-C capacity to mediate free cholesterol efflux (p=0.0015). These improvements result mainly from a decreased CETP activity after weight loss. In the adipose tissue (AT), the number of CD40+ cells significantly increased with obesity and in omental AT of obese subjects, while the number of CD163+ or CD206+ cells was marginally changed. A 2-fold decrease in the ratio of CD40/CD206 cells number in subcutaneous adipose tissue of obese subjects was observed 3 months after gastric surgery (p< 0.001). This was due to the concomitant increase of CD40+ and decrease of CD206+ cells counts. Interestingly, the improvement of HDL capacity to mediate free cholesterol efflux was strongly associated with the increased in M2 macrophage in subcutaneous adipose tissue.
Conclusion: In morbidly obeses women, we observe a relationship between the variation in M1/M2 macrophage balance and the improvement of HDL-C antiatherogenic properties after gastric by-pass induced weight loss.
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OP 17 Diabetes and pregnancy

97

Glycaemic control and perinatal outcomes of pregnancies complicated by type 1 diabetes: multiple daily injections vs continuous subcutaneous insulin infusion

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Background and aims: Several studies have demonstrated the superiority of continuous subcutaneous insulin infusion (CSII) compared to multiple daily injections (MDI) for improving metabolic control of type 1 diabetes (T1DM) subjects**,** but in pregnancy this remains unclear. Recent publications have reported an increased risk of ketoacidosis associated with the use of CSII.
The aim is to compare the degree of metabolic control and maternal and perinatal outcomes obtained in pregnancies of women with T1DM, treated with CSII or MDI.
Materials and methods: We analyzed 308 singleton pregnancies in women with T1DM consecutively attended at our centre. A total of 213 were treated with MDI (NPH insulin twice a day plus regular or lispro insulin before meals), and 95 were treated with CSII (using regular or lispro insulin). All patients were in the same modality of treatment before and throughout pregnancy.
Patient characteristics, variables of glycemic control and perinatal outcomes were analyzed using Chi-square test for categorical variables and non-parametric tests for quantitative variables.
Results: Patients in the CSII group were older and had a longer diabetes duration than those treated with MDI. The rate of prepregnancy consultation was also higher in the CSII group (92.9% vs 61.9%; p<0.05). Weight gain (14 vs 13.6 kg), incidence of hypoglycaemic comas (MDI 10% vs CSII 9.5%) and ketoacidosis (MDI 1.4% vs CSII 0%) were comparable between both groups. Mean blood glucose (MBG) and HbA1c levels were slightly higher at the 3 trimesters in the CSII-treated group (p<0.05). Perinatal outcomes as prematurity, large for gestational age infant (LGA), small for gestational age infant (SGA), macrosomia, major malformations, stillbirth, perinatal and neonatal mortality and neonatal hypoglycaemia, were comparable between groups.
Conclusion: Perinatal outcomes in women with T1DM treated with CSII or MDI were comparable, although the patients treated with CSII showed worse baseline characteristics and a slightly worse glycemic control. In our series, the use of CSII is not related with the presence of acute complications.

98

Prediabetes is associated with similar risks during pregnancy and delivery as obesity and overweight

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Background and aims: Diabetes that occurs during pregnancy (gestational diabetes GDM) can result in diabetic fetopathy, which is characterized by high birth weight and postnatal complications. In addition to GDM, obesity is an increasing problem. Obesity is associated with higher risk for complications during pregnancy and higher frequency of miscarriage, instrumental delivery and macrosomic births. Our aim was to study if the prediabetic women differed in characteristics during pregnancy and delivery from the women without diabetes who were over weighted, since obesity is a risk factor for developing gestational diabetes and type 2 diabetes
Materials and methods: The Swedish Birth Registry (Medicinska födelseregistret MFR) started 1973 and contains data regarding the health of the mothers during pregnancy and peri- and postnatal status of the infants. In a population based large cohort study including 23 524 women aged 35-75 yrs from the southern part of Sweden we have identified 802 women with diabetes. In the MFR we identified 30 559 pregnancies related to the women in our cohort. Using MFR and the cohort we could retrospectively identify the pregnancies of diabetic women, women developing diabetes later in life and women without diabetes. Overweight was defined as BMI >25. We compared characteristics between the prediabetic women (n=1008) and women without diabetes who were over weighted (n=2466). Normal weighted women without diabetes were considered a control group (n=42043).
Results: The prediabetic women were significantly younger compared to over weighted women (29.4 vs 31.5 years old; p<0.05).
Weight-gain: Prediabetic women and over-weighted women had a similar weight gain during pregnancy (13.3 vs. 13.1 kg; p=ns). This weight gain was significantly lower compared to controls (14.2 kg; p<0.05 and p<0.001). There was an inverse correlation between pre-pregnancy weight and the weight gain in both groups. For controls there was a positive correlation.
Weight of the infant: There was no difference regarding the mean weight between the infants of prediabetic women and over weighted women (3602 vs. 3659g; p=ns), but the infants were larger compared to controls (3493g; p=0,001 and p<0,001 respectively). The prediabetic women had a shorter gestational length (39.6 vs. 39.8 weeks; p<0.05) and the frequency of LGA-infants was higher in the group of prediabetic women (10.5% vs 7.1%; p=0.015).
Sectio: the prediabetic women had significantly lower frequency of caesarean deliveries than the over weighted women (4.8% vs. 8.2%; p=0.014). Both groups had a higher frequency compared to controls (2.2% p=0,001 and p<0,001 respectively).
Apgar: The infants of the prediabetic women had lower mean Apgar scores at 5 (9.5 vs. 9.7; p<0.001) and 10 minutes (9.6 vs. 9.8; p<0.001) compared to the infants of the over weighted women. Controls had significantly higher Apgar scores for 1, 5 and 10 minutes compared to both study groups (p<0,005).
Conclusion: Both prediabetic women and over weighted women without diabetes had less weight gain than controls. Despite this their infants had higher birth weight. The Apgar scores were lower and the frequency of caesarean section was higher in both groups, indicating that the deliveries were more complicated. Prediabetes seems to be associated with similar risks during pregnancy and delivery as over weight and obesity.

99

Phenotypic and metabolic characteristics of women with isolated hyperglycaemia in pregnancy

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Background and aims: Although ADA defines Gestational Diabetes Mellitus (GDM) by two abnormal values on a 3-h oral glucose test (OGTT), there are few studies that have shown that pregnant women with one abnormal value (OAV) in the OGTT present features different from those of normal women. The aim of this study is to examine in a large cohort of Greek pregnant women the phenotypic and metabolic characteristics of OAV women compared to normals and GDM, and also to test if the timing of the observed hyperglycaemia is related to any difference in their phenotype.
Materials and methods: 6671 pregnant women without known DM underwent a 100g OGTT in the third trimester of pregnancy. For GDM diagnosis the ADA 2000 criteria were used. Among these, 3384 (50.7%) were found with 4 normal values in the OGTT (Group NGT), 1107(16.6%) had one abnormal value (Group OAV), and the remaining 2180 (32.7%) had two or more abnormal values and were diagnosed as GDM (Group GDM). Further we subdivided the OAV group into three subgroups according to the time of hyperglycaemia: 315 had fasting OAV (28.5%), 471 had 1h OAV (42.5%) and 321had 2 or 3h OAV (29%). Age, height, pre-pregnancy weight and BMI, as well as family history of DM were recorded. Insulin sensitivity was measured using the insulin sensitivity index (ISI) of Matsuda and DeFronzo. For beta-cell function the index HOMA-B and the Stumvoll formulas for first and second phase insulin secretion were used. Finally the product of the first phase Stumvoll index and the ISI was calculated to obtain the insulin secretion-sensitivity index (ISSI).
Results: The characteristics of the three main groups NGT, OAV and GDM are shown in Table 1(superscripted a,b,c indicate difference from preceding group).There was also a significant difference among the three groups with regard to family DM history: mother's (NGT 7.6%, OAV 11.3%, GDM 18.2%, p<0.01), father's (9.6%, 12.9%, 14.7%, p<0.01 respectively).Regarding the three OAV subgroups: OAV-Fasting was heavier than the other two; there were no differences in ISI among them. On the other hand, the OAV-1h subgroup had the lower Stumvoll first and second phase compared to the others, and also the lowest ISSI.
Conclusion: In this large cohort of pregnant women studied the OAV group is clearly in- between the normal and GDM group in all examined parameters. Furthermore, isolated hyperglycemia seems to be heterogeneous. Fasting hyperglycemia is mainly characterized by impairment in basal insulin secretion, while OAV at 1-h presented significantly diminished stimulated insulin secretion. These data suggest that women with OAV need further attention during as well as after pregnancy.

Table 1

	NGT (n=3384)	OAV (n=1107)	GDM (n=2108)	p value
Age	30.0±5.5[a]	31.6±5.5[b]	33.1±5.3[c]	<0.01
BMI	24.7±4.5[a]	25.9±5.2[b]	27.3±6.0[c]	<0.01
Weight	64.4±12.8[a]	66.8±14.2[b]	69.8±16.3[c]	<0.01
Height	161.4±6.3[a]	160.5±6.2[b]	159.9±6.4[c]	<0.01
Glucose AUC	18.97±2.51[a]	22.70±2.09[b]	28.12±4.16[c]	<0.01
Insulin AUC	1237.5±634.9[a]	1472.4±708.8[b]	1705.1±769.6[c]	<0.01
ISI Matsuda	5.2±2.9[a]	4.1±2.7[b]	3.1±1.7[c]	<0.01
HOMA-B	266.0±415.3[a]	199.4±156.2[b]	166.3±179.0[c]	<0.01
Stumvoll first-phase	1420.2±552.8[a]	1306.0±638.3[b]	1081.3±612.3[c]	<0.01
Stumvoll second-phase	370.8±133.9[a]	348.3±155.3[b]	298.7±147.9[c]	<0.01
ISSI	6486.3±2339.2[a]	4480.2±1905.0[b]	2762.6±1458.9[c]	<0.01

100

Diet and weight gain characteristics of pregnant women with gestational diabetes

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Background and aims: The prevalence of gestational diabetes (GD) has increased worldwide in recent years. Nutritional therapy is the main treatment option for GD. The aim of the study was to compare food and nutrient intake of women with GD to unaffected women in the 8th gestational month and to examine the use of dietary supplements. Additionally the study illustrates associations of background characteristics with the risk of GD and weight gain during pregnancy.
Materials and methods: Food and nutrient intake in a series of 3732 pregnant women (70% of the women eligible to the study) was studied using food frequency questionnaires (FFQ).
Results: GD was reported in 4.7% of the participating women. The weight gain during pregnancy was smaller in women with GD compared to unaffected women (mean 9.4 kg vs. 12.6 kg, p<0.001). Women with GD had more milk products (84 vs. 76 g/MJ, p=0.002), cereal products (21 vs. 18 g/MJ, p<0.001), vegetables (32 vs. 22 g/MJ, p<0.001), meat (16 vs. 14 g/MJ, p<0.001) and fish (2.6 vs. 2.2 g/MJ, p<0.001) than unaffected women. The intake of total energy (10.8 vs. 11.6 MJ, p=0.012) and fat (8.8 vs. 9.3 g/MJ, p<0.001) was smaller while intake of protein (10.6 vs. 9.5 g/MJ, p<0.001) and fibre (3.1 vs. 2.4 g/MJ, p<0.001) was higher in women with GD. The quality of fatty acids in the diet was better as well in women with GD. The nutrient density was higher in women with GD regarding vitamin A, D and E, folate, calcium and iron.
Conclusion: The diet during late pregnancy in women with GD differed profoundly from the diet in unaffected women. Women with GD had larger BMI at the beginning of pregnancy but they gained less weight during pregnancy. These findings indicate that abnormal glucose tolerance during pregnancy encourages women to shape their dietary habits towards healthier food choices.
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101

Pregnancy outcomes in women with hyperinsulinaemia with impaired glucose tolerance prophylactically treated with metformin. A case control study

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Background and aims: To evaluate the effect of metformin on maternal and neonatal outcomes in pregnant women with hyperinsulinemia with normal and pathologic glucose tolerance.
Materials and methods: A prospective one year case-control study among 66 pregnant women with one unsuccessful pregnancy without previous glucose intolerance. 75 grams OGTTs were performed in all women during the second pregnancy at 12. gestational week (gw) and at 36. gw. The levels of blood glucose and immunoreactive insulin were measured at 0 min. 60 min. and 120 min. Women were divided into three groups according to results of initial OGTT: n_1 = 21 with normal OGTT without hyperinsulinemia, n_2 = 24 with impaired OGTT (IGT) with hyperinsulinemia and n_3 = 21 with gestational diabetes mellitus (GDM) with hyperinsulinemia. Women with IGT and GDM were placed on metformin (0.75 - 1.5 g/day) according to levels of immunoreactive insulin after glucose loading. Participants were matched for age, BMI, number of previous pregnancies with abortions. Maternal and neonatal complications were recorded. Statistical methods: ANOVA - with multiple comparison and chi square test have been used.
Results: No statistically significant difference in mean values for age and BMI. The blood glucose levels in early pregnancy measured before and during glucose loading were significantly higher in pregnant with GDM. No difference was observed in initial levels of insulin in early pregnancy (0 min. n_1 = 11.4 ± 8.5 mIU/ml, n_2 = 13.6 ± 4.5 mIU/ml, n_3 = 15.8 ± 9.8 mIU/ml, P=0.06), but stimulated insulin levels after glucose loading were higher in second and third groups (60 min. n_1 = 56.8 ± 20.8 mIU/ml, n_2 = 70.1 ± 41.9 mIU/ml, n_3 = 74.8 ± 44.1 mIU/ml, P=0.04). BMI in late pregnancy was statistically lower in pregnant with normal OGTT (BMI n_1 = 26.8 ± 3.6 kg/m^2 n_2 = 28. 3± 3.6 kg/m^2, n_3 = 29.5 ± 4.2 kg/m^2, P=0.001). There was statistically difference in mean levels of BMI compared in early and late pregnancy in all groups. The level of blood glucose in late pregnancy was lowest in second group [(basal: (n_1 = 5.9 ± 0.5mmol/l, n_2 = 5.6 ± 0.4mmol/l, n_3 = 5.8 ± 0.4mmol/l, P=0.04; (60 min. n_1 = 8.1 ± 0.8mmol/l, n_2 = 7.3 ± 0.7mmol/l, n_3 = 7.4 ± 0.7mmol/l, P=0.01); (120 min. n_1 = 7.4 ± 0.9mmol/l, n_2 = 6.5 ± 0.3mmol/l, n_3 = 6.7± 0.7mmol/l, P=0.01)]. The both initial and stimulated levels of insulin were highest in the first group [(0 min. n_1 = 20.2 ±11.5 mIU/ml, n_2 = 17.4 ± 5.8 mIU/ml, n_3 = 14.9 ± 6.1 mIU/ml, P=0.03); (60 min. n_1 = 88.4 ± 11.5 mUI/ml, n_2 = 66.2 ± 34.7 mIU/ml, n_3 = 69.6 ± 24.0 mIU/ml, P=0.01)] in late pregnancy. The levels of insulin in late pregnancy were statistically lower in n_2 and n_3 groups compared to same insulin levels in early pregnancy. No maternal complications and birth defects can be attributed to metformin. The newborns' body weight was similar in all groups (n_1 =3500.9 ± 443.5 g, n_2 = 3560.3 ± 408.9 g, n_3 = 3381.7 ± 267.2 g, P=0.6). Rate of spontaneous abortion was 13.6% (9/66). The frequency of miscarriages was highest in the first group 28.5% (6/21). The evaluation of risk factors established relationship between the high insulin levels in early pregnancy and abortions.
Conclusion: Metformin use during pregnancy improved metabolic markers and reduced spontaneous miscarried rates in women with hyperinsulinemia and impaired OGTT.

102

Osteocalcin in gestational diabetes mellitus (GDM): does bone cope with pancreatic beta cell dysfunction?

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Background and aims: Osteocalcin, an osteoblast derived protein involved in bone formation, was reported to exert endocrine function by increasing insulin secretion as well as insulin sensitivity, thereby preventing the development of diabetes mellitus in experimental animals. Furthermore, patients with type 2 diabetes (T2DM) were shown to have decreased levels of osteocalcin suggesting that osteocalcin deficiency could in fact play a role in the pathogenesis of T2DM. Since gestational diabetes (GDM) serves as a model for early changes in the pathophysiology of T2DM and since beta-cell dysfunction seems to be an essential feature of GDM, the aim of this investigation was to study osteocalcin in GDM pregnancy.
Materials and methods: 37 women with gestational diabetes (GDM) were compared to 37 women with normal glucose tolerance during pregnancy (NGT) matched for age and BMI (GDM: BMI:29±5,3 kg/m², age:34,4±6,3a; NGT: BMI:29,2±5,4 kg/m², age:32±5,4a). All women underwent an oral glucose-tolerance-test (oGTT) between 24-28 gestational weeks. Plasma

concentrations of osteocalcin, lipids, HbA1C were measured at fasting and glucose, insulin and c-peptide were assessed at 0, 30, 60, 90 and 120 minutes after ingestion of 75g glucose to calculate metabolic parameters (i.e.for insulin secretion, insulin sensitivity) by mathematical modeling. 12 GDM and 12 NGT were also restudied 8-10 weeks postpartum.
Results: Osteocalcin was significantly increased in GDM compared to NGT (15,6±5,6 vs. 12,8±4,3 ng/ml, p=0,0165) during pregnancy. We found a weak but significant correlation between osteocalcin and insulin secretion (p<0,02) as well as areas under the curve of glucose and c-peptide during oGTT. Furthermore, the adaptation index (OGIS*Total insulin secretion), which describes the capacity of the beta-cell to release increasing amounts of insulin to compensate for rising insulin resistance, was positively correlated with osteocalcin (p<0,02). At 8-10 weeks postpartum osteocalcin, as shown previously, was significantly increased, but was not different between NGT and GDM, who had already regained normal glucose tolerance.
Conclusion: Our data extend previous findings in women with GDM that osteocalcin could be involved in regulation of glucose metabolism potentially via stimulation of insulin secretion by an endocrine pathway. The fact that osteocalcin is elevated in GDM might suggest a compensatory mechanism in these women to cope with pancreatic beta-cell dysfunction.

OP 18 Molecular mechanisms of type 2 diabetes mellitus and obesity

103

Signatures of selection at the *FTO* (fat mass and obesity associated) locus in human populations
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Background and aims: Polymorphisms in the first intron of *FTO* (rs9939609) were identified in genome-wide association studies as a risk factor for obesity. In the Sorbs, a population resident in Eastern Germany, we verified this association. However, a SNP (single nucleotide polymorphism) in the third intron of *FTO* (rs17818920) showed even stronger effects on BMI in the Sorbs. This may indicate population genetic-relevant variants of *FTO* alleles. Since comprehensive data regarding *FTO* evolution are lacking, we initiated studies searching for signatures of selection in mammals and human populations.
Methods: First, we analysed the coding region of 13 mammalian *FTO* orthologs with Phylogenetic Analysis by Maximum Likelihood (PAML, $\omega=d_N/d_S$) to screen for signatures of selection between species. Second, in 34 non-related subjects of the Sorbian cohort 307 SNPs of the *FTO* locus (1 Mb) were extracted from the data obtained by genome-wide scans using 500K/6.0 Affymetrix GeneChips®. Additionally, phased (Phase version 2.1) SNP data of the HapMap (Europeans/CEU, Yoruba/YRI, Chinese/CHB, Japanese/JPT) and the Sorbs were analysed for signatures of selection with DnaSP version 4.5 and the Haplotter/PhaseII.
Results: The d_N/d_S analyses revealed that the *FTO* gene is highly conserved among the species analysed ($\omega<1$), hence being subjected to an overall purifying selection. Population genetic analyses provide significant evidences ($p<0.02$) for non-neutral evolution of *FTO* alleles in all investigated populations. Sliding-window analyses of phased SNP data show highly significant ($p<0.001$) signatures for recent selection specifically in the third intron of *FTO* in the Sorbs and other cohorts (Tajima's D in Sorbs: 2.77, CEU: 3.60, CHB: 2.73, JPT: 3.08, YRI: 3.46).
Conclusion: Population genetic analyses revealed signatures of recent selection at the *FTO* locus in Sorbs and other human populations. The strongest signal is observed in the third intron, a genomic region where the strong association with BMI in the Sorbs was found. Data provide some evidence to support the thrifty-gene theory where *FTO* variants were selected to accumulate energy more efficiently.

104

Association between fat intake, physical activity and mortality depending on genetiv variation in FTO
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Background and aims: Genetic variation in the fat mass and obesity associated gene (FTO) has been associated with obesity, appetite regulation and mortality. We recently observed interactions between FTO genotype and fat intake or physical activity on risk of obesity. The increase in BMI across genotypes was mainly restricted to individuals reporting high fat intake or low level of leisure-time physical activity. The aim was to examine the association between total mortality and FTO genotype and to examine the association between fat intake or physical activity and mortality depending on FTO genotype.
Materials and methods: In the population-based Malmö Diet and Cancer study 4,839 individuals were genotyped for the rs9939609 in FTO, and had information on dietary intake (from a modified diet history method), leisure time physical activity (from duration participants spent on 18 different physical activities) and direct anthropometric measures. Vital status during a mean follow-up of 12.5 years until 31 December 2005 was recorded. We used Cox proportion hazard regression model to estimate total mortality for FTO genotype and gender-specific tertiles of fat intake (energy percentage) or leisure-time physical activity. The analyses were adjusted for sex, age, BMI, diabetes status at baseline, alcohol habits, smoking, education, and fat intake or leisure-time physical activity. Interaction was assessed by introducing a multiplicative factor.

Results: A total of 462 deaths occurred during the follow-up time. The FTO genotype was not significantly associated with mortality (HR, 1.01; 95% CI, 0.83-1.22 for A-allele carriers compared to TT-carriers). However, among A-allele carriers, higher fat intake was associated with increased mortality (HR, 1.42; 95% CI,1.06-1.99; P-trend, 0.02 for highest compared to lowest tertile), whereas fat intake was not related to mortality among TT-carriers (P=0.80). A tendency of interaction between fat intake and FTO genotype on mortality was observed (P=0.16). The interaction was stronger (P=0.04) when excluding individuals reporting a substantial dietary change in the past (approximately 25 % of the study population). High leisure-time physical activity was associated with decreased mortality among TT-carriers (HR, 0.62; 95% CI, 0.42-0.93; P-trend, 0.02 for highest compared to lowest tertile), whereas the level of physical activity was not related to mortality among A-allele carriers (P=0.75). A significant interaction between physical activity level and FTO genotype on mortality was observed (P=0.05).
Conclusion: Our results indicate that FTO genotype may modify the association between fat intake or physical activity and mortality, and propose that risk genotype carriers may benefit from restriction of fat intake.
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105

Amino acid variants in the melatonin receptor type 1B associate with increased risk of obesity
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Background and aims: Common variants in the Melatonin Receptor type 1B (*MTNR1B*) locus increase fasting plasma glucose (FPG) and the risk of type 2 diabetes (T2D). The aim of this study was to evaluate if coding variation in *MTNR1B* associates with measures of T2D and obesity.
Materials and methods: The coding regions of *MTNR1B* were sequenced in 47 Maturity-Onset Diabetes of the Young (MODY) probands and in 51 probands with early-onset (<40 years) familial T2D. Six non-synonymous variants (L60R, V124I, rs8192552 (G24E), rs61746674 (R138C), rs8192553 (R231H) and rs61747139 (K243R)) were genotyped in the population-based Inter99 (n=6,000), T2D patients from the Danish ADDITION study (n=1,567) and additional non-diabetic individuals (n=618) and T2D patients (n=1,643) from the Steno out-patient clinic.
Results: Three novel non-synonymous variants (V124I, Y141C, R248Q) were identified in three unrelated probands with familial early-onset T2D. R248Q and Y141C did not co-segregate with diabetes in the families and were not detected in 384 Danish control individuals. V124I was identified in one obese (BMI 35 kg/m^2) T2D patient (age at diagnosis 36yrs). Among all genotyped individuals, the frequency of 124I was 0.4%. 124I associated with increased risk of overweight (OR=2.4 [1.3-4.5], *P*=0.006), obesity (OR=2.4 [1.2-4.9], *P*=0.02) and increased BMI (β = 1.9kg/m^2 [0.7; 3.1], *P*=0.002), weight (β = 5.2kg [1.5; 8.9], *P*=0.006) and waist circumference (β = 4.0cm [0.8; 7.1], *P*=0.01). No association with T2D was observed. Codon 24E associated with increased risk of overweight (OR=1.2 [1.1-1.3], *P*=0.004), obesity (OR=1.3 [1.1-1.5], *P*=0.0008) and with increased BMI (β = 0.4kg/m^2 [0.1; 0.7], *P*=0.004), weight (β =1.1kg [0.3; 2.0], *P*=0.006) and waist circumference (β = 0.9cm [0.3; 1.6], *P*=0.007). No association with T2D was observed. However, 24E associated with increased insulin response after an oral glucose load (BIGTT-AIR: β = 4% [0.9; 6.5], *P*=0.01) and decreased FPG (β = -0.032mmol/l [-0.063; -0.0017], *P*=0.04), an association that was strengthened when adjusting for the difference in BMI (*P*=0.003). L60R was only present in 6 of all genotyped individuals. 60R associated with decreased BMI (β = -4.5 kg/m^2 [-8.8;-0.3], *P*=0.04). Non-diabetic carriers of 60R had decreased acute insulin response, as measured by BIGTT-AIR (β = -60% [-106; -14], *P*=0.01) and the insulinogenic index (β = -85% [-151; -18], *P*=0.01). No associations with T2D or measures of obesity were observed for R138C, R231H, and K243R.
Conclusion: We demonstrate that non-synonymous variation in *MTNR1B* modulates the risk of obesity. 124I and 24E associate with increased the risk of overweight and obesity and associate with quantitative measures of obesity, while 60R associates with decreased BMI.
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106

Genome-wide expression profiling of human subcutaneous and visceral adipose tissue precursor stromal cells
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Background and aims: Multipotent precursor cells can be isolated from the stromal-vascular fraction (SVF) of adipose tissue specimens by collagenase digestion. In addition, it has been recently observed that mature adipocytes possess the ability to divide asymmetrically and produce progeny cells (Adipocyte-Derived Precursors, ADP), which share the same morphological, fibroblast-like appearance with the SVF-derived stem cells. The objective of this study was to investigate the biological features of these two different precursor cell populations.
Materials and methods: SVF and ADP were isolated from paired subcutaneous (SC) and visceral (V) fat biopsies of 4 subjects (1F/3M, age 65±1 years, BMI 24.9±1.4) with normal glucose tolerance, cultured up to 4th passage, and then studied. A DNA microarray-based, genome-wide differential gene expression analysis was performed on SVF and ADP obtained from SC and V human adipose tissue. The expression of specific differentiation markers was assessed by real-time RT-PCR.
Results: Both types of cells resulted to be positive for the adipogenic lineage markers CD105, CD44, CD49d, whereas they were negative for the endothelial and macrophage markers CD106, CD44, CD31 and CD11b, respectively. Of 25,000 genes assessed, microarray analysis identified 254 genes that were differentially expressed between SC-SVF and V-SVF (p<0.001), including members of the insulin-like growth factor binding protein family and of the sterol regulatory element binding transcription factors, and 188 genes that were found to be differentially expressed between SC-ADP and V-ADP (p<0.001), including phosphodiesterase 3B, cGMP-inhibited and signal transducer and activator of transcription 3 (STAT3). Interestingly, when the two precursor cell populations from the same fat depot were compared (SC-SVF vs SC-ADP and V-SVF vs V-ADP), the pairwise comparison yielded correlation coefficients ranging between 0.704 and 0.756, indicating a significant biological difference between SVF and ADP cells. Indeed, microarray analysis identified 191 genes differentially expressed between V-SVF and V-ADP (p<0.001), including the fat mass and obesity associated (FTO) gene, and 272 genes differentially expressed between V-SVF and V-ADP (p<0.001), including the wingless-type MMTV integration site family (WNT) gene.
Conclusion: These data demonstrate for the first time the existence of genetically heterogeneous fat-derived precursor cell populations, which additionally exhibit intrinsic depot-related gene expression profiles. These differences in fat cell progenitors may be relevant for the specific biological properties of SC and V adipose tissues in humans.

107

Gene expression analysis demonstrates presence of a non-coding RNA (*ANRIL*) in human islets and FACs sorted beta cells which has a potential regulatory role on *CDKN2B* expression
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Background and aims: Genome-wide association studies have identified a 200Kb region on chromosome 9 which hosts at least 2 independent signals for type 2 diabetes (T2D) [susceptibility being mediated through beta-cell dysfunction]. The closest protein-coding genes, *CDKN2A* & *CDKN2B* are tumour suppressors with primary actions as cyclin-dependent kinase inhibitors. In rodents, *Cdkn2a* over expression leads to islet hypoplasia and recapitulates the T2D phenotype, supporting a model whereby T2D-susceptibility in humans is mediated by variants which increase *CDKN2A/B* expression. Discovery of a large antisense noncoding RNA (ncRNA), *ANRIL,* transcribed from the region of maximal T2D association, and with the potential to regulate *CDKN2A/B*, raises the possibility that the T2D risk variants have primary effects on *ANRIL* expression or function, leading to tissue- & variant-specific effects on the magnitude and balance of *CDKN2A/B* transcripts. The aims of this study were to (i) determine the relative expression profiles of *CDKN2A*, *CDKN2B* and *ANRIL* in human islets & beta-cells (ii) determine the *ANRIL* transcripts present in human islets & (iii) investigate a potential regulatory role for *ANRIL* on *CDKN2A/B* expression.

Materials and methods: Relative gene expression profiles of *CDKN2A*, *CDKN2B* & *ANRIL* were determined in 22 human tissues including pancreatic islets and FACS sorted beta cells using a QRT-PCR approach on an ABI 7900HT platform. A combination of rapid amplification of cDNA ends (RACE), PCR and capillary sequencing on an ABI 3700 were used to determine the *ANRIL* transcripts present in human islets. *ANRIL* knockdown was performed using a custom designed siRNA against *ANRIL* exon 5 which was transfected into HuVec cells using Dharmafect. Transfection efficiency was determined using an siRNA to *GAPDH* and appropriate controls including a scrambled siRNA were used.
Results: *ANRIL*, *CDKN2A* and *CDKN2B* are widely expressed across a panel of 22 human tissues with high expression of all 3 in pancreatic islets and FACS sorted beta cells. RACE and direct sequencing of amplified transcripts demonstrated a single alternatively spliced *ANRIL* transcript of~1000bp consisting of 7 exons is present in human islets. Preliminary data demonstrates that a 60% [48.7%, 71.3%] *ANRIL* siRNA mediated knockdown leads to a 1.7 fold increase [1.3, 2.2; n=12] in *CDKN2B* expression with no detectable effect on *CDKN2A* expression levels.
Conclusion: Our data demonstrate that *CDKN2A*, *CDKN2B* and *ANRIL* are widely expressed with high levels of expression in human pancreatic islets and FACS sorted beta-cells. We have identified a single~1000bp *ANRIL* transcript which is present in human islets. Furthermore our data provide preliminary evidence for a regulatory role of the ncRNA *ANRIL* on *CDKN2B* expression supporting our hypothesis that T2D susceptibility may be mediated through *ANRIL*.
Supported by: the Medical Research Council (MRC)

108

Hyperactivity in adrenergic signalling via alpha2A receptors contributes to human type 2 diabetes
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Background and aims: Sequence variations in several genes have been demonstrated to contribute to the strong heredity of type 2-diabetes (T2D), but the exact pathogenic mechanisms remain largely unknown. Here we undertake a translational approach to couple genetic variations to detailed mechanistic studies.
Materials and methods: The GK rat is a model of T2D that harbours naturally occurring genetic variations that predispose to hyperglycemia. The major diabetes susceptibility locus in the GK rat, the *Niddm1* locus, is homologous to an important diabetes locus on human chromosome 10. Through repeated back-crossing of the GK rat with normoglycemic F344 rats we established congenic strains harbouring small segments of GK-derived Niddm1. These rats were investigated by insulin release measurements, high-resolution single-cell capacitance measurements of exocytosis, gene expression studies, and pharmacological and RNAi-mediated rescue experiments. 6000 individuals from the Botnia Study well-characterized for insulin secretion were genotyped. We also measured insulin secretion from human pancreatic islets.
Results: From investigations of congenic strains with different extent of GK genome we identified a 1.4 Mb locus within *Niddm1* that confers impaired insulin secretion *in vivo* and *in vitro* through defective pancreatic β-cell exocytosis. This locus contains five genes, one of which is *Adra2a* that encodes the alpha2A-adrenergic receptor. *Adra2a* was overexpressed by 59% ($P<0.01$) on the mRNA level and by 100% ($P<0.001$) on the protein level in congenic rats exhibiting impaired insulin secretion. Adra2a mediates adrenergic suppression of insulin secretion. Pharmacological receptor antagonism with yohimbine or silencing of receptor expression both led to complete normalization of insulin secretion in congenic islets. Blockade of the down-stream effector (calcineurin) of Adra2a signalling rescued β-cell function. Importantly, our results are not confined to rats. Thus, genotyping of 19 SNPs covering 500 000 bp upstream and downstream of the human *ADRA2A* gene in 6000 individuals identified a SNP rs553668 for which risk A allele carriers exhibited reduced fasting insulin secretion ($P=0.0004$), 30% decrease in insulin release in response to intravenous glucose ($P<0.001$) and 10% reduction in response to oral glucose ($P<0.01$), as well as increased risk of T2D. Moreover, human pancreatic islets from risk allele carriers secreted less insulin in response to glucose, an effect that was rescued by pharmacological inhibition of Adra2a.
Conclusion: The present study demonstrates that overexpression of the alpha2A-adrenergic receptor, due to variations in the *ADRA2A* gene, is an important mechanism for naturally occurring T2D and that this novel disease pathway is of significance for impaired insulin release and T2D in humans. In fact, this represents one of the first detailed pathogenic mechanisms for human T2D. Interestingly, rs553668 has previously been associated with increased stress sensitivity and high blood pressure in humans, which points to a possible common mechanism for components of the metabolic syndrome and T2D. The findings could open up exciting possibilities for specific diagnosis and therapeutic interventions tailored to the individual patient.
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OP 19 Tissue stress and response

109

Role of p66Shc in the modulation of oxidative stress in human liver cells

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Background and aims: Oxidative stress plays an important role in the pathogenesis of non-alcoholic and alcoholic fatty liver disease. The p66 Shc protein isoform (p66Shc), through its phosphorylation on Ser36, modulates the intracellular oxidative balance in part by regulating the expression of antioxidant genes via Forkhead transcription factors. Interestingly, p66Shc was shown to be overexpressed in human type 2 diabetes. In this study, the role of p66Shc in cellular responses to oxidative stress was studied in human HepG2 liver cells.
Materials and methods: Wild-type p66Shc and mutant p66Shc, in which Ser36 had been replaced by Ala (p66Shc-Ala36), were selectively overexpressed following infection with recombinant adenoviruses (HepG2/p66Shc and HepG2/p66Shc-Ala36, respectively). Expression and phosphorylation levels of specific signaling molecules were assessed by immunoblotting techniques. Intracellular ROS generation, in the presence of the DHE probe, was evaluated by fluorimetric analysis. Subcellular FoxO3a localization was investigated by confocal microscopy.
Results: Overexpression of wild-type p66Shc was associated with increased p66Shc phosphorylation on Ser36, both under basal conditions and following exposure to H_2O_2 (by 8.0- and 10-fold, respectively; $p<0.001$ vs mock-infected or non-infected control cells). HepG2/p66Shc cells showed increased ROS synthesis, both basally and after H_2O_2 exposure ($p<0.005$). In addition, Erk-1/2 and Akt phosphorylation was increased, and FoxO3a also showed increased phosphorylation levels and consequently reduced nuclear localization both basally and after H_2O_2 exposure ($p<0.001$ vs controls). Pretreatment of HepG2/p66Shc cells with the MEK inhibitor UO126 significantly reduced Erk-1/2 phosphorylation as well as p66Shc phosphorylation on Ser36 ($p<0.001$ vs controls), and this was associated with reduced basal and H_2O_2-stimulated ROS synthesis ($p<0.005$ vs controls). Then, HepG2 cells were examined following overexpression of mutant p66Shc, in which Ser36 had been replaced by Ala (HepG2/p66Shc-Ala36). In HepG2/p66Shc-Ala36 cells, the responses to oxidative stress were blunted, since following H_2O_2 exposure phosphorylation of Erk-1/2, Akt and FoxO3a was markedly reduced, and ROS synthesis was completely abolished ($p<0.001$ vs. HepG2/p66Shc cells).
Conclusion: p66Shc overexpression in HepG2 cells leads to inactivation of FoxO3a and an increase in intracellular oxidative stress both under basal conditions and following exposure to oxidants. This largely occurs via the Erk signaling pathway, which also feeds back to promote p66Shc phosphorylation and activation. Thus, the p66Shc/Erk/Foxo3a pathway represents a novel modulator of oxidative stress-induced injury in liver cells, which might be relevant for the development of liver abnormalities in the metabolic syndrome and type 2 diabetes.

110

Endoplasmic stress is induced by lipopolysaccharides and high glucose and is alleviated by salicylates in cultured primary human adipocytes

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Background and aims: Obesity and type 2 diabetes mellitus (T2DM) are closely associated with chronic inflammation. Adipose tissue may have a significant role in obesity associated inflammation but, the mechanisms underlying the pathogenesis of obesity induced inflammation remains unclear. Recent findings indicate that endoplasmic reticulum (ER) stress is critical to the initiation and integration of pathways of inflammation and insulin action. The ER stress occurs when there is an accumulation of unfolded/misfolded proteins, in addition to other factors. This results in activation of the unfolded protein response (UPR) to restore functional integrity which, leads to upregulation of genes such as PERK, IRE1α, ATF6 and other protein chaperones. In turn, PERK and ATF6 interlink the JNK/NFkB pathways, known to be key mediators in obesity induced inflammation. To date, no study has examined the relationship between ER stress and inflammation in human adipocytes. Therefore, our aims were to determine the existence and causes of ER stress in human adipose tissue and primary human adipocytes along with therapeutic interventions.
Methods: Human abdominal subcutaneous (AbSc) adipose tissue (AT) was obtained from a Caucasian non-diabetic population (BMI: 27.9±7.3 kg/m^2: age 36-49 yrs; n=40; all female subjects) that underwent elective liposuction surgery, as part of the well established AT collection program. The human preadipocytes were isolated from stromal fraction, grown and fully differentiated into human adipocytes (n=5). Well differentiated adipocytes were then treated with LPS (100ng/ml to 10μg/ml), tunicamycin (750ng/ml), high glucose (30mM) and in combination with 20mM salicylate, salicylate alone and with and without DMSO (controls). To characterise protein expression of the key markers relevant to ER stress, inflammation and insulin signalling pathway, total protein and RNA was extracted from adipose tissue and cultured adipocytes using standard protocol. Western blots and Real-Time RT-PCR were performed to examine protein and RNA expression levels.
Results: The expression of ER stress proteins Calnexin1, BiP1, Ero-1α, PDI, IRE1α and Phospho-PERK were significantly increased in AbSc AT from obese compared to lean subjects (n=4; $p<0.05$ to $p<0.001$). Additional data has identified upregulation of inflammation markers NFκB, TRAF6 and myd88 in AbSc AT of obese compared to lean individuals. ER stress proteins phospho-eIF2α (n=5; $p<0.005$) and calnexin (n=5; $p<0.02$) were both significantly induced by LPS, tunicamycin and high glucose proving the existence of ER stress in differentiated human adipocytes. In the same adipocytes ER stress was significantly reduced when treated with anti-inflammatory compound salicylate. Similarly, phospho-Akt (S473) (n=5; $p<0.002$), which is an important member of insulin signalling pathway was also activated when ER stress was down-regulated by salicylate.
Conclusion: Our findings highlight, for the first time, ER stress in human AbSc AT from obese subjects as compared with lean subjects. From this study we also conclude that salicylates improve insulin sensitivity and alleviate ER stress induced by LPS and high glucose in cultured primary human adipocytes. Our future aim is to investigate the mechanisms of ER stress pathways and ER stress induced inflammation pathways in fully differentiated human adipocytes.
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111

ASK1 specifically in adipocytes is constituent of adipose tissue stress response in central obesity: potential role of oxidative stress and inflammatory cytokines

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Background and aims: Ask1 is a MAP3K known in certain cells to mediate the activation of p38MAP kinase and JNK in response to oxidative stress, inflammatory signals (TNF-R, Toll-like receptors) and endoplasmic reticulum stress. We previously reported that in human obesity omental fat exhibits activation of JNK and p38MAP kinase in correlation with the degree of obesity-related cardio-metabolic risk. The aim of the present study was to assess the possible role of Ask1 specifically in adipocytes in adipose tissue stress response.
Materials and methods: We utilized two independent cohorts, in Leipzig, Germany, and in Beer-Sheva, Israel, from whom paired omental (OM) and subcutaneous abdominal (SC) fat samples were obtained. The protein expression and phosphorylation of Ask1 and its mRNA levels were assessed. To investigate Ask1 activation specifically in adipocytes we separated adipocytes from the stromal-vascular fraction (SVF) by collagenase digestion, and utilized 3T3-L1 adipocytes.
Results: Obesity was associated with elevated expression of Ask1 in OM at both the mRNA (~1.5-fold, $p<0.05$) and protein levels (~2-fold, $p<0.05$), as compared to the paired SC depot, and as compared to OM fat from lean people. Ask1-mRNA levels correlated in OM more strongly than in SC with fasting insulin levels and the glucose infusion rate during euglycemic-hyperinsulinic clamp ($r=0.349$ and $r=-0.510$, respectively, both $p<0.001$, n=196), and with triglycerides and non-esterified fatty acid levels. Moreover, Ask1 phosphorylation on its auto-phosphorylation site, which confers activation, was elevated 2-2.5 fold in OM of obese women, and correlated with the phosphorylation of the MAP2K MKK4 ($r=0.474$, $p<0.01$, n=19) and that of p38MAP kinase ($r=0.596$, $p<0.01$, n=19). To verify the specific contribution of adipocytes to the increased OM Ask1, collagenase digestion of fat biopsies was performed, and Ask1-mRNA was assessed separately in OM adipocytes versus OM SVF. Whereas in lean persons SVF had 6-fold higher expression of Ask1, persons with predominantly subcutaneous obesity (determined by CT) had ~2.5-fold higher expression in the adipocytes, with no change in the SVF.

In persons with predominantly intra-abdominal obesity Ask1 mRNA levels increased 4.3-fold and 1.5-fold in adipocytes and SVF fraction, respectively (both $p<0.05$) compared to the lean group. Furthermore, the Ask1-MKK4-p38MAPkinase cascade could be activated in 3T3-L1 adipocytes by TNFα, IL-1β and by exposure to H_2O_2.
Conclusion: Ask1, specifically in adipocytes, is a MAP3K activated in intra-abdominal fat in human obesity, being regulated at both the expression and phosphorylation levels. In cultured adipocytes it can be activated by pro-inflammatory cytokines and by oxidants, potentially reflecting on the stress-type and stress response developing in intra-abdominal fat in obesity.
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112

Skeletal muscle oxydative capacity is increased in Erk1 deficient mice
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Background and aims: Skeletal muscle exhibits metabolic flexibility. Under fed condition muscle uses glucose whereas during fasting it oxidizes lipids. In type 2 diabetic patients, the failure of a such switching is associated to intramuscular triglyceride accumulation (lipotoxicity) and insulin resistance. Some members of the MAPK family are implicated in the downregulation of insulin action and signaling. We have shown that Erk1-/- mice are resistant to high fat diet-induced obesity and protected from insulin resistance. Moreover, these mice display a higher post-prandial metabolic rate and are protected against lipotoxicity. Here, we investigate Erk1-/- skeletal muscle oxydative capacity to determine whether Erk1 invalidation could protect from lipotoxicity.
Materials and methods: Ten weeks old Erk1-/- and WT mice are sacrified after 16h of fasting. Protein expression is determined by Western Blotting and enzymatic activity with commercial kits. Electron microscopy and immunofluorescence studies were performed on isolated muscles in order to investigate mitochondria phenotype and muscular fiber composition, respectively.
Results: Under fasting, a situation rising fatty acid flux, Erk1-/- muscles display a higher expression of proteins involved in fatty acid oxidation (M-CPT1, LCAD, MCAD) and in oxydative phosphorylation (complexes 1, 2, 4; cytochrome C; UCP3). Mitochondrial complex activity and expression of transcription factors regulating fatty acid oxidation steps (PGC1, PPARa, ERRa, MEF2, FOXO1) as well as some of their targets (TFAM, MHC1) are also increased in Erk1-/- muscles. Furthermore, mitochondrial content and fiber composition are modified in Erk1-/- mice. AMPK is an important energy sensor which activation promotes fatty acid oxidation by phosphorylating Acetyl CoA Carboxylase (ACC). Phospho AMPK and phospho ACC expression is increased in Erk1-/- muscles as compared to WT mice muscle.
Conclusion: Erk1 invalidation seems to induce an increased oxidative capacity that could explain that Erk1 deficient mice are protected from lipotoxicity and display an improveed insulin sensitivity. Thus, targeting specifically Erk1 may represent a new pharmacological strategy to fight obesity and insulin resistance.

113

The Tpl2 kinase is up-regulated in adipose tissue in obesity and mediates IL-1beta and TNFalpha effects on ERK activation and lipolysis
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Background and aims: Cytokines impair insulin action, increase lipolysis and alter adipocytes biology taking part in the development of insulin resistance and diabetes. Activation of the ERK pathway in adipocytes by inflammatory cytokines such as TNF-α and IL-1β could be an important component in the development of the alterations of adipose tissue function that participate in the insulin resistance. Thus, the identification of regulatory proteins that govern the activity of ERK specifically in response to inflammatory cytokines may provide important insights into mechanisms that promote metabolic diseases.MAP kinase and IKK/NFkappaB pathways often act synergistically to mediate cytokine action. It is thus possible that in adipocytes proteins that control cytokine-induced ERK activation are regulated by IKK/NFkappaB pathway. One interesting candidate could be MAP kinase kinase kinases (MAP3K) that regulate ERK through the phosphorylation and activation of MEK. Therefore, the aim of present study was to identify upstream kinase specifically involved in ERK activation by inflammatory cytokines and to address its implication in the alteration in adipocytes biology.
Materials and methods: Pharmacological inhibitors were used to inhibit IKKβ or Tpl2 activation and small interfering RNA (siRNA) was used to silence Tpl2 in 3T3-L1 adipocytes. Kinase activation and expression were analyzed by Western Blot. Gene expression was measured by real-time PCR in 3T3-L1 adipocytes and in adipose tissue of obese and morbidly obese patients and of obese mice. Lipolysis was monitored through the glycerol release in cells medium.
Results: IKKβ inhibition prevented MEK and ERK1/2 activation in response to IL-1β and TNFα, suggesting that IKKβ regulates a MAP kinase kinase kinase (MAP3K). We found that the MAP3K8 Tpl2 is expressed in both 3T3-L1 and human adipocytes and is activated by IL-1β and TNFα and that IKKβ inhibition prevented its activation in adipocytes.Both in 3T3-L1 adipocytes or in human adipocytes pharmacological inhibition of Tpl2 or its silencing prevented MEK/ERK1/2 activation by IL-1β and TNFα but not by insulin highlighting its involvement in ERK1/2 activation specifically in response to inflammatory cytokines. Prolonged treatment of 3T3-L1 adipocytes with IL-1β or TNFα markedly up-regulated the expression of Tpl2 via an IKKβ dependent-pathway, and Tpl2 mRNA level is increased in adipose tissue of obese mice and obese patients and is correlated with TNFα level. Finally, Tpl2 silencing or its pharmacological inhibition reduced lipolysis in adipocytes.
Conclusion: Our results identify Tpl2 as an inflammatory kinase specifically involved in inflammatory cytokines-induced ERK activation in adipocytes and reveal its deregulated expression in human obesity. Our data also raise the possibility that pharmacological targeting of Tpl2 could prevent the deleterious effect of cytokines on adipocytes biology.
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114

Adiponectin and skeletal muscle: pathophysiological implications during metabolic stress
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Introduction: Adiponectin (ApN) is a hormone exclusively secreted by adipocytes under normal conditions. This adipokine exhibits insulin-sensitizing and anti-inflammatory properties as well as modulatory effects on oxidative stress thereby thwarting several disorders belonging to the metabolic syndrome. We have recently shown that ApN was paradoxically induced in skeletal muscle of obese and insulin-resistant *ob/ob* mice and related to oxidative stress. We hypothesized that this paradoxical upregulation of muscular ApN in obese mice could be viewed as a local protective mechanism to counteract oxidative damage.
Objective: To test this hypothesis, ApN-knockout (ApN-KO) mice and wild-type (WT) mice were rendered obese and insulin-resistant by administration of a western diet (WD; high-fat/high sucrose). We examined whether muscles of ApN-KO mice exhibited higher degree of oxidative stress, inflammation and apoptosis than those of WT mice when challenged by a WD and whether these abnormalities could be corrected by local administration of ApN.
Methods: ApN-KO mice (with virtually undetectable circulating ApN) and WT mice were fed with a WD or a standard laboratory diet (SD) for 6 wks. Next, mice were killed and *tibialis anterior* muscles were collected for immunohistochemistry. In additional experiments, after 2 wks on WD, muscle transfer of ApN gene was performed in anesthetized ApN-KO mice by injection of ApN cDNA containing-plasmid followed by electroporation in one *tibialis anterior*, the contro-lateral muscle being electroporated by a control plasmid. Mice were maintained on the WD for 4 additional wks. ApN was overexpressed only in the muscle injected with ApN cDNA without any rise in circulating levels of the adipokine.
Results: We first extended our data in *ob/ob* mice and showed that WT mice rendered obese by the WD diet, unlike those receiving the SD, displayed a positive labelling for ApN in myocytes. When challenged by the WD, ApN-KO mice were more obese and insulin-resistant than WT mice. Muscles of ApN-KO exhibited myotube degeneresccence when compared to WT mice,

especially after the WD-induced challenge. We searched for the underlying mechanisms. Even in basal conditions (SD), myotubes of ApN-KO mice already displayed a positive immunolabelling for 2 markers of oxidative stress (peroxiredoxin 3/5) as well as for a lipid peroxidation product (hydroxynonenal). The pro-inflammatory cytokine (TNF-alpha) and a marker of apoptosis (caspase-6) were also already present in muscle of these mice, but not in those of WT mice. After the WD challenge, immunoreactivity for these markers was much stronger in muscle of ApN-KO mice, while being detected at a lesser extent in WT mice. When compared to WT mice (whatever the diet), nuclear immunolabelling for NF-kB doubled in myocytes of ApN-KO fed the SD and further increased when these mice were on the WD. Eventually, transfer of ApN gene prevented muscle stress in ApN-KO mice challenged by the WD (reduction of oxidative stress and inflammatory markers compared to the contro-lateral untreated muscle).
Conclusion: Induction of muscular ApN may be triggered by conditions associated with obesity and insulin resistance. This induction in muscle appears to be crucial to counteract locally oxidative stress, inflammation and subsequent apoptosis. These results may have repercussions in the pathophysiology of the metabolic syndrome.

OP 20 Beta cell biology

115

Fluorescence based real-time analysis of stimulus-secretion coupling in mouse beta cells

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Background and aims: Glucose is the main stimulus of insulin secretion in pancreatic beta cells. Expression of the high capacity GLUT2 glucose transporter in beta cells facilitates glucose uptake close to the extracellular concentration. Glucokinase (GK) phosphorylates glucose in the first and rate limiting step of glycolysis and plays the pivotal role of a glucose sensor in beta cells. Thus, glucose recognition by cooperative action of GK and GLUT2 is vital for the subsequent machinery of insulin secretion. Recently we could establish a fluorescence resonance energy transfer (FRET) based glucose specific nanosensor (FLIPglu) to monitor the intracellular glucose concentration. Therefore the aim of this study was to characterize in real-time glucose uptake and metabolism in primary mouse beta cells in comparison to insulin-secreting MIN6 cells and with respect to insulin secretion.
Materials and methods: FLIPglu expression in primary mouse beta cells was achieved by transduction with a recombinant adenovirus. In addition an insulin-secreting MIN6 cell clone stably expressing FLIPglu was used. Using fluorescence microscopy, the dynamic changes in the intracellular glucose concentration were analyzed in a perifusion chamber in the absence and presence of glucose and selective inhibitors of glucose uptake and metabolism, respectively. FRET calculations as an enhanced yellow fluorescence protein (EYFP)/enhanced cyan fluorescence protein (ECFP) emission intensity ratio upon ECFP excitation were performed offline. Insulin was analyzed in the perifusion medium by ELISA.
Results: Glucose homeostasis in primary mouse beta cells could be depicted by calculating the rates of uptake and metabolism. Glucose uptake in mouse beta cells was significantly higher than in MIN6 cells upon addition of Krebs Ringer medium containing 10 mM glucose. However, the increase of the EYFP/ECFP ratio was accompanied by a subsequent increase in insulin secretion. Interestingly, glucose consumption which was calculated as a decrease of the EYFP/ECFP ratio after glucose removal was comparable in primary beta cells and MIN6 cells. Incubation of cells in medium supplemented with 10 mM 3-O-methylglucose caused in the presence of 10 mM glucose a significant decrease in the glucose uptake compared to incubation at 10 mM glucose alone. While the glucose uptake capacity was reduced by 50 % in mouse beta cells, the reduction was 80 % in MIN6 cells. When mouse beta cells and MIN6 cells were perifused with a mixture of 10 mM glucose and 10 mM mannoheptulose glucose metabolism was reduced by 50 % due to inhibition of GK.
Conclusion: Glucose metabolism mediated by GK in MIN6 cells is comparable to that in primary mouse beta cells, while glucose uptake is altered possibly due to a lower expression of the GLUT2 glucose transporter. The adenoviral FLIPglu sensor construct is a useful tool to analyze in future studies glucose homeostasis of primary beta cells in type 2 diabetes. This may provide new insights into the defects in the beta cell stimulus-secretion coupling in the diabetic state.

116

Disruption of TFB1M impairs insulin secretion in beta cells

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Background and aims: The nucleus encoded mitochondrial transcription factor B1 (TFB1M), a protein homologous to bacterial di-methyltransferases, has recently been shown to be crucial for mitochondrial translation. Using gene microarray analysis, we previously found that TFB1M gene expression is reduced in whole pancreatic extracts from diabetic GK compared to non-diabetic Fisher rats. This suggests a possible role for TFB1M in insulin secretion, given that maintenance of the mitochondrial proteome is essential for mitochondria-based stimulus secretion coupling. Here, we examined gene expression of TFB1M in islets from GK rats, and the effects on insulin secretion and mitochondrial ATP production following TFB1M knock down in INS-1 832/13 clonal beta-cells.

Materials and methods: RNA expression of TFB1M was analysed in pancreatic islets from GK and Fisher rats and in clonal 832/13 β-cells, using TaqMan qRT-PCR. Knock down was achieved by RNA interference. TFB1M was detected by immunoblotting. Insulin secretion was determined by RIA. ATP levels were detected by luciferase assay.
Results: Islets from the GK rat strain, a diabetic rat mode, showed a 50 % reduction in TFB1M mRNA expression compared with the normoglycemic F344 rat (P = 0.006). qRT-PCR analysis and detection of TFB1M by immunoblotting confirmed knock down of TFB1M by RNA interference in INS-1 832/13 beta-cells. TFB1M knock down resulted in a significant decrease in basal and glucose-stimulated insulin secretion (GSIS; 175 vs. 114 ng/mg/h; P<0.05). Impaired GSIS was accompanied by a decrease in mitochondrial and increase in glycolytic ATP production.
Conclusion: We show reduced expression of TFB1M in islets from the diabetic GK rat strain. Reduced expression of TFB1M in INS-1 832/13 clonal beta-cells impairs insulin secretion and alters ATP production. These findings indicate that: (1) TFB1M knock down causes profound changes in mitochondrial function/structure and metabolism in INS-1 832/13 cells; (2) the knock down of TFB1M in INS-1 832/13 cells represents a suitable model to study the cellular mechanisms causing impaired insulin secretion. Studies of TFB1M in relation to mitochondrial function and structure also opens a new avenue for research in pancreatic islet cell biology.
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117

Metabolite profiling of clonal (832/13) beta cells after glucose stimulation
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Background and aims: The pancreatic beta-cell secretes insulin in response to elevated blood sugar levels. Two different pathways, the triggering and the amplifying pathways, have been identified as responsible for this stimulus-secretion coupling. Whereas signaling in the triggering pathway has been well studied, the identity of the signals in the amplifying pathway has remained essentially unresolved. Here, we have profiled the beta-cell metabolome following glucose stimulation. By monitoring the dynamics of beta-cell metabolism, we aimed for an unbiased identification of possible metabolic signals.
Materials and methods: INS-1 832/13 cells were starved for two hours at 2.8 mM glucose, followed by a 16.7 mM glucose stimulation. The cell cultures were subsequently quenched at different times in the interval of 0 to 30 min. Metabolites were extracted, derivatised, and subsequently analysed by gas chromatography/mass spectrometry (GC/MS). Orthogonal projection to latent structures (OPLS) was used to correlate the metabolic profile with the time of quenching.
Results: Three OPLS models were calculated to account for metabolic alterations during the first 15 min, the last 15 min and the whole time interval. The corresponding loading plots showed that proteolytic amino acids, exemplified by hydroxyproline, were continuously down-regulated, whereas serine, being an important precursor of many amino acids, purines, and pyrimidine was up-regulated. Several amino acids also showed an initial decrease followed after 15 min by a clear stagnation, which probably indicated a switch from protein catabolism to anabolism. Also ribose-5-phosphate, a pentose phosphate pathway intermediate, was up-regulated during the last 15 min investigated, which indicates increased biosynthesis. Succinate exhibited a late rise, which could reflect a higher level during starvation due to the entrance of amino acids proximal to succinate in the TCA cycle. All other glycolytic and TCA cycle intermediates were strongly up-regulated during the whole interval. The model was confirmed by raw data analysis and t-tests.
Conclusion: We suggest that a putative metabolic coupling signal should have a response curve that is correlated or inversely correlated with the insulin secretion profile. In the present investigation, several metabolites were identified with levels which were tightly coupled to the extracellular glucose level. Experiments are in progress to investigate the stimulation response during a shorter time span, as well as the alterations evoked by a drop in glucose levels.
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118

Expression and possible roles of NOX family NAD(P)H oxidases in pancreatic islets of NOX-specific knockout mice
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Background and aims: Elevated production of reactive oxygen species (ROS) has been proposed to contribute to pancreatic beta-cell impairment in diabetes. Although the presence of phagocyte-like NOX family NAD(P)H oxidases, a potential source of ROS, has been studied in rat pancreatic islets and beta-cell lines, their role in islet function remains poorly characterized due to lack of specific inhibitors and reliable antibodies. Here, functional analysis of NOXs in islets was carried out using NOX isoform-specific knockout mice.
Materials and methods: Expression of NAD(P)H oxidase components in C57BL/6J mouse and human islets was tested by RT-PCR. Next, islets isolated from wild-type (WT) and knockout mice for specific NOX isoforms (koNOX-1, -2 and -4), were used to establish correlation between glucose-stimulated insulin secretion (measure by RIA) and NOX-derived superoxide generation (measured by NBT). In koNOX-2, islet morphology, beta-cell mass and insulin contents were determined (immunostaining) and glucose homeostasis was examined by ipGTT, plasma insulin levels (ELISA) and HOMA test. Additionally, effects of non-selective NOX inhibitor DPI on glucose-stimulated insulin secretion was evaluated on WT and koNOX-1, -2 and -4 mouse islets to test for specificity.
Results: Both mouse and human pancreatic islets expressed membrane-associated NOX-2 and -4, NOX-2 being the predominant isoform. NOX-1 and cytosolic components, such as p22phox, p40phox, p47phox, p67phox, NOXO-1, NOXA-1 were also detected in mouse islets. A negative correlation (r=-0.96, p<0.05) was found between glucose-induced insulin secretion and NOX-derived superoxide generation comparing WT and koNOX-1, -2 and -4 islets. Compared to WT, koNOX-2 islets stimulated with 22.8 mM glucose exhibited potentiated insulin release (+213%, p<0.005) associated with lower superoxide production (-37%, p<0.05). No differences in islet organisation, beta-cell mass and insulin contents were observed. The NOX inhibitor DPI exhibited a general inhibitory effect on insulin secretion in WT and all koNOX islets, showing the lack of specificity of this inhibitor. Mice deficient for NOX-2 exhibited glucose intolerance (p<0.05) associated with slightly higher plasma insulin levels, suggesting insulin resistance. This was corroborated by the observed elevated HOMA index (+62%, p<0.01).
Conclusion: NOX-2 is the most abundant NAD(P)H oxidase in human and mouse islets. NOX knockouts represent a valuable experimental model for the study of NAD(P)H oxidases and show that studies using non-specific NOX inhibitors might be misleading. NAD(P)H oxidases positively contribute to glucose-induced ROS generation and negatively regulate insulin secretory function.
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119

Characterization of the insulin secretory profile in islets isolated from beta cell specific glutamate dehydrogenase knockout mice
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Background and aims: Glutamate dehydrogenase (GDH) is a mitochondrial enzyme that catalyses the reversible transformation of glutamate to alpha-ketoglutarate. GDH might play a role in glucose-induced amplifying pathway through generation of glutamate and/or as an amino acid sensor triggering insulin release upon glutamine stimulation in conditions of GDH allosteric activation. Here, we investigated the role of GDH in a beta-cell specific GDH knockout mouse model, named ßGlud1-/-, recently generated in our laboratory.
Materials and methods: Islets were isolated from wild type (WT) and beta-cell specific GDH null mice (ßGlud1-/-), cultured overnight and used for perifusion experiments or static incubations to measure insulin secretory responses. Reintroduction of GDH expression was achieved using the adenovirus AdGDH. Amino acids levels were measured in islet extracts by HPLC and gene expression analysed by quantitative RT-PCR.
Results: In perifusion experiments, islets from WT mice increased insulin secretion 2-fold in response to 11.8 mM glucose and 3-fold upon 22.8 mM

glucose stimulation. Islets isolated from ßGlud1-/- beta-cell specific GDH knockout responded normally to 11.8 mM glucose, but the response to 22.8 mM glucose was reduced by 56%. The secretory response to glucose could be significantly rescued (p=0.01) when GDH knockout islets were simultaneously exposed to 22.8 mM glucose and 5mM dimethyl-glutamate, added as a cell permeant glutamate precursor. When testing the amplifying pathway by combination of KCl (30 mM) / diazoxide (0.25 mM) plus 22.8 mM glucose, the sustained insulin release observed in WT islets was inhibited by 75% in beta-cell GDH knockout islets. Glutamine stimulation is strictly GDH dependent and elicits insulin release only upon GDH allosteric activation, for instance using BCH. In accordance with this model, ßGlud1-/- islets were totally unresponsive to glutamine (5 mM) / BCH (10 mM) stimulation. The response to glutamine/BCH was partially rescued in GDH knockout islets transduced with an adenovirus encoding for GDH (50% of the WT response, p=0.02). Amino acids related to glutamate pathways were reduced in GDH knockout islets stimulated with 22.8 mM glucose (glutamate -38%, glutamine -49%, aspartate -40%, and alanine -37%, all p<0.05). Moreover, in ßGlud1-/- islets we measured decreased expression of the mitochondrial glutamate carrier GC1 responsible for glutamate transport across the mitochondrial membrane (-41%, p=0.05).

Conclusion: The present study took advantage of a unique model of beta-cell specific GDH knockout to question the role of this enzyme in islet function. Data show that GDH is essential for sustained insulin secretion at optimal glucose concentration. In these conditions, lower glutamate concentrations seem to be responsible for reduced secretory response as provision of cell permeant glutamate restored the secretory response. The lack of GDH also impacted on related glutamate pathways, pointing to GDH as a master switch linking glucose and amino acid metabolisms.

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120

On role of transglutaminase 2 in insulin secretion: sub-cellular localization in human endocrine pancreas and identification of transamidation substrates in INS1E

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Background and aims: Transglutaminase 2 (TG2) is a multifunctional and ubiquitous enzyme that belongs to the transglutaminase family. Mutational analysis of human TG2 gene in subjects affected by familial forms of type 2 diabetes led us to the identification of three different missense mutations, M330R, I331N and N333S, that determine a reduced TG2 transamidation activity in vitro. Hence, to better investigate the role of TG2 transamidation activity in insulin secretion, we labeled INS1E rat insulinoma cells in vivo with a biotinylated TG2 specific substrate and studied transamidation activity before and after (2', 5', 8' and 15') the application of a 15.5 mmol/L glucose stimulus, in order to mimic first-phase insulin secretion.

Materials and methods: TG2 transamidation kinetics in INS1E (basal condition versus glucose-stimulated) was studied by labeling cells with 1 mM EZ Link 5 (biotinamido) pentylamine and following the appearance/disappearance of specific biotinylated substrate/s. Bi-dimensional (2D) polyacrylamide gel electrophoresis followed by Western blot, performed with streptavidin HPRT, was employed for kinetic experiments. For substrates identification, TG2 biotinylated proteins were purified on avidin sepharose column before 2D gel separation followed by MALDI MS/MS.

Results: Our data indicate that 2' after glucose stimulus, corresponding to the highest intracellular Ca2+ concentration, TG2 transamidation function is fully activated as demonstastrated by 2d gel analysis of INS1E proteins. By MALDI MS/MS, we then identified some TG2 natural substrates, and among them: cytoplasmic actin 1 and 2, GRP78, HSP90, mitogen activated protein kinase 1, tropomyosin alpha 4 and gamma 3 chain. All these proteins are involved either in insulin secretion or in glucose metabolism, or both. Western blot with an anti-cytoplasmic actin 1 polyclonal antibody showed that actin is indeed one of the biotinylated protein spots identified with 2D. In addition, we also demonstrated that cytoplasmic actin co-immunoprecipitates with TG2. Ultrastructural microscopy indicated that in normal human pancreas TG2 is localized in the cytoplasm and 2D electrophoresis showed that TG2 stays in INS1E cytoplasm during the entire period of glucose stimulus (2'-15') and beyond (up to 30').

Conclusion: We conclude that TG2 transamidation activity has a direct role in insulin secretion. This action is probably mediated by cytoplasmic actin 1 and 2 which are known to be involved in the remodeling of cytoskeleton, one of the fundamental events in insulin exocytosis.

OP 21 Insulin action - animal models

121

Adenoviral gene transfer of PLD1-D4 results in enhanced insulin sensitivity in skeletal muscle of *ped/pea-15* transgenic mice

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Background and aims: Ped/pea-15 is a gene commonly overexpressed in tissues from type 2 diabetic individuals and in First Degree Relatives. Indeed, overexpression of the ped/pea-15 gene in mice impairs glucose tolerance and leads to diabetes in association with high-fat diets. Several studies demonstrated that PED/PEA-15 binds phospholipase D1 (PLD1), stabilizing and enhancing its activity in the cell. This effect is mediated by the C-terminal D4 domain of PLD1. Both in cells and in skeletal muscle tissue from PED/PEA-15 transgenic mice (TgPED), the increase of PED/PEA-15/PLD1 interaction induces insulin-resistance, at least in part, by impairing PKC signal transduction. Indeed, PLD1 stabilization induces the constitutive activation of PKCα, determining the inhibition of insulin-induced activation of PKCζ, an important kinase involved in insulin-induced glucose uptake. In this work, we sought to test the hypothesis that D4 overexpression could represent a potential tool to rescue insulin-sensitivity and glucose tolerance in TgPED.
Materials and methods: We generated a recombinant adenoviral vector containing D4 cDNA (Ad-D4) to induce D4 expression in TgPED. 6-months old TgPED and control mice were intravenously injected with 1x1011 viral particles of either control Ad-GFP or Ad-D4. The effects of Ad-D4 were evaluated after 7-10 days of infection.
Results: After infection, D4 is efficiently expressed in skeletal muscles of TgPED and control mice. In TgPED skeletal muscles, D4 expression decreases PED/PEA-15/PLD1 interaction. This is accompanied by a decrease of PKCα constitutive activity and the recovery of insulin effect on PKCζ activation. In TgPED, Ad-D4 infection restores insulin sensitivity (ITT. AUC control mice Ad-GFP: 7930±4062.3, control mice Ad-D4: 8408±779.6; TgPED Ad-GFP: 11155±2893.8, TgPED Ad-D4: 6532,8±2683.3, p<0.001 vs TgPED Ad-GFP) and ameliorates glucose tolerance (GTT. AUC control mice Ad-GFP: 20155±5040, control mice Ad-D4: 18676±3046; TgPED Ad-GFP: 31127±6617, TgPED Ad-D4: 15619±2490, p<0,0001 vs TgPED Ad-GFP). Furthermore, D4 expression in TgPED decreases fasting insulin levels (TgPED Ad-GFP: 1.04±0.29 ng/ml, TgPED Ad-D4: 0.44±0.04 ng/ml, p<0.01), FFA (TgPED Ad-GFP: 0.87±0.28 mmoles/l, TgPED Ad-D4: 0.57±0.14 mmoles/l, p<0.01) and tryglycerides (TgPED Ad-GFP: 22.2±0.16 mg/dl, TgPED Ad-D4: 15.9±0.10 mg/dl, p<0.01). Finally, D4 expression in TgPED increases insulin-induced glucose uptake in skeletal muscles.
Conclusion: In conclusion, adenoviral delivery of PLD1-D4 rescues insulin sensitivity and glucose tolerance in vivo. Thus, interfering with PED/PEA-15/PLD1 interaction represents a major strategy for developing new molecules for the treatment of type 2 diabetes.
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122

SIRT1 deacetylase activity is essential for calorie restriction-induced enhancement of skeletal muscle insulin sensitivity

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Background and aims: Calorie restriction (CR) manifests numerous metabolic and physiological benefits including enhanced insulin sensitivity and increased longevity. The NAD^+-dependent protein deacetylase, sirtuin 1 (SIRT1), is induced by CR, and pharmacological activation of SIRT1 mimics many of the beneficial health outcomes of CR, including improved insulin sensitivity. However, the precise role of SIRT1 in regulating insulin sensitivity in skeletal muscle is unclear. The purpose of the present study was to determine whether SIRT1 is essential for the CR-induced enhancement in skeletal muscle insulin sensitivity.
Materials and methods: Mice with knockout of SIRT1 deacetylase activity in skeletal muscle were generated using Cre recombinase methodology. Similar to their floxed/wildtype (WT) littermates, the SIRT1 protein is still present in the knockout (KO) mice, however, exon 4 of the SIRT1 gene (which encodes the catalytic domain responsible for deacetylase activity) is deleted. At 13 wks of age, we measured insulin-stimulated 2-deoxyglucose uptake (2DGU) in isolated extensor digitorum longus (EDL) and soleus muscles, as well as isolated epididymal adipocytes after ad libitum (AL) or CR (20 d at 60% of AL) feeding. We also measured the gene expression of SIRT1, protein tyrosine phosphatase 1B (PTP1B) and cytochrome c oxidase (COXIV) in skeletal muscle.
Results: Insulin-stimulated 2DGU at a physiological insulin concentration (60uU/mL) was not different in EDL and soleus muscles in WT vs. KO mice after AL feeding. After 20 d of CR, insulin-stimulated 2DGU was significantly enhanced ~3-fold in EDL and soleus muscles from WT mice. Interestingly, in KO mice, CR did not enhance insulin-stimulated 2DGU in EDL, and only partially increased (70%) insulin-stimulated 2DGU in the soleus. Importantly, these effects were muscle specific, as CR significantly enhanced (~3-fold) insulin-stimulated 2DGU in isolated adipocytes equally in KO and WT mice. Preliminary data suggests that the gene expression of PTP1B, COXIV and SIRT1 in skeletal muscle was unaffected by genotype or diet.
Conclusion: These results demonstrate that, 1) loss of SIRT1 deacetylase activity does not impair skeletal muscle insulin sensitivity and 2) SIRT1 deacetylase activity is essential for the full effect of CR to enhance skeletal muscle insulin sensitivity.
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123

Hepatic inflammatory gene expression and the development of insulin resistance in IL-1 receptor 1 (IL1-R1) knockout mice

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Background and aims: The Metabolic Syndrome (MetS) is characterised by obesity, insulin resistance and a sub-actute chronic pro-inflammatory state. It is well known that impairing different components of the inflammatory system (TNFα, NF-κB, IKK, JNK, etc) either due to genetic or dietary manipulations can reduce the risk of the MetS. Relatively little is known in relation to IL-1RI signaling and the MetS. Preliminary work has demonstrated that IL-1R deficient ($IL\text{-}1R1^{-/-}$) mice are protected against diet-induced obesity-related insulin resistance. In addition obese $IL\text{-}1R1^{-/-}$ mice also had improved lipid profiles wherein plasma TAG and NEFA are significantly lower. Elevated TAG are another characteristic of the MetS. Therefore the present study sought to elucidate how lack of IL-1R1 improves hepatic lipid metabolism.
Materials and methods: Detailed hepatic gene expression analysis of key markers of lipid metabolism and insulin sensitivity was preformed using real time PCR and western blot.

Results: An 8 week high-fat diet induced a phenotype characteristic of the MetS, with insulin resistance and elevated TAG and NEFA concentrations in WT compared to $IL\text{-}1R1^{-/-}$ mice. Gene expression analysis showed a reduction in hepatic SREBP-1c mRNA expression in $IL\text{-}1R1^{-/-}$mice ($P<0.05$)(fig 1). SREBP-1c is a key transcription factor that regulates both lipid and glucose metabolism. In terms of understanding the role of adipokines in hepatic insulin resistance and inflammation we focused on adiponectin, which is secreted from WAT but mediates peripheral insulin sensitivity. Plasma levels of adiponectin decrease the in $IL\text{-}1R1^{-/-}$mice. In the liver the expression of the receptor adipoR1 was equally increased in both WT and $IL\text{-}1R1^{-/-}$mice, however there was a marked increase in adipoR2 in $IL\text{-}1R1^{-/-}$mice only. As expected, inflammatory marker TNFα mRNA expression showed a significant reduction in $IL\text{-}1R1^{-/-}$mice only ($P<0.05$). Finally, western blot analysis demonstrated that JNK and NF-κB p65, critical regulators of insulin action, were significantly lower in $IL\text{-}1R1^{-/-}$mice ($P<0.05$) which might partially confirm the anti-diabetic phenotype in reference to the high-fat diet.
Conclusion: The observed results underlie the link between inflammation and metabolic deregulation. Our study provides further, but not conclusive evidence that IL-1 is a crucial mediator pro-inflammatory and abrogation of its receptor may have potential therapeutic benefits.

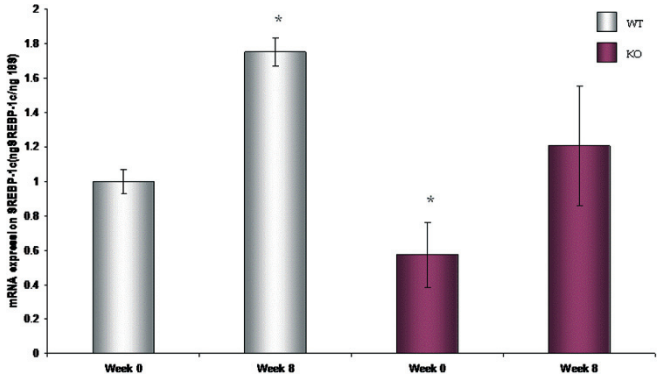

Figure 1. mRNA expression hepatic SREBP-1c was significantly increased in WT mice at week 8 compared to week 0 (P value= 0,0001). Hepatic SREBP-1c mRNA expression was significantly higher in WT mice at baseline compared KO mice at week 0 (P=0,05). Values represent means ±SEM. Significantly different *p<0.05 mRNA expression was normalised to 18S and expressed relative to WT mice at week 0
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124

FAAH$^{-/-}$ are metabolically flexible despite whole body insulin resistance
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Background and aims: Endocannabinoid (eCb) signaling is pivotal in modulating several physiological processes including: feeding, obesity, fertility, inflammation and pain. Fatty acid amide hydrolase (FAAH), a 63 kDa integral membrane enzyme, is responsible for degrading eCbs, such as anandamide and 2-arachidonoly glycerol. FAAH$^{-/-}$ mice are obese, and obesity in general is usually associated with insulin resistance and metabolic inflexibility. This study characterized metabolic flexibility and tissue-specific insulin resistance for the FAAH$^{-/-}$ mouse.
Materials and methods: FAAH-/- mice, generated from a C57BL6 strain and C57BL6 mice (C57 wt) were used for the study. Glucose disposal was assessed with a stable isotope GTT (SiGTT) using [6,6-^{2}H]-glucose. Hepatic glucose production (HGP) in the fasted state and in vivo lipolysis was determined by constant infusion of [U-$^{13}C_6$]-glucose, 2-^{13}C-glycerol by Alzet minipump respectively. Metabolic inflexibility was assessed by indirect calorimetry, using an 8-chamber Oxymax system (Columbus Instruments, Columbus, OH).
Results: FAAH $^{-/-}$ mice were obese (35±3.4 *vs.* 29±1.6 grams, p<0.002, at 4 months of age) and their food consumption/day was higher than C57 wt (4.4 *vs.* 3.5 g, p<0.01). FAAH$^{-/-}$ mice have hepatic insulin resistance, as fasting insulin (15h overnight fast) was 1.351±0.3 *vs.* 0.474±0.3 ng/ml for FAAH$^{-/-}$ *vs.* C57 wt (p<0.001). There was no difference in basal glucose levels, or HGP. FAAH$^{-/-}$ mice have adipose insulin resistance because glycerol production, reflecting *in vivo* lipolysis, was increased by 42% (p<0.01) in fasted FAAH$^{-/-}$ mice, despite increased fasting insulin. FAAH$^{-/-}$ mice have skeletal muscle insulin resistance, as measured during the SiGTT. The insulin AUC was 3 fold higher for FAAH$^{-/-}$ mice (p≤0.05). The AUC for D2 glucose (which mainly reflects glucose disposal by muscle during the GTT), however, was similar to the C57 wt, reflecting insulin resistance. Indirect calorimetry showed no difference (p>0.05) in substrate utilization during a diurnal cycle. Thus, metabolic flexibility was preserved, even though FAAH$^{-/-}$ mice have lower activity in the fed state (p<0.05).
Conclusion: Despite obesity, and flux analysis indicating hepatic, skeletal muscle and adipose insulin resistance,, FAAH$^{-/-}$ mice still have normal metabolic flexibility. Since decreases in metabolic flexibility are thought to accompany the progression of insulin resistance in the development of Type 2 DM, endocannabinoid signaling, may play a complex role on the whole body level in the linkage, as well as compensation, of insulin resistance and metabolic flexibility.
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125

The *PED/PEA-15* diabetogene as a potential thiazolidinedione target
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Background and aims: Thiazolidinediones (TZDs) represent a class of insulin-sensitising drugs currently used for treating type 2 diabetes (T2D); however, resistance to TZDs sometimes occurs. The TZD target is PPARγ, a nuclear hormone receptor able to modulate gene expression upon ligand binding. Ligand-mediated activation of PPARγ has been linked to glucose homeostasis.
The phosphoprotein enriched in diabetes/phosphoprotein enriched in astrocytes (*PED/PEA-15*) gene is over-expressed in tissues from T2D subjects and from first-degree relatives of T2D subjects. Furthermore, transgenic mice ubiquitously over-expressing *ped/pea-15* feature impaired glucose tolerance and insulin resistance. In cultured cells and *in vivo*, *ped/pea-15* over-expression impairs insulin activation of protein kinase C zeta (PKCζ) and glucose disposal. Recently, we have obtained evidence that TZDs down-regulate *ped/pea-15* expression. This study aims at investigating whether and how TZDs ameliorate insulin sensitivity by modulating *ped/pea-15* gene expression.
Materials and methods: *ped/pea-15* expression was analyzed by real-time quantitative polymerase chain reaction (PCR) and Western blotting in tissues from C57BL/6J mice and in L6 myotubes, used as a cellular model system of skeletal muscle. L6 myotubes were also used for glucose transport assays. Gene Reporter Assays, Electrophoretic Mobility Shift Assays (EMSA) and Chromatin Immuno-Precipitation Assays (ChIP) were performed in HeLa cells.
Results: In L6 skeletal muscle cells, rosiglitazone (RGTZ) decreased both *ped/pea-15* mRNA and protein levels. Consistent with the *in vitro* data, muscle tissue from mice treated with RGTZ for 10 days showed a 40% decrease of ped/pea-15 protein levels.
We obtained evidence that *PED/PEA-15* expression is modulated by AP-1 activators, such as 12-O-tetradecanoyl-phorbol-13-acetate (TPA). In fact, when treated with TPA, HeLa cells showed an increase in *PED/PEA-15* mRNA levels; TPA also induced the activation of *PED/PEA-15* promoter activity. Interestingly, the TPA effect was almost completely reverted by RGTZ in a concentration dependent-manner and *via* PPARγ. Specifically, the CRE-like site within the 200bp regulatory region of *PED/PEA-15* was involved in both TPA and RGTZ effects. EMSA and ChIP assays further demonstrated that TPA caused an increase in the binding of c-JUN to the CRE-like site (a putative AP-1 binding site) on the *PED/PEA-15* promoter, and the binding was reduced by 50% in presence of RGTZ. Taken together, these results indicated that AP-1 induces *PED/PEA-15* transcription by the binding to the CRE-like site. The AP-1 activity is interfered by PPARγ and it might be due to a physical interaction between activated PPARγ and c-JUN. In addition, in L6 skeletal muscle cells the down-regulation of *ped/pea-15* expression by TZDs was associated with an increase in PKCζ activation and in glucose up-take.
Conclusion: The results of this study identified *PED/PEA-15* gene as a potential target of TZDs in T2D treatment. More interestingly, the down-regulation of *ped/pea-15* expression represents a direct effect of TZDs on skeletal muscle, in addition to the first TZD action on adipose tissue. Therefore, *PED/PEA-15* gene might represent a potential marker for resistance to TZDs in T2D treatment.

126

Cox6a2 deficient mice are protected against high fat diet-induced obesity and insulin resistance
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Background and aims: Mitochondrial dysfunction has repeatedly been associated with the etiology of obesity and type 2 diabetes. Therefore, we investigated the metabolic profile of mice deficient in the respiratory chain gene *Cox6a2*. Cox6a2 is a subunit of the cytochrome c oxidase, and it is believed to play a role in thermogenesis at rest and in protection against the formation of reactive oxygen species (ROS).

Materials and methods: Glucose tolerance (2.5 mg glucose/g body weight) was assessed in mice of 14 weeks of age and after 9 and 22 weeks of high fat diet (HFD) feeding. Insulin sensitivity was measured using hyperinsulinemic, euglycemic clamps. To investigate energy expenditure, mice were monitored in metabolic cages for three consecutive days. Affymetrix Mouse 1.0 ST Gene Arrays as well as qRT-PCR were used to assess differences in gene expression. ROS levels in skeletal muscle were measured using the fluorescent probe dihydroethidium and quantified using fluorescence microscopy. To study skeletal muscle fiber type composition, muscle sections were stained for myosin ATPase. Resistance to fatigue of isolated muscles was examined by repetitive stimulation for 12 min with decreasing rest intervals of muscles which were mounted on a force transducer and placed in oxygenated Krebs-Henseleit buffer.
Results: Glucose tolerance was comparable in wild type and knockout mice on regular diet, although fasting blood glucose was lower in Cox6a2$^{-/-}$ mice ($p < 0.001$). Cox6a2$^{-/-}$ mice were completely resistant to body weight gain after HFD feeding, in contrast to WT mice which became progressively obese and glucose intolerant. Weight gain in WT mice was mainly due to increased adiposity, and adipocytes were smaller in Cox6a2$^{-/-}$ mice ($p < 0.02$). Better glucose tolerance in Cox6a2$^{-/-}$ mice on HFD was explained by higher sensitivity to insulin ($p < 0.01$). Protection against obesity could be explained by increased energy expenditure and thermogenesis in Cox6a2$^{-/-}$ mice, which was independent of circulating thyroid hormone levels, but correlated with elevated expression of uncoupling proteins in metabolically active tissues such as skeletal muscle, heart, and white and brown adipose tissue. This upregulation was associated with increased ROS formation in skeletal muscle of Cox6a2$^{-/-}$ mice. Furthermore, we found a compensatory coordinated increase in expression of respiratory chain genes and a fiber type switch of skeletal muscles of Cox6a2$^{-/-}$ mice to a more oxidative fiber profile. These changes resulted in improved resistance to fatigue of isolated skeletal muscle. Finally, we found that female Cox6a2$^{-/-}$ mice lost more body weight during a short period of fasting ($p < 0.005$), suggesting that *Cox6a2* may be a thrifty gene which helps to efficiently store and utilize energy.
Conclusion: We conclude that Cox6a2 is involved in whole-body energy metabolism and thermogenesis. This may indicate that genetic variations in the human *COX6A2* gene could influence the susceptibility to develop insulin resistance and obesity.
Supported by: GOA, IUAP, EURODIA, JDRF

OP 22 Responses to GLP-1-based therapy

127

A pilot study examining the effect of *GLP1R* polymorphisms on insulin secretion in response to glucose and GLP-1 infusion in non-diabetic subjects
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Background and aims: Glucagon-Like Peptide-1 (GLP-1) interacts with its transmembrane receptor (encoded by *GLP1R*) to produce insulin secretion in a glucose-dependent manner. The effect of genetic variation at *GLP1R* on the response to GLP-1 is unknown. Variation in response may affect the response to incretin-based therapy. We therefore assessed the effect of *GLP1R* polymorphisms on insulin secretion in response to glucose and GLP-1 infusion in non-diabetic subjects.
Materials and methods: We studied 81 healthy individuals (48 females and 33 males, age=26.1±0.6 years, BMI=24.9±0.4 kg/m^2, fasting glucose < 4.82 ± 0.03 mmol/L) using a hyperglycemic clamp. A variable infusion maintained peripheral glucose concentrations at 150 mg/dl for 240 minutes. At 120 minutes an intravenous infusion of GLP-1 was started and maintained till the end of the study (0.75pmol/kg/min from 121-180 minutes, 1.5pmol/kg/min from 181-240 minutes). Insulin secretion was measured using a C-peptide minimal model modified to account for the effect of GLP-1 on secretion. This assumes that insulin secretion is made up of a dynamic component, proportional to rate of glucose change through the dynamic responsivity index, Φ_d (10^{-9}), and a static index, proportional to glucose through the static responsivity index, Φ_s (10^{-9} min^{-1}). Genotyping of 21 tag single nucleotide polymorphisms (tag SNPs) and 4 non-synonymous SNPs (nsSNPs) in the extended *GLP1R* locus was undertaken and their relationship with insulin secretion indices examined.
Results: The nsSNP, rs6923761, altered insulin secretion indices (Φ_{Total}, Φ_d and Φ_s) and early insulin secretion in response to GLP-1 infusion ($P < 0.05$). The tag SNPs rs4714210 and rs13202369 were also weakly associated with a blunted response to intravenous GLP-1.
Conclusion: In young, healthy subjects variation in *GLP1R* alters response to intravenous GLP-1. It remains to be determined whether these observations are replicated in a larger cohort and in diabetic subjects with impaired insulin secretion.

ISR = Insulin Secretion Rate (pmol/l per min)									
	rs6923761 ($P < 0.05$)			rs13202369			rs4714210 ($P < 0.05$)		
	1,1	1,2	2,2	1,1	1,2	2,2	1,1	1,2	2,2
ISR at 180min	364±24	346±29	337±21	351±23	373±27	278±16	306±39	324±23	395±26
ISR at 240min	519±35	455±40	420±38	483±24	499±35	360±42	418±51	476±35	500±37
Peak ISR	851±76	678±53	647±61	813±74	729±47	541±37	567±63	769±70	780±60
	rs6923761 ($P < 0.05$)			rs13202369 ($P < 0.05$)			rs4714210		
	1,1	1,2	2,2	1,1	1,2	2,2	1,1	1,2	2,2
Φ_{Total} 180min	104±10	82±7	77±7	102±12	93±7	67±8	82±12	93±3	100±11
Φ_{Total} 240min	152±13	117±10	111±11	149±16	131±10	92±14	121±18	137±13	139±15
Peak Φ_{Total}	160±13	126±11	118±11	157±17	141±10	97±14	126±19	146±13	148±15

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128

Impact of endogenous and exogenous GLP-1 on glucagon secretion in type 1 diabetic patients with and without residual beta -cell function
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Background and aims: Effect of secreted and infused GLP-1 on glucagon secretion and glucose excursions during a mixed meal was investigated in type 1 diabetic patients with and without residual insulin secretion.

Materials and methods: Eight diabetic patients with stimulated C-peptide concentration of 0.46 ± 0.1 nM and eight without residual insulin secretion

underwent three mixed meal tests with infusion of either the GLP-1 receptor-antagonist, exendin 9-39 (ex 9-39) (300 pmol/kg/min), GLP-1 (1.2 pmol/kg/min) or saline. Eight healthy matched (age, sex and BMI) subjects with normal glucose tolerance were included as controls. Infusions were started 30 minutes prior to the meal and patients injected half of their usual fast acting insulin-dose shortly before ingesting the meal. To assess gastric emptying rate, we added 1 g of acetaminophen to the meal. Postprandial glucose, glucagon, insulin secretion and gastric emptying rate were evaluated from incremental area under the plasma concentration curves from -30 to 180 minutes and presented as mean ± SEM.

Results: The injected dose of insulin tended to be lower in the C-peptide positive compared with the C-peptide negative subjects: 2.6 ± 0.5 vs. 3.9 ± 0.3 IE, p=0.06. Ex 9-39 increased glucagon levels in the three groups compared with saline: 2,541 ± 249 vs. 566 ± 179 and 3,244 ± 470 vs. 696 ± 213 and 1,824 ± 122 vs. 644 ± 196 pmol/l x min for the C-peptide negative, positive and the control subjects respectively, p<0.01. GLP-1 decreased glucagon concentrations in the three groups compared with saline: -409 ± 119 vs. 566 ± 178, -152 ± 109 vs. 696 ± 213 and -590 ± 114 vs. 644 ± 196, p<0.05. Glucose excursions were strongly decreased with GLP-1 compared with saline: -137 ± 141 vs. 930 ± 184, -273 ± 105 vs. 731 ± 137 and -7 ± 27 vs. 84 ± 31 mM x min, p< 0.01. In both diabetic groups, postprandial glucose levels with GLP-1 did not differ from control subjects receiving saline. Ex 9-39 increased glucose levels in the C-peptide positive group: 1,072 ± 103 vs. 731 ± 137, p=0.02, but the increment did not reach statistical significance in the C-peptide negative or control subjects: 1,168 ± 132 vs. 930 ± 184 and 141 ± 25 vs. 84 ± 31, p=NS. However, in the diabetic subjects as a whole group, ex 9-39 did significantly increase blood glucose levels (p=0.002). Compared with saline, GLP-1 decreased gastric emptying rate in both diabetic groups (p<0.01), whereas ex 9-39 increased gastric emptying rate in the diabetic subjects (p<0.05). In the C-peptide positive subjects, insulin secretion was significantly lower with both GLP-1 and ex 9-39 compared with saline: 40.6 ± 17.9 and 56.4 ± 22.0 vs. 69.8 ± 25.6, nM x min p=0.02 and 0.05 respectively, but with GLP-1, C-peptide was transiently increased from the beginning of the infusion until start of the meal.

Conclusion: Infusion of GLP-1 is able to control blood glucose excursions during a mixed meal in patients with type 1 diabetes regardless of residual beta-cell function. This is probably due to decreased glucagon levels and slowed gastric emptying. Blocking the endogenous GLP-1 action strongly increases glucagon levels leading to increased postprandial glucose excursions in type 1 diabetic patients.

129

The effect of LY2189265 (GLP-1 analogue) once weekly on HbA_{1c} and beta cell function in uncontrolled type 2 diabetes mellitus: the EGO study analysis

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Background and aims: LY2189265, a novel, long-acting glucagon-like peptide 1 (GLP-1) analog, consists of a dipeptidyl peptidase-IV (DPP-IV)-protected GLP-1 analog covalently linked to a human IgG4-Fc heavy chain by a small peptide linker. This randomized, placebo-controlled, double-blind study investigated the effect of dose titration of LY2189265 in overweight/obese patients with type 2 diabetes mellitus (T2DM) taking 2 classes of oral antihyperglycemic agents (OAH). The impact of LY2189265 on metabolic outcomes and measures of physiologic function of β-cell function and insulin sensitivity was evaluated.

Materials and methods: Following a 2-week placebo run-in, 262 patients (49% female) with T2DM (mean duration 8.3±6.4 years) and elevated BMI (mean 33.9±4.1 kg/m^2) and HbA1c (mean 8.2±0.9%) were randomized to once-weekly subcutaneous injections of either placebo (16 weeks), 1 of 2 titrated doses of LY2189265 (0.5 mg for 4 weeks followed by 1.0 mg for 12 weeks; or 1.0 mg for 4 weeks followed by 2.0 mg for 12 weeks), or LY2189265 1.0 mg (16 weeks). Patients were stratified with similar numbers randomized across the strata to the 4 treatment arms. The primary measure was glycemic control, as measured by HbA1c change from baseline at 16 weeks. Secondary measures were fasting serum glucose (FSG); solid mixed meal test postprandial glucose (PPG) excursion; body weight; and β-cell function (%B), insulin sensitivity (%S) and insulin resistance (IR), as measured by the Homeostasis Model Assessment (HOMA2) using c-peptide concentrations.

Results: Statistically significant decreases in glycemic and weight measures and increases in β-cell function were observed compared to placebo in response to LY2189265 treatment, as shown in the following table:

Outcome Measures (units)	Change from Baseline***			
	Placebo	LY2189265		
		0.5/1.0 mg	1.0/1.0 mg	1.0/2.0 mg
HbA1c (%)	-0.27	-1.28**	-1.29**	-1.52**
FSG (mmol/L)	-0.49	-2.09**	-2.04**	-2.64**
Test Meal PPG AUC	36.36	30.71**	32.21**	28.24**
Test Meal PPG AUC Excursion	10.94	8.85*	9.92*	8.16*
Weight (kg)	-0.07	-1.58*	-1.40*	-2.51**
HOMA%B	1.04	39.20*	44.26**	45.61**
HOMA%S	2.46	0.46	-1.65	3.58
HOMA-IR	-0.23	-0.20	0.01	-0.23

*p<0.05 vs placebo
**p<.0.001 vs placebo
***HbA1c, FBG, and weight reported as Least-Squares Mean; HOMA%B, %S, and IR reported as Mean; Test Meal PPG AUC and Test Meal PPG AUC Excursion reported as Mean for test meal at endpoint.

The incidence of hypoglycemic episodes was not statistically significantly different across treatment groups. Nausea (13%), diarrhea (8.8%) and abdominal distension (8.0%) were the most frequently recorded treatment-emergent adverse events.

Conclusion: In conclusion, in patients with T2DM sub-optimally controlled by OAHs, adjunctive administration of once-weekly LY2189265 resulted in significant reduction of HbA1c, fasting and postprandial glucose and an improvement in β-cell function with a favorable effect on body weight.

130

The long-acting GLP-1-receptor agonist albiglutide improves glycaemic control in type 2 diabetes: time-course analysis

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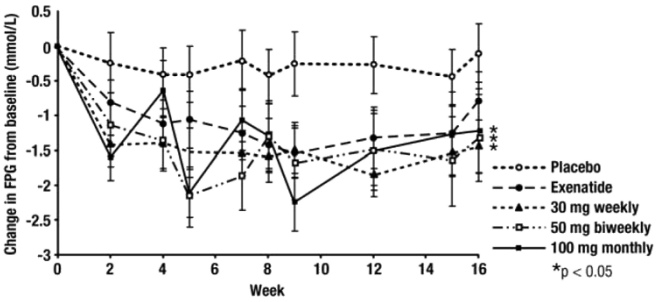
Background and aims: To assess early responses to pharmacologic therapies, fasting plasma glucose (FPG) is a more accurate reflection of immediate glucose control than is HbA_{1c}, and variations in FPG levels may in part be due to underlying pharmacokinetics (PK). Albiglutide is a GLP-1-receptor agonist with a half-life of ~5 days, having the potential for weekly or less-frequent dosing. Accordingly, in a Phase 2 study designed to determine optimal dosing and scheduling of albiglutide, a time-course analysis was conducted to examine optimal effects on key measures of glycemic control. Exenatide was included as a clinical reference for twice-daily GLP-1-receptor agonist therapy.

Materials and methods: A randomized, multicenter, double-blind, parallel-group study in 356 subjects (mean age 54 years, BMI 32.1 kg/m^2) with type 2 diabetes (mean duration 5 years) previously treated with diet and exercise or metformin (mean baseline HbA_{1c} 8.0%) was conducted. Patients received subcutaneous placebo, albiglutide [weekly (4, 15 or 30 mg), every other week (biweekly; 15, 30 or 50 mg) or monthly (50 or 100 mg)], or non-blinded exenatide twice daily (per label, in metformin-treated patients) over 16 weeks. FPG was assessed throughout the study so that the time course of these effects could be evaluated.

Results: Improvements in FPG were seen as early as the first assessment (2 weeks) in all albiglutide groups. Among top doses within each schedule, the lowest FPG fluctuations were seen for 30 mg weekly albiglutide, followed by 50 mg biweekly dosing. The 100 mg monthly albiglutide dose produced the greatest FPG fluctuations between dosing, with attenuated FPG reductions as albiglutide exposure decreased. PK/PD modelling showed a clear relationship between albiglutide exposure and FPG. Despite greater fluctuations in FPG for monthly dosing, similar HbA_{1c} improvements were observed with the highest dose of each albiglutide regimen at week 16 (-0.87, -0.79 and -0.87% by 30 mg weekly, 50 mg biweekly and 100 mg monthly dosing, respectively, vs PBO -0.17%, p < 0.005. Exenatide reduced HbA_{1c} by -0.54%).

Conclusion: Albiglutide at all doses and schedules improved HbA_{1c} in a stable manner in patients with type 2 diabetes over 16 weeks. The 30 mg weekly dose generated the least fluctuation in FPG, followed by 50 mg biweekly albiglutide. The stability of the FPG profile over time among patients in the

weekly and biweekly albiglutide groups may have important therapeutic benefits and is likely related to the reduced PK fluctuations compared with monthly administration.



Supported by: GlaxoSmithKline

131

Post-meal pharmacodynamic profile of AVE0010, a once-daily GLP-1 receptor agonist, in patients with type 2 diabetes inadequately controlled on metformin

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Background and aims: AVE0010 is a new GLP-1 receptor agonist under development for the treatment of type 2 diabetes mellitus (T2DM). In a 13-week, double-blind, placebo-controlled study, AVE0010 given once- (QD) or twice-daily (BID) provided dose-dependent improvements in HbA_{1c} (-0.5% to -0.8% from a baseline of 7.5%), reductions in body weight of approximately 2-4 kg, and was well tolerated. Here, we report the results of a post-meal pharmacodynamic (PD) evaluation carried out in a subset of patients from that study.
Materials and methods: Patients (n=542) inadequately controlled on a stable dose of metformin for at least 3 months (1.6-1.9 g/day; HbA_{1c} ≥7%) were randomised to subcutaneous AVE0010 at doses of 5, 10, 20, and 30 µg QD or BID, or matching placebo. At selected sites, patients (n=201) received a standardised breakfast meal challenge 30 min after drug administration to evaluate fasting, 2-h post-meal, and $AUC_{[0\text{-}4h]}$ values of plasma glucose, serum insulin, proinsulin, proinsulin/insulin ratio, C-peptide, and glucagon levels, at baseline and Week 13.
Results: Dose-dependent decreases in mean 2-h post-meal glucose and glucose $AUC_{[0\text{-}4h]}$ from baseline were seen for both QD and BID dosing. Insulin $AUC_{[0\text{-}4h]}$, 2-h C-peptide, 2-h insulin, and 2-h proinsulin were also dose-dependently reduced with QD dosing (Table). Glucagon levels at 2 h and glucagon $AUC_{[0\text{-}4h]}$ were significantly decreased at all doses (except the 2-h value at 5 µg QD), but no dose-response relationship was evident. The 2-h proinsulin/insulin ratio was unchanged with AVE0010, but the fasting ratio was significantly reduced by -0.4 to -1.4 relative to placebo at higher doses (20 and 30 µg QD and 10 to 30 µg BID), suggesting improved β-cell function.
Conclusion: AVE0010 given QD or BID was associated with dose-dependent improvements in post-meal glucose levels, and this was accompanied by reduced glucagon and insulin levels, consistent with a glucose-dependent effect on insulin secretion. These results highlight the promising post-meal glucose control profile of this new once-daily GLP-1 receptor agonist in T2DM.

132

Once-daily saxagliptin added to metformin is well tolerated and provides sustained glycaemic control over 102 weeks in patients with type 2 diabetes

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Background and aims: Saxagliptin (SAXA) is a potent selective dipeptidyl peptidase-4 (DPP-4) inhibitor specifically designed for extended inhibition of the DPP-4 enzyme. The long-term efficacy and safety of SAXA added to metformin (MET) were assessed in patients with type 2 diabetes (T2D) and inadequate glycemic control (HbA_{1c} ≥7.0%–≤10.0%) on MET alone.
Materials and methods: For the double-blind (DB) short-term (ST) treatment (tx) period, 743 patients (baseline HbA_{1c} 8.0%) were randomized and treated 1:1:1:1 to SAXA 2.5, 5, 10mg or PBO od+stable MET dose (1500–2500mg/day) for 24wks. Patients who met prespecified glycemic rescue criteria during the ST tx period received open-label pioglitazone (pio) 15–45mg+blinded study medication and entered the DB 42mo long-term extension (LTE). Patients completing the ST tx period without rescue were also eligible to enter the 42mo LTE; pio rescue therapy was also available during the LTE based on prespecified glycemic criteria. Efficacy analyses (ANCOVA, LOCF) reflect data before rescue; safety analyses reflect data regardless of rescue.
Results: At 102wks PBO-subtracted HbA_{1c} changes from baseline (n/N) for SAXA 2.5, 5, and 10mg were −0.62 (181/192; 95%CI: −0.84, −0.40), −0.72 (184/191; 95%CI: −0.94, −0.50), and −0.52 (177/181; 95%CI: −0.74, −0.30), respectively. The proportion of patients (n/N) discontinued for lack of glycemic control or rescued for meeting prespecified glycemic criteria was lower for SAXA 2.5- (58.3% [112/192]), 5- (51.8% [99/191]), and 10mg (56.9% [103/181]) vs PBO+MET (71.5% [128/179]). SAXA+MET was generally well tolerated; AE frequencies for SAXA 2.5, 5, and 10mg were 89.6%, 78.0%, and 86.7%, respectively, vs 78.8% for PBO+MET. The proportions of patients with hypoglycemia events (all) were 10.4%, 8.9%, and 11.0% for SAXA 2.5, 5, and 10mg vs 10.1% for PBO+MET. Confirmed hypoglycemia (symptoms with glucose ≤50mg/dL) was infrequent (≤1.1%, all tx groups). Small decreases in mean body weight at wk102 (before rescue, LOCF) vs baseline were observed in all tx groups.
Conclusion: In patients with T2D inadequately controlled on MET alone, SAXA added to MET provided sustained, clinically meaningful glycemic improvements over 102wks vs control and was generally well tolerated with no increase in hypoglycemia or weight.
Supported by: BMS and AZ

Least-squares mean changes±SD in postprandial parameters from baseline to Week 13

	Placebo	AVE0010 5 µg QD	AVE0010 10 µg QD	AVE0010 20 µg QD	AVE0010 30 µg QD	AVE0010 5 µg BID	AVE0010 10 µg BID	AVE0020 20 µg BID	AVE0010 30 µg BID
2-h glucose (mmol/L)	-0.4±0.5	-2.1±0.7*	-3.6±0.6*	-3.7±0.7*	-4.3±0.7*	-2.0±0.6*	-3.5±0.6*	-4.1±0.7*	-4.6±0.7*
2-h glucagon (ng/L)	+9.1±4	-5.3±6	-4.5±5*	-13.0±6*	-6.8±6*	-6.9±5*	-9.6±5*	-12.6±6*	-8.7±6*
2-h insulin (pmol/L)	+36±38	-22±57	-80±50	-102±53*	-134±56*	-82±51	27±51	-83±55	-148±53*
2-h proinsulin (pmol/L)	+0.2±5	-6.8±8	-10.8±7	-17.9±8*	-27.0±8*	-14.5±7	-5.5±7	-19.3±8	-26.4±8*
2-h C-peptide (nmol/L)	-0.1±0.1	-0.1±0.2	-0.1±0.2	-0.6±0.2*	-0.6±0.2*	-0.2±0.2	0±0.2	-0.2±0.2	-0.7±0.2
Glucose $AUC_{[0\text{-}4h]}$ (h•mmol/L)	-2.4±1.4	-7.6±2.3*	-11.0±2.0*	-11.5±2.2*	-14.1±2.2*	-6.6±1.9	-11.7±2.1*	-14.1±2.4*	-15.8±2.2*
Glucagon $AUC_{[0\text{-}4h]}$ (h•ng/L)	+34±14	-23±22*	-41±19*	-49±20*	-42±21*	-24±19*	-26±20*	-29±22*	-39±21*
Insulin $AUC_{[0\text{-}4h]}$ (h•pmol/L)	+42±76	-7±115	-100±101	-228±104*	-302±112*	-168±101	+87±106	-122±118	-383±109*

*p<0.05 vs placebo, step-down linear trend test on change from baseline to Week 13, adjusted for baseline and country

Supported by: sanofi-aventis

OP 23 Epidemiology of type 2 diabetes mellitus and cardiovascular risk

133

Prediction of coronary heart disease in a general, prediabetic and diabetic population during 10 years of follow-up: accuracy of the Framingham, SCORE and UKPDS functions. The Hoorn Study

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Background and aims: Risk of coronary heart disease (CHD) can be estimated using prediction formulas that combine values for different risk factors. We compared the validity of the Framingham, SCORE and UKPDS risk function in the prediction of risk of first or fatal CHD in populations with normal glucose tolerance (NGT), intermediate hyperglycemia (IH) and diabetes mellitus (DM).
Materials and methods: We used prospective data of 1482 Caucasian men and women, 50 to 75 years of age, who participated in the Hoorn Study. The predictive accuracy of the three risk functions was estimated using calibration (the ability to predict the number of observed events during follow-up) and discrimination appraisals (the ability to distinguish between those who experience a CHD event during follow-up from those who do not). Calibration of the models was visually checked by plotting the predicted probabilities against the observed proportion of CHD events. The discriminatory ability of the models was assessed by calculating the area under the receiver operating characteristic curve (AUROC) using the 10-year risk estimates predicted by the three models. The discriminatory power of the models is graded as low for an AUROC between 0.5-0.7, moderate between 0.7-0.9 and high if >0.9. All analyses were stratified for glucose status.
Results: During 10 years of follow-up, a total of 197 CHD events of which 43 were fatal, were observed in this population, with the highest percentage of CHD events in the diabetes group. The Framingham and UKPDS prediction models overestimated the risk of first CHD in the NGT, IH and DM sub-group. Overall, all prediction models had a low to moderate discriminatory capacity, showing better discrimination when fatal CHD was used as the predicted outcome. The SCORE risk function was the best predictor of fatal CHD events in the NGT group (AUROC=0.79 (95% CI, 0.70-0.87)), whereas the UKPDS performed better in the IH group (0.84 (0.74 (95% CI, 0.56-0.93) when estimating fatal CHD risk. In the diabetic sub-group all prediction models had low discriminatory ability when estimating the risk of first CHD.
Conclusion: The use of the Framingham function for prediction of first CHD is likely to be inappropriate due to the overestimation of persons' absolute CHD risk. In CHD prevention, the application of the SCORE risk function in diabetic patients and application of the SCORE and UKPDS risk functions in persons with normal glucose metabolism or intermediate hyperglycemia might prove useful to estimate relative changes in the absence of a more valid tool.

Discriminatory ability of the Framingham, SCORE and UKPDS risk functions: AUROC (95% CI))

	Normal glucose tolerance	Intermediate hyperglycemia	Type 2 diabetes mellitus
First CHD			
Framingham	0.68 (0.63-0.74)	0.60 (0.47-0.74)	0.63 (0.50-0.76)
SCORE	0.71 (0.66-0.76)	0.70 (0.60-0.79)	0.66 (0.54-0.79)
UKPDS	0.71 (0.66-0.75)	0.70 (0.60-0.80)	0.66 (0.56-0.78)
Fatal CHD			
Framingham	0.71 (0.61-0.82)	0.76 (0.65-0.88)	0.61 (0.37-0.86)
SCORE	0.79 (0.70-0.87)	0.70 (0.50-0.89)	0.74 (0.56-0.93)
UKPDS	0.77 (0.68-0.86)	0.84 (0.74-0.94)	0.72 (0.55-0.89)

Supported by: the Netherlands Organization for Health Research and Development (ZonMw)

134

Comparison of the impact of glycaemic status, HbA_{1c}, fasting and 2-hour glucose on aortic stiffness: a 5-year follow-up from The Whitehall II study

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Background and aims: HbA_{1c} is being considered as a diagnostic tool for diabetes. Direct evidence regarding its association with early stages of atherosclerosis is essential, however compared to fasting or 2-hour glucose is scarce. Our aim was to compare the relation between glycaemic status, HbA_{1c}, fasting and 2-hour glucose with aortic stiffness 5 years later in a middle-aged, non diabetic population, accounting for potential confounders.
Materials and methods: We examined 1818 participants (1214 men) of the Whitehall II cohort without known diabetes (mean age: 60.8 years, SD:5.8; mean BMI: 25.9, SD:3.8), who had a clinical assessment including a 75g oral glucose tolerance test and HbA_{1c} measurement at study phase 7 (2003-2004, baseline for the current analysis) plus measurement of carotid-femoral pulse wave velocity (PWV) as an indicator of aortic stiffness at study phase 9 (2007-2009). We present results from linear regression models adjusted for age, sex and mean arterial pressure (MAP), and the degree of attenuation on further adjustment for cardiovascular risk factors.
Results: Mean PWV was 8.3 m/s (SD:2.0). PWV did not differ between those with isolated Impaired Fasting Glycaemia (IFG) and the reference group with normoglycemia (β:-0.02 m/s; 95%CI:-0.5;0.5). Those with isolated Impaired Glucose Tolerance (IGT), combined IFG/IGT and newly detected diabetes had higher PWV [β (95%CI): 0.9 (0.3;1.5), 0.5 (0.2;0.7) and 0.7 (0.3;1.2) respectively], indicating stiffer arteries. Figure 1 shows age, sex and MAP adjusted differences in PWV by quintile of HbA_{1c}, fasting and 2-hour glucose, compared to the lowest quintile. Further adjustment for cardiovascular risk factors (smoking, HDL cholesterol, triglycerides, statin therapy, antihypertensive therapy) attenuated the differences by up to 23, 27 and 33% for HbA_{1c}, fasting and 2-hour glucose respectively. Differences in the upper two HbA_{1c} quintiles and the upper quintile of fasting and 2-hour glucose remained statistically significant. Across the range of values, PWV increased by 0.19 m/s (95%CI: 0.11;0.28), 0.19 m/s (0.11;0.27) and 0.25 m/s (0.17;0.33) per standard deviation increase in HbA_{1c}, fasting and 2-hour glucose respectively, adjusted for age, sex and MAP.
Conclusion: Our findings show similar impact of HbA_{1c}, fasting, and 2-hour glucose levels on aortic stiffness (an early marker of systemic atherosclerosis). Although 2-hour glucose has a stronger impact on PWV in the highest ranges, its overall effect is explained to a larger degree by other cardiovascular risk factors. HbA_{1c} may therefore be expected to perform at least as well as direct glucose measures in the prediction of cardiovascular events.

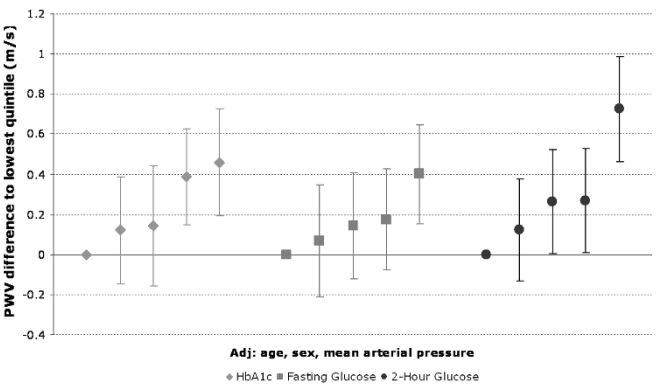

Figure 1.

135

Central obesity markers, but not BMI, are associated with cardiovascular risk in type 2 diabetic patients: results from the ADVANCE study

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Background and aims: The relative importance of anthropometric markers [body mass index (BMI), waist circumference (WC) and waist-to-hip ratio (WHR)] for cardiovascular risk in people with type 2 diabetes (T2D) has not been well established. These analyses examine the strength of the associations between baseline anthropometric markers and cardiovascular risk in T2D patients participating in the ADVANCE (Action in Diabetes and Vascular Disease: PreterAx and DiamicroN MR Controlled Evaluation) Study.
Materials and methods: 11,140 patients followed-up for 5 years were included in the present report. Cox proportional hazard models were used to determine the hazard ratios (HR) and 95% confidence intervals (95% CI) for one standard deviation (SD) change in baseline BMI, WC and WHR after adjustment for age, sex, ethnicity, current smoking and treatment allocation.
Results: Mean age was 66 years (SD = 6), 43% were female and 32% had macrovascular disease at baseline. Mean BMI was 28 kg/m² (SD = 5), WC was 98 (13) cm and WHR was 0.93 (0.08). During follow-up, 1147 major cardiovascular, 647 major coronary, 484 major cerebrovascular events and 542 cardiovascular deaths were recorded. A positive linear trend was observed for WC with cardiovascular, coronary events (both p-values ≤ 0.05) and cardiovascular death (p = 0.07). A similar pattern was observed for WHR with cardiovascular, coronary events and cardiovascular death (all p-values ≤ 0.001) and cerebrovascular events (p = 0.07). Multivariate HR (95% CI) associated with one higher SD for cardiovascular, coronary events and cardiovascular death were 1.10 (1.03-1.18), 1.13 (1.03-1.24) and 1.08 (0.98-1.19) for WC, respectively and 1.12 (1.05-1.19), 1.17 (1.08-1.28) and 1.19 (1.09-1.31) for WHR. BMI was not linearly related to any of the cardiovascular outcomes (all p-values > 0.6), but there was some suggestion of an inverse association with cerebrovascular events (p = 0.04), such that for every SD increment in BMI the risk of stroke was 0.92 (0.83-1.02). Sensitivity analyses excluding patients with macrovascular disease at baseline did not materially alter these results.
Conclusion: Data from this large, prospective, well characterised study indicate that markers of central obesity, but not BMI, are positively and continuously associated with cardiovascular risk in older individuals with T2D and to a lesser extent with cerebrovascular outcomes. This lack of association with BMI is not unexpected in a population limited to individuals who already have long-standing diabetes. There was some suggestion of an inverse association between BMI and the risk of stroke which warrants further investigation.
Supported by: NHMRC & Servier Int.

136

Differences in height and waist circumference explain gender differences in 2-hour plasma glucose levels - the Inter99 study

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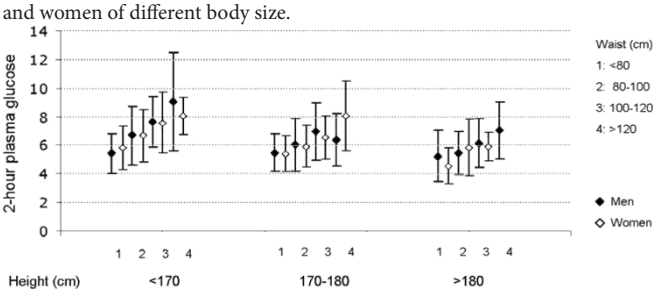
Background and aims: The prevalence of impaired glucose tolerance (IGT) is higher in women than in men. Currently, it is debated whether this higher prevalence in women is caused by hormonal factors or whether it is simply due to differences in body size between men and women and thereby differences in the ability to dispose of the fixed amount of 75 g glucose during an oral glucose tolerance test (OGTT). Therefore, the aim of this study was to test the hypothesis that men and women of same height and waist circumference have similar 2-hour post OGTT plasma glucose (2hPG) levels.
Materials and methods: We used baseline data from 3,007 non-diabetic men and 3,104 non-diabetic women of the Inter99 study with available information on height, waist circumference (WC), and 2hPG levels. Univariate analyses between 2hPG and height and between 2hPG and WC were performed stratified by gender. Furthermore, a linear regression model with 2hPG level as response variable and height, WC, and gender as explanatory variables was tested.
Results: The mean ± SD 2hPG levels were 5.99 ± 1.83 for men and 6.17 ± 1.69 for women (p<0.001 for diff.). In both men and women we found associations between 2hPG and WC (p<0.001) and inverse associations between 2hPG and height (p<0.001). When height, WC, and gender were included as explanatory variables, there was no difference in 2hPG levels between men and women (p=0.881), whereas both height and WC were still significantly associated with 2hPG levels (p<0.001). The figure illustrates 2hPG levels for men and women by height and WC strata.
Conclusion: Gender differences in the prevalence of IGT can be explained by differences in body size between men and women. This finding questions the validity of using the same standard 75 g-OGTT for classifying IGT in men and women of different body size.



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137

Prevalence and incidence of atrial fibrillation among patients with and without type 2 diabetes: a confluence of two epidemics?

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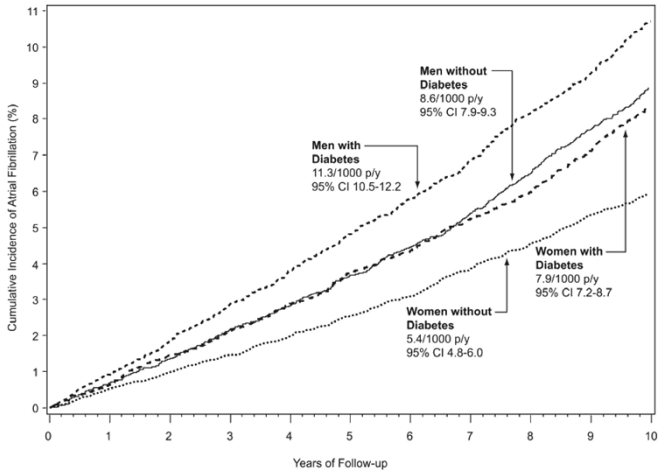
Background and aims: Diabetes is a known risk factor for cardiovascular disease (CVD), but the relationship between diabetes and atrial fibrillation (AF) merits further evaluation. We sought to estimate and compare the prevalence and incidence of AF among patients with and without diabetes.
Materials and methods: From the electronic medical records of Kaiser Permanente Northwest, a group model HMO, we selected 10,213 patients who were members of the diabetes registry as of January 1, 1999, and 7,159 patients who entered the registry by December 31, 2004 (total=17,372). Index date was defined as January 1, 1999 or date of registry entry. We then matched these patients to KPNW members without diabetes on year of birth and sex. All patients were then followed until they died, left the health plan, or until December 31, 2008, whichever came first. We estimated the prevalence of AF at baseline, then followed patients without prevalent AF to estimate and compare the incidence of AF among those with and without diabetes, controlling for age, sex, race, body mass index, systolic blood pressure, cigarette smoking, and comorbidities including ischemic heart disease, stroke, valvular disease, hypertension, and heart failure.
Results: Mean age of the study sample was 58.4±11.5 years and 51.2% were men. The prevalence of AF was significantly greater among patients with diabetes (3.6% vs. 2.5%, p<0.0001). Prevalence increased with age in both groups, but did so more dramatically among diabetes patients. Over a mean follow-up of 7.2±2.8 years, the 16,741 diabetic patients without prevalent AF at baseline developed AF at an age/sex adjusted incidence rate of 9.6/1000 person-years (95% CI 9.0-10.2), compared to 6.9 (6.5-7.4) among the 16,930 non-diabetic patients. Thus, the relative risk of AF was 39% (29-50%) greater among persons with diabetes. Among men, the age-adjusted incidence of AF per 1,000 person-years was 11.3 (10.5-12.2) and 8.6 (7.9-9.3), respectively, for patients with and without diabetes, producing a 32% (19-46%) greater relative risk among men with diabetes. Among women, age-adjusted AF incidence was 7.9 (7.2-8.7) and 5.4 (4.8-6.0), respectively, with a higher relative risk attributable to diabetes of 48% (32-66%). After further controlling for other known risk factors, patients with diabetes were at 18% greater risk of developing AF (hazard ratio 1.18, 95% CI 1.07-1.30). AF risk associated with diabetes remained substantially higher among women (1.23, 1.07-1.43) than men (1.14, 1.01-1.29).

Conclusion: Atrial fibrillation is significantly more prevalent, and develops at a greater rate, among patients with diabetes. Although women are at a lesser absolute risk of AF than men, diabetes imparts a greater relative risk of AF among women than men. A potential mechanistic link between these two important disease conditions, and the differential effects of gender, warrants further investigation.



138

Hypoglycaemia and risk of myocardial infarction in U.S. veterans with diabetes

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Background and aims: Hypoglycemia is a common and serious complication of diabetes treatment. There have been reports that hypoglycemia may increase risk of cardiovascular disease (CVD) and mortality, a concern heightened by recent trial reports of adverse outcomes in patients randomized to intensive glycemic control. These risks are not well understood, however, and further research is needed.
Materials and methods: We conducted case control studies of hypoglycemia among diabetes patients in the U.S. Veterans Health Administration (VA) to assess CVD risks, using the Diabetes Epidemiology Cohorts, a national linked database of all veteran VA diabetes patients in the U.S. Among diabetes patients in 2000-2004 with no history of myocardial infarction (MI), unstable angina, or cardiac surgery, we identified 63,519 cases with first MI and 1,009,746 controls with no MI. Controls were randomly matched to cases at a 12:1 ratio and assigned the index date of the case (hospital admission date for MI). Using diagnosis codes from inpatient and outpatient encounters, we identified hypoglycemic events prior to the index date (excluding the day of admission for MI and the day before to minimize potential ascertainment bias). MI risk was estimated using logistic regression with adjustment for CVD risk factors, other diabetes complications, other medical conditions, medications prescribed, laboratory test results, socio-demographic factors, and health care use. Analyses were stratified by insulin use. We also conducted a case-time-control analysis, comparing hypoglycemic event rates prior to the MI with rates occurring one year earlier, using a similar comparison in the controls to account for increasing age and severity of disease over the year.
Results: Hypoglycemic events in the year before the index date were more common in cases (9.6%) than controls (4.2%) with the greatest difference in the weeks before the index date. After adjusting for potential confounders, the odds ratios and 95% confidence intervals for MI associated with hypoglycemic events in the 2 weeks before the index date were 1.60 (1.41-1.81) for those not using insulin, 1.53 (1.33-1.75) for insulin users, and 1.94 (1.28-2.94) for new insulin users. MI risks were lower for hypoglycemia between 2 weeks and 6 months before the index date and there was no elevated risk for hypoglycemia occurring more than 6 months ago (0.96 (0.90-1.01) for the prior year in insulin users). MI risks were particularly high for hypoglycemia in a recent hospital stay prior to admission for the MI. We found similar elevated risks in the case-time-control analysis suggesting that the results are not explained by unmeasured confounding.
Conclusion: Hypoglycemia occurred more often than expected prior to MI in diabetes patients. It remains to be determined whether hypoglycemia is simply a marker for increased risk of imminent MI or has a direct effect in causing the infarction. Clinicians should be cognizant of these potential risks in managing diabetes patients.
Supported by: CVD Risks Associated with Hypoglycemia - sanofi-aventis

OP 24 Effects of maternal diabetes and childhood obesity in diabetes

139

Reduced renal reserve in non-diabetic adults exposed in utero to maternal type 1 diabetes

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Background and aims: Experimental animal studies support that fetuses exposed to hyperglycemia display impaired nephrogenesis and reduced global angiogenesis. We tested if such a phenomenon could be observed in humans.
Materials and methods: We studied renal microcirculation of non-diabetic Offspring from Type 1 Diabetic Mothers (OT1DM) compared to similar subjects born from Type 1 Diabetic Fathers (OT1DF). As Renal Functional Reserve (RFR) is a surrogate marker of nephron number and renal vasculature, we measured simultaneously Glomerular Filtration Rate (GFR, ^{51}Cr-EDTA clearance), Effective Renal Plasma Flow (ERPF, ^{123}I-Hippurate clearance), Mean Arterial Pressure (MAP, Dynamap®) and calculated Filtration Fraction (FF = GFR / ERPF), and Renal Vascular Resistances (RVR = MAP / ERPF), both at baseline, and during Amino Acid (AA) infusion
Results: The OT1DM (n = 19) and OT1DF (n =18) were similar for age (median 27 (range 18 - 41) vs 26 (18 - 37) years), sex ratio (14 F / 5 M vs 9 F / 9 M), BMI (23.1 ± 3.7 vs 22.6 ± 2.3 Kg/m^2), and birth weight (3288 ± 550 vs 3440 ± 489 g). During AA infusion, GFR rose from 103 (9) to 122 (14) ml/min in OT1DF, vs from 102 (12) to 110 (12) in OT1DM (inter-group comparison p<0.01), ERPF rose from 511 (72) to 591 (90) ml/min in OT1DF vs from 507 (57) to 530 (66) in OT1DM (p<0.01), and MAP varied from 85 (7) to 84 (5) mmHg in OT1DF versus from 89 (6) to 91(8) in OT1DM (p<0.01). RVR decreased less in OT1DM (from 0.18 (0.03) to 0.17 (0.03) mmHg/ml/min) than in OT1DF (from 0.17 (0.03) to 0.15 (0.02); p<0.001). In addition, GFR changes were related to birth weights in OT1DM (r=0.62,p<0.01), not in OT1DF(r=-0.08,NS), while the ranges of birth weights were similar in the 2 groups.
Conclusion: Fetal exposure to maternal diabetes is associated with reduced RFR (as assessed by comparative changes in GFR, ERPF and RVR), which may reflect a reduced number of nephrons and/or reduced renal microvascularisation. Thus, offspring of T1D mothers may be predisposed to glomerular and cardiovascular diseases later in life.
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140

Perinatal outcome is worse in male newborns of women with pregestational diabetes mellitus

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Background: Male sex is a well-known risk factor for unfavorable perinatal outcome that has only occasionally been assessed in diabetic pregnancy .
Aim: To assess perinatal outcome in infants of mothers with pregestational diabetes mellitus in relation to fetal sex.
Methods: Retrospective review using a prospectively collected database including all diabetic pregnancies attended in the Diabetes and Pregnancy Clinic of the Center from 1st January 1981 to 31st December 2006. The analysis was limited to single pregnancies of women with pregestational diabetes mellitus (381 with Type 1 DM and 69 with Type 2 DM). Evaluated maternal characteristics were: anthropometrics, diabetes duration and mean HbA1c in the three trimesters. Outcomes measured: stillbirth, neonatal mortality, perinatal mortality, major malformations, newborns small/large for gestational age, preterm birth, and a composite outcome including all of the former. Statistics: Chi-square test, Student T-test or Mann-Whitney U-test corresponding to qualitative variables, normally distributed quantitative variables and non-normally distributed quantitative variables. Significance was set at a bilateral p <0.05.
Results: Baseline and metabolic characteristics did not differ in mothers of male and female newborns. A consistent trend for worse outcome in male newborns was observed for most variables in both Type 1 and Type 2 DM without reaching statistical significance. Nevertheless the composite outcome was more frequent in male fetuses (56.2 vs 46.1%, p <0.05).
Conclusion: In women with pregestational diabetes mellitus, perinatal outcome is worse in male newborns despite similar maternal characteristics.

141

Anthropometric characteristics at 11 years in children exposed to maternal gestational diabetes mellitus or mild gestational hyperglycaemia in France: DIAGEST 2 study

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Background and aims: Intrauterine exposure to gestational diabetes (GDM) may promote offspring overweight or obesity and cardiometabolic risk. It has been shown that there was a relationship between maternal glucose tolerance during pregnancy and offspring obesity or systolic blood pressure at 3 years. The objective of this study was to determine the anthropometric measurements at 11 years in children exposed to gestational diabetes, mild gestational hyperglycaemia (MGH) compared to children whose mothers had a normal glucose tolerance (C) during pregnancy in a French population (466 GDM, 322 MHG and 221 C).
Materials and methods: Each child whom mother had participated in a previous study on the screening and diagnosis of GDM (DIAGEST study) underwent follow-up evaluations at a median age of 11 years. Measures included BMI, BMI z-score, waist circumference, skinfold thicknesses, documentation of medical history, dietary and physical activity habits. Childhood overweight and obesity were established using age-specific reference ranges.
Results: Five hundred fifteen children were assessed at 11 years of age, so the adherence to follow-up was 49.4%. We examined 257 offspring of GDM mothers, 150 offspring of MGH and 108 offspring of control mothers (49.9%, 29.1% and 21 % of the whole population respectively). According to the IOTF criteria, the rate of overweight and obesity were respectively 22.8% and 8.2% in the whole population. The percentages of fat mass were significantly higher among children born from GDM mothers or MGH mothers compared to children born from control mothers (25.7% vs 26.1% vs 23.7%; p< 0.05 respectively). In univariate analysis, there were a significant increase of the BMI z-score (1.80 vs 1.05, p<0.05), the waist circumference (71.64 cm vs 68.38 cm, p<0.05), the percentages of fat mass (27.15% vs 24.95%, p<0.05) among children born with a macrosomia compared to those without. In multivariate analysis, the only factor related to an increasing BMI z-score (p=0.0012), waist circumference (p=0.011) and percentage of fat mass (p=0.009), is macrosomia at birth in children born from mother with an abnormal glucose tolerance.
Conclusion: This study confirms that there is an association of maternal glucose tolerance during pregnancy with offspring adiposity at age 11 years. This association is dependent of the presence of macrosomia at birth. Exposure to a diabetic environment in utero predisposes to metabolic disturbances later in life. Maternal diabetes is associated with programming of overweight and obesity in offspring.
Supported by: DIAGEST Group's members: NovoNordisk, LifeScan

142

Prevalence and predictors of overweight in offspring of mothers with gestational diabetes

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Background and aims: Recently we have shown, that maternal type 1 diabetes (T1D) is not an independant risk factor for overweight in offspring of type 1 diabetic mothers, but that factors associated with maternal T1D, such as short breastfeeding duration and high birth size, predispose children to overweight during childhood. We now studied the prevalence and predictors of overweight in offspring of mothers with gestational diabetes (GDM) and evaluated the influence of Body mass index (BMI) on the development of insulin resistance.

Materials and methods: Data on child's weight and height were collected at age 2 and 8 years from 331 children born between 1989 and 2000 participating in the prospective German GDM offspring study. All children had a mother with GDM. Overweight was defined as BMI percentile ≥90, adjusted for sex and age. Prevalence of overweight was compared to a prospective cohort of offspring of mothers with T1D (n=912) born between 1989 and 2000 participating at a similar follow-up study (BABYDIAB). Data on birth weight/height, maternal BMI before pregnancy, insulin requirement and smoking during pregnancy were collected at birth, breastfeeding data at age 3 months, 9 months and 2 years. To identify predictors of overweight, a multiple logistic regression analysis was performed. As an indicator for insulin resistance, HOMA-IR was determined on fasting venous blood samples at age 8 years (n=18).
Results: Prevalence of overweight in offspring of mothers with GDM was 17.2% at age 2 years and 20.2% at age 8 years and was increased compared to offspring of mothers with T1D (14.6% at age 2 years and 11.5% at age 8 years; p=0.3 and p=0.04 respectively). Multivariate analysis including birth size, maternal BMI and maternal insulin requirement during pregnancy as covariates indicated that risk for overweight was increased throughout childhood in offspring of mothers with GDM who were born large for gestational age (HR 6.3, 95%CI:0.9-41.9, p=0.06 at 2 years of age and HR 5.2, 95%CI:1.2-23.0, p=0.03 at 8 years of age) and in offspring of mothers with a maternal BMI>30 kg/m² before pregnancy (HR 16.8, 95%CI:1.2-234.8, p=0.04 at age 2 years and HR 14.1 95%CI:1.4-147.3, p=0.03 at age 8 years). Smoking during pregnancy, duration of full breastfeeding and insulin requirement during pregnancy had no effect on weight development in offspring of mothers with GDM. At age 8 years, HOMA-IR was significantly increased in overweight offspring of mothers with GDM compared to normal weight offspring (p=0.003).
Conclusion: Prevalence of overweight during childhood, which is associated with higher risk of insulin resistance, is increased in offspring of mothers with GDM compared to offspring of mothers with T1D indicating that maternal diabetes type affects overweight risk. The finding that overweight risk is predicted by maternal obesity and high birth size suggests that both, genetic predisposition as well as exposure to maternal diabetic environment contribute to childhood growth in offspring of mothers with GDM.
Supported by: JDRF

143

Could insulin sensitivity predict growth restricted infants?
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Background and aims: The "Barker Hypothesis" suggests that low birth weight predicts future risk of developing obesity, cardiovascular disease and type 2 diabetes. Identification of the causes of fetal growth restriction (FGR) is critical for preventive and management strategies. It has been observed that post-glucose-load plasma insulin and glucose levels are lower in women with FGR fetuses than in women with a fetus with a normal growth. This seems to indicate that maternal carbohydrate metabolism might be involved in the pathogenesis of FGR. The aim of our study was to evaluate, in a large number of normotensive pregnant women with normal glucose tolerance, the effect of insulin sensitivity (evaluated with fating index QUICKI and dynamic index OGIS)and maternal anthropometric and demographic characteristics on fetal growth.
Materials and methods: 1814 Caucasian pregnant women with a pre-pregnancy BMI between 19 and 25, and singleton pregnancy were tested with a 75g 2-hour glucose load in the period between 24 and 28 weeks of gestation. FGR was defined by a birth weight below the 5th percentile for gestational age and developed in 99 (5.5%) pregnant women..
Results: Women with FGR showed lower weight gain (7.1±vs12.8±3.2 p<0.001), lower FPI (4.2±3.9vs 6.7±6.6;p0.007), AUC Glu (p<0.000) and AUC ins(p<0.000) higher QUICKI(0.46±0.14vs 0.39±0.06;p<0.000)and OGIS (469±71 vs444±65;p<0.0002) with respect to women with normal weight babies. Birth weight percentiles showed a positive correlation with weight gain (r=0.56, p<.01) and a negative correlation with IS_{QUICKI} (r=-65, p<.006). In multiple logistic regression analysis, IS_{QUICKI} and weight gain were shown to be statistically significant independent predictors of FGR. The adjusted odds ratios for FGR were 2.1 (95% CI 1.2-2.5) for each 0.05 IS_{QUICKI} increment in $IS_{QUICKI,}$ and 1.2 (95% CI 1.1-1.9) for each 500 g decrement in weight gain.
Conclusion: In this study we demonstrated that women with FGR pregnancies have higher insulin sensitivity than women with normal fetal growth. So we could suggest that increased insulin sensitivity leads to a reduction in metabolic substrates for fetal growth.

144

Factors determining childhood adiposity and cardiometabolic risk over a 5 year period - the Da Qing children study
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Background and aims: Childhood adiposity is increasingly recognized as a significant predictor of cardiometabolic risks in later life. There are very few longitudinal studies of childhood adiposity, especially in Asian populations. We investigated various factors, including TV viewing time, birth weight, fasting insulin, glucose and triglycerides, and blood pressure, and their association with change in childhood adiposity over a 5 year period.
Materials and methods: 424 five-year-old Chinese children enrolling into four public elementary schools in Da Qing city, China, were recruited for this cohort study in 1998. Birth weight, TV viewing time, blood pressure, anthropometric measurements, fasting insulin, glucose and triglycerides levels were measured. Percentage ideal weight for height (WFH) was used as a measure of adiposity. All children were reassessed 5 years later. The children were ranked and classified into tertiles based on WFH at 5 years, or change in WFH from 5 to 10 years old. General linear model for analysis of variance was used to compare variables of interest in each tertile group. Multiple stepwise regression analysis was used to assess the association of TV time, birth weight, WFH at 5 years and WFH at 10 years. The study was approved by the Ethics Committee of the China-Japan Friendship Hospital and the Public Health Ministry of China.
Results: Significant associations were found in TV viewing time, birth weight and fasting insulin**.** (1) At 5 years of age, total TV viewing time was significantly longer in boys with higher WFH at 5 years (WFH5)(p<0.05), but not in girls(p=0.09). Change in WFH was significantly correlated with TV viewing time in both boys (top tertile 5.31±0.28 *vs.* lowest tertile 4.18±0.28 hrs/week, p<0.01) and girls (top tertile 5.31±0.29 *vs.* lowest tertile 4.41±0.29 hrs/week, p<0.05). An increase of 5 hours/week of TV viewing time led to 1.1 kg/m² increase in BMI over the 5 years follow-up. (2) Birth weight was significant greater in the top tertile of WFH5 in both boys (p<0.01) and girls (p<0.05), but not with change in WFH 5 years later. (3) Fasting insulin (FI) and HOMA-IR were significantly higher in the top tertile of WFH5 in boys (p<0.01) and girls (p<0.05). In addition, FI, HOMA-IR, systolic and diastolic blood pressures, and triglycerides remained significantly higher in the top tertile of change in WFH 5 years later.
Conclusion: Although both TV viewing time and birth weight affected adiposity during early childhood, TV viewing time may be the stronger determinant during later childhood and with change in WFH over 5 years. Greater change in WFH (from 5-10 years) may confer risk of development of cardiometabolic disease. Thus, reducing TV viewing time in young children may help to prevent the development of obesity and decrease risk of cardiometabolic disease.

OP 25 Cognitive function and type 2 diabetes mellitus

145

A longitudinal study on cognition in type 2 diabetes: no accelerated cognitive decline

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Background and aims: Type 2 diabetes mellitus (T2DM) is associated with an increased risk of cognitive dysfunction. In a previous cross-sectional study we showed that patients with T2DM (mean duration: 8 years) performed worse than controls on the domains memory, information processing speed and attention and executive functioning. Patients and controls were reexamined after a 4-year interval.
Materials and methods: Of the 178 participants at baseline (122 T2DM, 56 controls) 106 (60%; 68 T2DM, 38 controls) participated in the follow-up examination. Cognitive status of individuals that did not attend follow-up as ascertained with the Telephone Interview for Cognitive Status was slightly lower than in those that did attend (p=0.07). Performance on 11 neuropsychological tasks, covering 5 cognitive domains, was expressed in standardized z-scores. ANOVA for repeated measures was used to examine the effect of Group, Time and the Group×Time interaction.
Results: The T2DM and control groups were similar in age, estimated IQ and sex distribution. There was a significant effect of Time on the domains abstract reasoning (mean difference in z-scores between T1 and T2 -0.16, p=0.03) and attention and executive functions (-0.29, p<0.001). Significant between-group effects were found on information-processing speed (mean difference in z-scores for T2DM compared to controls: -0.37, p<0.05) and attention and executive functions (-0.25, p=0.04), and a trend in the same direction on memory (-0.16, p=0.07). There were no significant Group×Time interactions.
Conclusion: In this sample of non-demented patients with T2DM we found modest decrements in cognitive functioning compared to controls. Between-group differences at follow-up were similar to those found in the baseline examination. There was no accelerated decline in the T2DM group. Apparently, cognitive decrements in T2DM develop slowly, over a much longer time frame than the 4 years sampled in the current study.
Supported by: the Dutch Diabetes Research Foundation

146

Risks of cardiovascular disease and severe hypoglycaemia according to cognitive function in people with type 2 diabetes in ADVANCE

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Background and aims: The relationship between cognitive dysfunction, cardiovascular (CV) disease and premature death is not well established in people with type 2 diabetes. We assessed the effects of cognitive dysfunction in 11,140 patients with type 2 diabetes who participated in the Action in Diabetes and Vascular disease: Preterax and Diamicron Modified Release Controlled Evaluation (ADVANCE) trial. We also tested whether cognitive function at baseline influenced the beneficial effects of blood pressure lowering and glycaemic control in the trial.
Materials and methods: Cognitive function was assessed using the Mini Mental State Examination at baseline, and defined by scores 28-30 ('normal', n=8689), 24-27 ('mild dysfunction', n=2231), and <24 ('severe dysfunction', n=212). Risks of CV events (non-fatal myocardial infarction, non-fatal stroke and CV death), all cause mortality, CV death, severe hypoglycaemia and interactions with treatment, were assessed using Cox proportional hazards analysis.
Results: Compared with normal cognitive function, both mild and severe cognitive dysfunction at baseline significantly increased the multiple-adjusted risks of major CV events (HR 1.27 [95% confidence interval, 1.11-1.46] and 1.42 [1.02-2.00]; both p<0.05), CV death (1.41 [1.16-1.71] and 1.57 [1.00-2.48]; both p≤0.05) and all-cause mortality (1.34 [1.16-1.55] and 1.52 [1.07-2.15]; both p<0.02). Severe, but not mild, cognitive dysfunction increased the risk of severe hypoglycaemia (HR 2.20 [1.19-4.06]; p=0.012). There was no evidence of heterogeneity of randomised treatment effects on these outcomes in subgroups defined according to baseline cognitive function.
Conclusion: Cognitive dysfunction is an independent predictor of clinical outcomes in people with type 2 diabetes but does not alter the effects of more intensive blood pressure or glucose lowering treatment on the risks of major CV events or death.
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147

Correlates of cognitive function measured in frail elderly patients with diabetes mellitus

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Objective: Among elderly populations, impaired cognition imposes a heavy burden in terms of dementia-related quality of life, morbidity and mortality. Preventing or delaying cognitive decline would have considerable clinical and socioeconomic benefits. In this study, we investigated the relationship between cognitive function, glycaemic control and insulin treatment among frail elderly patients with diabetes mellitus.
Methods: Acute Geriatric Management and Remobilisation Units (AG/R) are specialized inpatient hospital wards introduced in Austria in recent years. AG/R provide interdisciplinary rehabilitation and mobilisation services for frail elderly patients. In AG/R Units Geriatric Comprehensive Assessment (GCA) is routinely used to assess the functional status of frail elderly patients and to guide the geriatric therapeutic process. In our analysis, we performed a record linkage between GCA data and a diabetes quality improvement dataset (FQSD). Multiple linear regression models were fitted in order to investigate correlations between cognitive function, measured by Mini Mental State Exam (MMS), and several potential correlates of cognitive function including age, sex, BMI and glycaemic control.
Results: Between November 2002 and November 2006, 316 consecutive patients with diabetes mellitus were admitted to AG/R unit at the Hoergas hospital. Mean age was 77 ± 9 years, with mean diabetes duration of 9 ± 10 years. 69% of patients were female, mean BMI was 26 ± 5 kg/m^2, mean HbA1c was 6.7 ± 1.2 %, and mean fasting plasma glucose (FPG) was 147 ± 54 mg/dl. Insulin was used by 26%. Median MMS score was 26 (IQR 23 - 28) points. In linear models, a relationship between MMS and age, BMI (non-linear) and stroke was established. Gender and insulin treatment did not show a significant correlation with MMS scores and were eliminated from the model. Impact of glyceamic control was explored by adding gylceamic control to the previous model: FPG and HbA1c were added in a stepwise fashion as predictors. An increase in 1 mg/dl of FPG resulted in a predicted decrease of 0.01 MMS score points (p=0.008). An increase of 1% HbA1c resulted in predicted MMS score decrease of 0.33 points (p=0.08). Further results are given in figure 1.
Discussion: In our sample, cognitive function was negatively associated with older age and stroke, and positively with moderate obesity. Inferior glyceamic control was associated with impaired cognitive function with FPG being a better predictor than HbA1c. Prospective controlled studies are needed in order to determine if the decline in cognitive function can be reversed, arrested or delayed with tight glyceamic control.

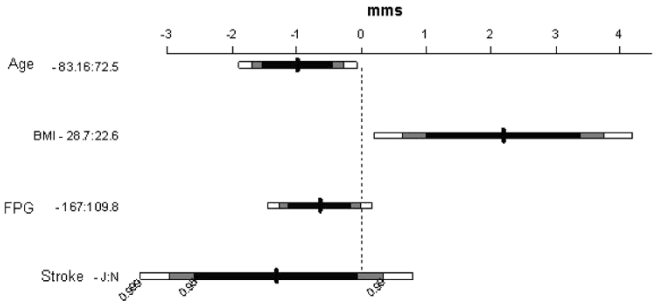

Figure 1: Impact of change of independent predictors from lower to upper quartile on predicted MMS scores. Black, grey and white bars represent 95%, 99% and 99.9% confidence intervals, respectively.
Supported by: Novo Nordisk Pharma Austria

148

Rosiglitazone and cognitive stability in older persons with type 2 diabetes and mild cognitive impairment

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Background and aims: Type 2 diabetes mellitus (T2DM) has been consistently associated with a higher risk of cognitive decline, especially Mild Cognitive Impairment (MCI). Such findings suggest that T2DM can predict cognitive impairment through mechanisms affecting the amyloid accumulation or indirect mechanisms, namely cerebrovascular disease. Considering that insulin resistance (IR) is a critical component in the causal pathway to cognitive impairment, the objective of our study was to test whether an improvement in IR could explain cognitive performance variations in older person with MCI and T2DM that had recently began anti-diabetic oral or diet treatment over a 9 month observation period.
Materials and methods: Ninety-seven older persons (mean age: 76 ± 6 yrs) from a group of approximately two hundred individuals with MCI and T2DM in poor metabolic control that recently (less than 2 months) began anti-diabetic oral treatment with: metformin (1000mg/day) (n=30), or metformin (500mg/day) + rosiglitazone (4mg/day) (n=32) or in diet therapy (n=35) agreed to participate in study protocol. Exclusion criteria included: dementia, cardiovascular disease, severe hypertension, stroke, heart failure, MCI>6 months, or severe depression. All participants underwent a neuropsychological battery that consisted of the Mini Mental State Examination (MMSE), Rey Verbal Auditory Learning total recall (RAVLT), Trail Making Tests (TMTA, TMTB, DIFFBA) which was performed at baseline and every 12 weeks for 9 months along with biochemical and clinical testing (including electrocardiograms). Echocardiography and myocardial perfusion scintigraphy tests were performed in all participants at baseline and at the last follow-up.
Results: Baseline data: There were not any significant differences found between groups at baseline in any clinical or neuropsychological parameters. The mean values of the following parameters were found in the entire population at baseline: HbA_{1c}= 7.5 ± 0.5 %, FPG = 8.6 ± 1.3 mmol/l, FPI=148 ± 74 pmol/l, MMSE=24.9 ± 2.4, TMTA=61.6 ± 42.0, TMTB=162.8 ± 78.7, DIFFBA=101.2 ± 58.1, RAVLT=24.3 ± 2.1. Follow-up data. No participant had experienced any cardiovascular event during the study period. ANOVA models were created to test metabolic control parameters (fasting plasma insulin (FPI), fasting plasma glucose (FPG), HbA_{1c}) within and between groups over time. Metabolic control parameters (HbA_{1c}, FPG, FPI) significantly improved in the metformin and the metformin+rosiglitazone groups only (p for trend<0.05 in both groups). ANCOVA repeated models were performed to test cognitive performance variability independently of common confounders over time and showed that the metformin+rosiglitazone group maintained cognitive stability on all cognitive tests, the diet controlled group maintained stability on the MMSE test only and showed a decline in TMT-B (p for trend=0.024), executive efficiency (DIFFBA) (p for trend= 0.026) and RAVLT memory test (p for trend=0.011), while the metformin group maintained stability on MMSE, TMTs, but showed a decline in the RAVLT (p for trend=0.011). Using linear mixed effects models, the decline in FPI levels was independently associated with cognitive stability on the RAVLT memory test for the metformin + rosiglitazone group only (β=-3.333; p<0.001).
Conclusion: These findings suggest that rosiglitazone, may protect against cognitive decline in older persons with T2DM and MCI.

149

MRI correlates of cognitive decline in patients with type 2 diabetes

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Background and aims: Type 2 diabetes (DM2) is associated with an increased risk of cognitive dysfunction and dementia. In a previous cross-sectional study we demonstrated cognitive decrements and brain abnormalities in DM2 patients compared to controls. Patients and controls were re-examined after 4 years to study changes over time. The present study focuses on brain MRI abnormalities underlying cognitive decline in patients with DM2.
Materials and methods: Cognitive functioning was assessed twice in 54 DM2 patients without dementia, with a 4-year interval. 1.5T MRI scans were obtained at both time-points. Automated volumetric measurements of sub-cortical structures, cortical gray-matter, lateral ventricles, white-matter lesions and cerebrospinal fluid volume were performed and expressed as a percentage of intracranial volume. Cognitive assessment consisted of 11 neuropsychological tasks, covering 5 cognitive domains. Only the 3 cognitive domains which showed a group difference at baseline were used for the present analyses (i.e. memory, information processing speed, attention and executive functioning). A regression based index, using the control group as a reference, was calculated to assess changes in cognitive test performance over time, adjusted for age, estimated IQ and sex. Changes in brain volume in DM2 patients with the 15% greatest decline in cognitive performance on any of the three domains (n=22) were compared to the other DM2 patients (n=32).
Results: There were no significant differences in any of the baseline parameters between patients that attended (n=54) and patients that did not attend follow up (n=44). DM2 patients within the 15% of greatest decline on any of the three domains showed a significant increase in white matter lesion volume (mean difference±S.E.: 0.12%±0.05; p<0.05) and ventricular volume (0.21%±0.06; p=0.001) over the 4-year period compared to DM2 patients without this reduction in cognitive performance. The groups did not differ on changes in sub-cortical structures (0.01%±0.20) or cortical gray-matter volumes (-0.34%±0.29).
Conclusion: These results indicate that white matter lesions and subcortical atrophy in DM2 may underlie the cognitive decline observed in a subgroup of DM2 patients.
Supported by: the Dutch Diabetes Research Foundation

150

The lifestyle intervention reversed cognitive function in aged people with diabetes mellitus, two year follow-up

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Background and aims: Type 2 diabetes is associated with poor performance on various cognitive tests, especially with aging. Cognitive impairment, with or without features of overt dementia, is a major cause of the disease burden in an aging population. However, regarding decreased cognitive function in elderly people with DM; it remains unclear whether the cognitive decline in elderly DM people is reversible. To clarify the reversibility of cognitive decline in elderly people with type 2 diabetes.
Materials and methods: In August 2006, we recruited 129 people aged older than 75 years who participated in free health screening offered by the Health Care Center of Tosa town in Japan. Written informed consent was obtained from participants. Before and after two years of lifestyle intervention, 55 people older than 75 years with DM or IGT defined as 75g OGTT and 74 control people were evaluated for cognitive function including MMSE, HDS-R and Koh's block design test.
Results: The DM group had significantly lower MMSE and HDSR scores in baseline than that of the NGT group (NGT vs. IGT vs. DM = 27.1±0.7 vs. 26.1±1.0 vs. 24.5±1.4 points, p<0.01, 26.7±0.8 vs. 26.4±1.1 vs. 23.8±1.6

points, $p<0.01$). The quantity of the MMSE and HDS-R score change between baseline and follow up was significantly larger in the DM group than in the control group (NGT vs. IGT vs. DM = -0.0±0.6 vs. -0.0±0.9 vs. 1.9±1.2 points, $p<0.01$, 0.0±0.9 vs. -0.1±0.9 vs. 1.7±1.6 points, $p<0.01$). The delayed recall function was lower in the IGT and DM group than in the NGT group at baseline, but no difference was found among the three groups after intervention (Baseline: NGT vs. IGT vs. DM = 2.6±0.3 vs. 2.1±0.3 vs. 1.6±0.3 points, $p<0.05$; After: NGT vs. IGT vs. DM = 2.6 ±0.2 vs. 2.3±0.2 vs. 2.2±0.5 points). Koh's block design test did not change before and after intervention. Stepwise regression analyses showed that the quantity of cognitive function changes independently in relation to DM group membership and HbA1c in baseline (standard $\beta = 0.3$, $p<0.05$, standard $\beta = 0.2$, $p<0.01$, respectively).
Conclusion: The lifestyle intervention involving glycemic control has a beneficial effect on selective aspects of cognitive decline in elderly people with type 2 diabetes mellitus.

OP 26 Mechanisms and markers of cardiovascular disease

151

High liver fat content is associated with impaired myocardial perfusion and metabolism in patients with uncomplicated type 2 diabetes
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Background and aims: Cardiovascular disease (CVD) is the leading cause of mortality in type 2 diabetes (T2DM). Central obesity and hepatic steatosis, both hallmark abnormalities in T2DM, have been related to increased CVD. The aim of the present study was to investigate whether T2DM patients without clinical ischemic heart disease, as assessed by dobutamine-stress echocardiography, with high versus low liver fat content differ with respect to myocardial function and metabolism.
Materials and methods: In sixty-one T2DM patients (mean age±SE 56.8±0.7 yr, BMI 28.7±0.5 kg/m^{-2}, HbA_{1c} 7.1±0.1%) myocardial perfusion and substrate metabolism were assessed by positron-emission tomography using [^{15}O]water, [^{11}C]palmitate under fasting conditions and [^{18}F]-2-fluoro-2-deoxy-D-glucose under euglycaemic hyperinsulinaemic conditions. Additionally whole-body insulin sensitivity (M/I) was determined. Moreover, myocardial left ventricular (LV) systolic and diastolic function and dimensions were measured by magnetic resonance (MR) imaging and in a subgroup (n=25) the PCr/ATP ratio was assessed by [^{31}P]-MR spectroscopy. Patients were divided according to the liver median in a T2DM-low (n=30, liver fat ≤ 8.6%) versus T2DM-high (n=31, liver fat > 8.6%) liver fat group.
Results: Median (IQR) liver fat in T2DM-low was 3.1 (1.5-5.4) % and 20.3 (12.9-29.2) % in T2DM-high patients. Patients in both groups were well matched for age, HbA_{1c} and hemodynamics, but T2DM-high versus T2DM-low had a higher BMI (30.2±0.6 versus 27.1±0.6 kg/m^2, P<0.001). T2DM-high relative to T2DM-low showed decreased myocardial perfusion (P<0.016) and glucose uptake (P=0.002), PCr/ATP ratio (P=0.011) and M/I (P<0.001), whereas myocardial fatty-acid metabolism and LV diastolic and systolic function and dimensions were similar. Significant associations were found between hepatic fat and myocardial glucose uptake (Spearmans' rho r=-0.396, p=0.015), PCr/ATP ratio (r=-0.467, p=0.019) and M/I (r=-0.634, p<0.001). Myocardial glucose uptake was correlated with M/I (r=0.517, p<0.001) and PCr/ATP (Pearsons r=0.481, p=0.015). No correlations were found between myocardial metabolism and LV function.
Conclusion: High liver fat content in T2DM patients is associated with impaired whole-body insulin sensitivity which is paralleled by altered myocardial perfusion, substrate and high-energy phosphate metabolism.
Supported by: Eli Lilly, the Netherlands

152

Which cardiac fat depot is the best cardiometabolic risk marker?
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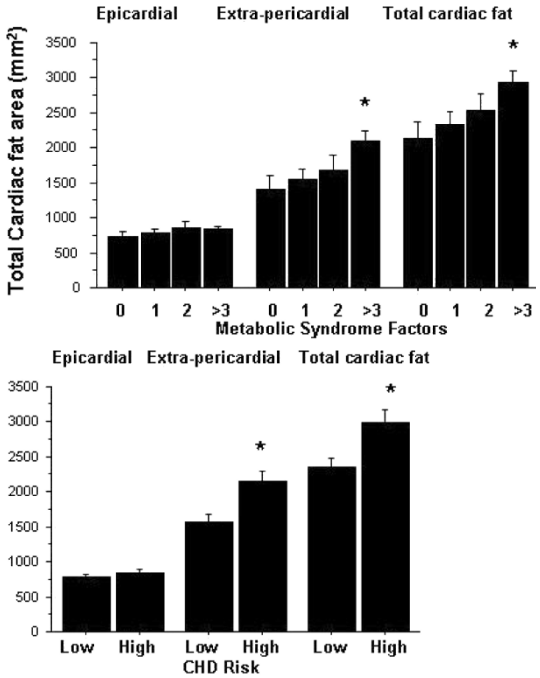
Background and aims: Fat around the heart (mediastinal fat) accumulates in both the epicardial (EPI) and extrapericardial (Ex-PER) or intrathoracic area. Most of published work focused on EPI, now recognized as a cardiometabolic risk factor, and only few studies measured Ex-PER, despite most of the fat is accumulated in this region. Thus, we investigated the associations between EPI, Ex-PER and mediastinal fat with metabolic dysfunction and coronary heart disease (CHD) risk in healthy subjects.
Materials and methods: We studied 113 subjects (94M/19F; age: 18-74 years, BMI: 15-40 kg/m^2) and measured total cardiac, Ex-PER, EPI, visceral (VF), subcutaneous (SC) fat depots, as well as cardiac function, by MRI. We also measured blood pressure, insulin sensitivity evaluated by QUICKI and OGIS, triglycerides (TG), cholesterol (total and HLD), glucose (FPG), insulin concentrations and 10-year CHD risk by Framingham score.

Results: Most of cardiac fat was constituted of Ex-PER (~77%). However, in this group we observed a wide range of variability for both cardiac fat depots (EPI ranged 172-2008 mm^2 and Ex-PER ranged 100-5056 mm^2). Both EPI and Ex-PER correlated significantly with BMI (r=0.34 and r=0.42), waist circumference (r=0.41 and r=0.53), visceral fat (r=0.40 and r=0.50), subcutaneous fat (r=0.40 and r=0.27). Only Ex-PER correlated significantly with mean blood pressure (r=0.29), insulin sensitivity (r=-0.22) and concentration of triglycerides (r=0.27). Only mediastinal fat and Ex-PER, but not EPI, could discriminate between subjects with more than 3 metabolic syndrome factors (ie, increased waist circumference, TG, FPG, blood pressure and/or low HDL) or medium to high CHD risk score (figure). However, no correlation was found with cardiac function parameters (ejection fraction or left ventricular volume and mass). After correcting for BMI and waist, CHD risk remained associated with Ex-PER (partial r=0.24, p=0.003) or mediastinal fat (partial r=0.24, p=0.004) but not EPI (partial r=0.08, p=ns).
Conclusion: Increased Ex-PER or mediastinal fat, but not EPI, at least when measured by MRI, are better markers of altered metabolic profile and CHD risk.



153

Effects of acute manipulation of serum NEFA concentrations on left ventricular (LV) energy metabolism in patients with heart failure
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Background and aims: This study was designed to test the hypothesis whether acute changes in circulating energy substrates may be detrimental for the LV energy homeostasis in humans with heart failure.
Materials and methods: Cardiac function and the relative content of LV phosphocreatine (PCr) and ATP were assessed non-invasively using in vivo MR-imaging (MRI) and 31P MR-spectroscopy (MRS) in 10 individuals with chronic heart failure (age: 74±9 ys, BMI: 25.6±1.9 kg/m^2, NYHA class: 2 (1-3), ejection fraction (EF): 35±7%, fasting glucose: 96±11 mg/dl; fasting insulin: 6.3±3.0 μU/ml) in two experimental conditions 7 days apart. In Study 1 MRI and 31P-MRS data were acquired before and 3 to 4 hours after the administration of a i.v. bolus + continuous heparin infusion designed to achieve a serum NEFA concentration of 1.2-1.5 mM. In Study 2 the same procedure was performed before and 3 to 4 hours after the oral administration of Acipimox designed to achieve a serum NEFA concentration of 0.1-0.2 mM. Acquisitions were acquired in the fasting and resting state.
Results: During the studies serum glucose and insulin concentration did not significantly change; serum NEFA increased from 0.65±0.24 to 1.21±0.47 mM during Study 1 and dropped from 0.68±0.25 to 0.15±0.06 mM during Study 2. The PCr/ATP ratio did not change during Study 1 (from 1.70±0.22 to 1.75±0.34; P=0.49) meanwhile during Study 2 it dropped from 1.81±0.23 to 1.53±0.32 (P=0.016) and the percent change was also different (102±12% vs. 85±15%; P=0.009). In parallel the LVEF did not change during Study 1 (from 35±13% to 34±13%; P=0.21) meanwhile it dropped from 36±12% to 33±11% (P=0.03) during Study 2
Conclusion: In individuals with chronic heart failure acute reduction of serum NEFA concentration impaired LV energy metabolism meanwhile a two-fold increment did not induce any detectable change. These data demonstrate that deprivation of energy substrates in the acute setting may be detrimental for cardiac metabolism
Supported by: an EFSD Clinical Research Grant

154

Role of HDL glycation in counteracting the inhibitory effect of oxidized LDL on endothelium-dependent vasorelaxation
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Aims/hypothesis: In healthy normolipidaemic and normoglycaemic control subjects, HDL are able to reverse the oxidized LDL-induced inhibition of vasodilation. We have previously shown that in type 1 diabetic patients, HDL do not protect against inhibition of endothelium dependant vasorelaxation induced by oxidized LDL. This defect was not explained by abnormalities in HDL composition, size or paraoxonase activity. The aim of this study was to analyse the role of glycation of HDL on this vasodilation effect.
Methods: Blood samples were collected from healthy patients. Extracted HDL particles, separated by ultracentrifugation, were glycated in vitro using a procedure with a highly concentrated glucose solution, mimicking the "in vivo" conditions of glycation in type 1 diabetes. Vasoreactivity was evaluated by the relaxation response to acetylcholine of rabbits aorta rings pre-contracted with noradrenaline, before and after two hours incubation with or without different lipoprotein fractions (Krebs'buffer; ox-LDL; normal HDL; normal HDL + ox-LDL; glycated HDL; glycated HDL + ox-LDL).
Results: Lipid composition of normal and glycated HDL was similar. The difference between the two kinds of HDL was glycation. Indeed the mean of the ratio Fructosamine/ApoA1 was 17.58 μmole /g of protein for normal HDL and 48.67 μmole /g of protein for glycated HDL. Oxidized LDL inhibited endothelium vasodilation (maximal relaxation (Emax) = 53.34 ± 27.06 vs 98.67 ± 10.16 % for incubation in Krebs'buffer, p<0.005). Normal HDL were able to counteract the oxidized LDL-induced inhibition of vasorelaxation (Emax = 78.75 ± 19.72 vs 53.34 ± 27.06 %, p=0.003), whereas glycated HDL had no effect (Emax = 46.64 ± 21.72 vs 53.34 ± 27.06 %, p=0.35).
Conclusion: Our data indicate that glycation of HDL is responsible for the inability of HDL particles to counteract the oxidized LDL-induced inhibition of endothelium-dependant vasorelaxation. Glycation of HDL is likely to be one important factor explaining the absence of vasodilation effect of HDL particles in patients with diabetes.

155

Higher plasma sRAGE levels are associated with incident cardiovascular morbidity and mortality as well as all-cause mortality in type 1 diabetes: a 12-yr follow-up study
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Background and aims: Plasma soluble receptor for advanced glycation end-products (sRAGE) is a putative indicator of RAGE expression, which is associated with vascular complications. Studies so far have shown contradictory results and whether sRAGE is associated with incident cardiovascular disease (CVD) and mortality is unknown. We have therefore addressed this question in a prospective cohort study of individuals with type 1 diabetes (T1D) and the extent to which any such association could be explained by renal dysfunction (estimated GFR, eGFR).

Materials and methods: We studied 358 individuals (187 with diabetic nephropathy and 171 with persistent normoalbuminuria, 216 men, mean age at baseline of 41±10 yrs) who were followed for a median period of 12.3 (7.1-12.5) years. Plasma sRAGE was measured at baseline by means of ELISA; eGFR was estimated by the MDRD equation; data on non-fatal CVD events were obtained from patient and hospital files, and data on mortality were retrieved from the Danish National Register. We used multiple Cox regression analyses, first adjusted for age, sex, duration of diabetes and HbA1c, and further for other CVD risk factors (i.e. total cholesterol, systolic BP and smoking, and also eGFR) to investigate the association between sRAGE and the incidence of fatal and non-fatal CVD and all-cause mortality. Results are expressed by HR and respective 95% CI.
Results: During the follow-up 100 (27.9%) patients suffered a fatal or non-fatal CVD event and, considering all causes, 91 (25.4%) died. The incidence of fatal and non-fatal CVD and of all-cause mortality increased with increasing levels of sRAGE (Figs. 1A and 1B): HR=1.50 (95%CI: 1.27 to 1.77, p<0.001) and HR=1.46 (1.25 to 1.70, p<0.001) per 1 SD increase in plasma sRAGE, respectively. These associations were attenuated after adjustments for CVD risk factors [HR=1.30 (1.09 to 1.54), p=0.003 for fatal and non-fatal CVD events, and HR=1.30 (1.10 to 1.52), p=0.002 for all-cause mortality], but were further attenuated after adjustment for eGFR [HR=1.16 (0.96 to 1.40), p=0.125 for fatal and non-fatal CVD events and HR=1.18 (0.99 to 1.41), p=0.073 for all-cause mortality].
Conclusion: Higher levels of sRAGE are associated with incident fatal and non-fatal CVD and all-cause mortality in T1D. Although we cannot exclude the possibility that eGFR is a confounder in the examined association, our findings suggest that renal dysfunction could mediate, at least in part, the association between plasma sRAGE and the high risk of CVD in T1D.

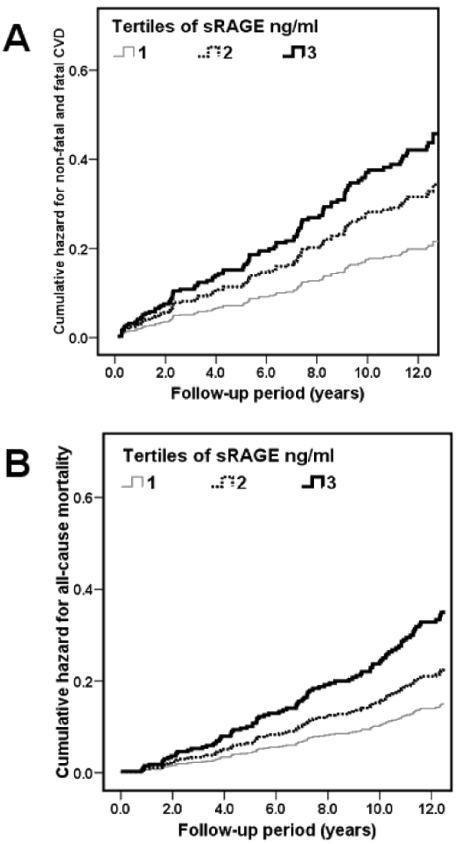

Figure 1: Cummulative hazard for non-fatal and fatal cardiovascular events (A) and all-causemortality (B) across tertiles of sRAGE. Data are adjusted for age, sex, duration of diabetes and HbA1c.

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156

Type 2 diabetes significantly modulates the impact of TCF7L2 rs7903146 variant on the risk of coronary atherosclerosis

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Background and aims: Variations in the transcription factor 7-like 2 (TCF7L2) gene, particularly rs7903146, increase the risk of type 2 diabetes (T2DM). Coronary artery disease (CAD) is the most frequent cause of death in T2DM patients, and CAD shares common risk factors with T2DM. Potential associations between TCF7L2 variant rs7903146 and coronary atherosclerosis are unknown.
Material and methods: We addressed the association between rs7903146 and CAD in a large cohort of 1595 consecutive Caucasian patients undergoing coronary angiography for the evaluation of stable CAD. An additive model of inheritance was used; significant CAD was diagnosed in the presence of coronary stenoses ≥50%.
Results: The prevalence of T2DM significantly increased from homozygous carriers of the frequent allele over heterozygous subjects to those who were homozygous for the rare allele (20.3%, 24.9%, and 31.3%; p_{trend}=0.001). In the total study cohort, variant rs7903146 was significantly associated with the presence of significant CAD (adjusted odds ratio (OR) 1.27 [1.06-1.51]; p=0.008). Importantly, subgroup analyses with respect to the presence of T2DM showed a strong and significant association between variant rs7903146 and significant CAD in T2DM patients (n=373; OR=1.84 [1.27-2.68]; p=0.001), whereas in non-diabetic subjects (n = 1222), variant rs7903146 was not associated with significant CAD (OR=1.08 [0.88-1.32]; p=0.446). An interaction term T2DM x rs7903146 was significant (p=0.004), indicating that this variant had a significantly stronger impact on CAD in patients with T2DM than in non-diabetic individuals.
Conclusion: We conclude that T2DM significantly modulates the impact of TCF7L2 rs7903146 variant on angiographically characterized coronary atherosclerosis.

OP 27 The secretory machinery

157

Defective insulin crystallisation in beta cells in the absence of ZnT8

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Background and aims: In beta cells, zinc co-crystallises with insulin in dense-core secretory granules, but the exact role of zinc in insulin biosynthesis, storage and secretion is unknown. Polymorphism in the ZnT8 gene confers susceptibility to T2D, and autoantibodies to ZnT8 protein are observed in T1D. The aim of this study was to assess the role of ZnT8 in beta cells using mice that are deficient in ZnT8 expression (*ZnT8*$^{-/-}$ mice).
Materials and methods: *ZnT8*$^{-/-}$ mice have a deletion of the promotor region and first exon. ZnT8 expression was assessed by RT-PCR, Western blots, and immunohistochemistry. Beta cell ultra-structure was investigated by transmission electron microscopy. Glucose tolerance was measured at age 6, 12, 25 and 52 weeks and after 10 weeks on high fat diet. Insulin processing was analysed via ^{35}S pulse chase experiments and *in situ* mass spectral tissue profiling of pancreatic islets. Insulin release from isolated islets was measured in perifusion experiments, and zinc exocytosis from islet cell clusters was monitored by total internal fluorescence microscopy.
Results: *ZnT8*$^{-/-}$ mice were completely negative for the expression of islet ZnT8 mRNA and protein. Absence of ZnT8 expression caused loss of zinc release upon stimulation of exocytosis, indicating that ZnT8 is the only zinc transporter responsible for efflux of zinc to the secretory granules. Also dense-core insulin granules were absent in *ZnT8*$^{-/-}$ beta cells (92.2 ± 6.6 vs 7.0 ± 2.7 %, p<0.0001); instead, immature, pale insulin "progranules" were detected, which were larger (433±83 vs 375±84 nm, p<0.001) than insulin granules in control cells. This phenotypic change was associated with loss of *in situ* and *in vitro* staining of islets with dithizone and disappearance of the bright light reflection of isolated islets. In contrast, rates of insulin biosynthesis, insulin content and insulin release after glucose stimulation were well preserved in *ZnT8*$^{-/-}$ mice. Moreover, when *ZnT8*$^{-/-}$ mice were fed a normal diet, glucose tolerance was preserved. However, when fed a high fat diet, the *ZnT8*$^{-/-}$ mice had the tendency to gain more weight and became glucose intolerant.
Conclusion: This study shows the importance of beta cell secretory granule ZnT8 transporter in the formation of insulin crystals which -in turn- explains islet dithizone staining and the bright light reflection of isolated islets. Our *in vivo* data also suggest that zinc-insulin crystals allow a better insulin packaging efficiency in beta cell granule stores, which is relevant for resisting abnormal glucose homeostasis under the metabolic stress of a high fat diet.
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158

Metabolic amplification of insulin secretion in mouse islets: role of beta cell microtubules and microfilaments

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Background and aims: Two pathways control glucose-induced insulin secretion. The triggering pathway involves closure of KATP channels in the beta cell membrane, depolarization, influx of Ca^{2+} and a rise in the cytosolic concentration of ionized Ca^{2+} ($[Ca^{2+}]c$), which then triggers exocytosis of insulin granules. The amplifying pathway augments insulin secretion without further increasing $[Ca^{2+}]c$. It is not known how this metabolic amplification augments the efficacy of Ca^{2+} on exocytosis. In the present study, we tested a previously proposed hypothesis suggesting that amplification results from the acceleration of insulin granule translocation from a reserve pool to the exocytotic sites at the plasma membrane. Such translocation is thought to involve granule movement along microtubules, followed by their transfer across a sub-membrane web of microfilaments.
Materials and methods: After 18h culture, normal mouse islets were treated for 90 min with agents known to interfere with microfilament function (cytochalasin B, latrunculin B or jasplakinolide), with microtubule function (taxol or vinblastine) or both (taxol and cytochalasin B). The amplifying effect of glucose was tested by comparing insulin secretion and $[Ca^{2+}]c$ in low (1-3mmol/l) or high (15mmol/l) glucose after $[Ca^{2+}]c$ was raised by a high concentration (500µmol/l) of tolbutamide (KATP channels closed) or by 30mmol/l KCl in the presence of 100µmol/l diazoxide (KATP channels open).
Results: In control islets, glucose amplified insulin secretion in the presence of tolbutamide (~ 4x) and of KCl (~ 2x). Depolymerization of microfilaments by cytochalasin B or latrunculin B augmented (2-3x) tolbutamide- or KCl-induced insulin secretion in low glucose without affecting $[Ca^{2+}]c$, and did not impair the amplifying action of high glucose. Amplification in the presence of KCl was even larger (~ 4x). Surprisingly, stabilization of microfilaments by jasplakinolide also augmented tolbutamide- and KCl-induced insulin secretion in low glucose (~1.5x) independently of $[Ca^{2+}]c$ changes, and did not affect the amplification of secretion by high glucose. Both stabilization (taxol) and depolymerization (vinblastine) of microtubules inhibited (~1.4x) insulin secretion in low glucose + KCl or tolbutamide, and unexpectedly attenuated the $[Ca^{2+}]c$ rise induced by both stimuli. Nevertheless, this did not impair the amplifying effect of high glucose. Amplification of insulin secretion was also unaltered in islets treated by a combination of cytochalasin B and taxol.
Conclusion: Pharmacological disruption of beta cell microfilaments markedly augments insulin secretion independently of $[Ca^{2+}]c$ changes, which supports the concept of the inhibitory role of microfilaments in exocytosis. In contrast, the inhibition of insulin secretion by agents affecting microtubules may partly be attributed to a previously unreported lowering of $[Ca^{2+}]c$. Finally, metabolic amplification does not appear to require functional microfilaments or microtubules. The results, therefore, do not support the hypothesis suggesting that the process corresponds to mobilization of insulin granules from a reserve pool, but point to a more distal step of stimulus-secretion coupling.

159

Glucose- and hormone-induced cAMP oscillations in intact prancreatic islets

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Background and aims: Cyclic AMP is an important messenger in insulin and glucagon secretion from pancreatic β- and α-cells. However, little is known about the kinetics of cAMP signals in islet cells. In the present study we investigated the effects of adenylyl cyclase-activating hormones and glucose on the cAMP concentration beneath the plasma membrane ($[cAMP]_{pm}$) of cells within intact isolated mouse islets.
Materials and methods: Islets were isolated from the pancreata of C57/Bl mice and infected with adenovirus expressing a fluorescent translocation biosensor for $[cAMP]_{pm}$. After attachment of the islets to coverslips, biosensor fluorescence changes were recorded from the superficial islet cells adhering to the glass using ratiometric evanescent wave microscopy. β-cells were identified based on their large size and $[cAMP]_{pm}$-lowering in response to adrenaline, while α-cells were smaller and reacted to adrenaline with an increase of $[cAMP]_{pm}$.
Results: In islets exposed to 3 mM glucose, $[cAMP]_{pm}$ was low and stable. Addition of 1-100 nM GLP-1 or glucagon induced prompt elevation of $[cAMP]_{pm}$ with oscillations synchronized among β-cells identified by $[cAMP]_{pm}$ lowering after exposure to 5 µM adrenaline. The GLP-1 and glucagon response patterns were dose-dependent with increasing duration of each $[cAMP]_{pm}$ oscillation with concentration of the stimulus. The hormone-induced $[cAMP]_{pm}$ elevations were markedly enhanced by a rise of the glucose concentration to 11 mM. Elevation of the glucose concentration to 11 or 20 mM in the absence of hormone stimuli induced a marked increase of $[cAMP]_{pm}$ and slow oscillations (frequencies range 0.13-0.59/min) both in α-cells reacting to subsequent addition of adrenaline with elevation of $[cAMP]_{pm}$ and in cells where adrenaline suppressed $[cAMP]_{pm}$. The glucose-induced $[cAMP]_{pm}$ oscillations persisted but with diminished amplitudes after removal of extracellular Ca^{2+}.
Conclusion: Glucagon, GLP-1 and glucose stimulation is associated with pronounced changes of cAMP concentration beneath the plasma membrane of individual cells within intact pancreatic islets. Such oscillations should contribute to the pulsatile release of islet hormones. The glucose-induced elevation of $[cAMP]_{pm}$ in α-cells may underlie a recently identified Ca^{2+}-independent stimulatory action of the sugar on glucagon secretion.

160

Subplasmalemmal Ca^{2+}measurements in pancreatic beta cells support the existence of an amplifying effect of glucose on insulin secretion

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Background and aims: Triggering and amplifying pathways are thought to underlie glucose (G) stimulation of insulin secretion by β-cells. In the triggering pathway, G metabolism leads to a K_{ATP} channel-dependent depolarization that promotes Ca^{2+} influx via L-type Ca^{2+} channels, with subsequent increase in the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) and triggering of exocytosis. The cellular mechanisms of the K_{ATP} channel-independent amplifying pathway are still elusive. One proposal is that G metabolism augments the efficacy of cytosolic Ca^{2+} on exocytosis without a further increase in $[Ca^{2+}]_c$. However, G has been reported to modulate the properties of Ca^{2+} channels. It is thus possible that $[Ca^{2+}]$ changes confined to the exocytotic domain beneath the plasma membrane escaped detection during measurements of global $[Ca^{2+}]_c$. Therefore, we investigated whether an increase in subplasmalemmal $[Ca^{2+}]$ ($[Ca^{2+}]_{SM}$) might explain the apparent amplifying effect of G.
Materials and methods: We generated an adenovirus encoding the Ca^{2+} indicator D3-cpv (not targeted) and expressed it in clusters of β-cells from NMRI mice. LynD3-cpv (targeted to plasma membrane) was selectively expressed in β-cells within intact islets by the combination of LoxStopLox-LynD3-cpv and RIP-Cre adenoviruses. $[Ca^{2+}]_{SM}$ changes were monitored using total internal reflection fluorescence microscopy. In some experiments insulin secretion was measured in parallel.
Results: When β-cell clusters expressing D3-cpv were stimulated by increasing [G] from 3 to 15mmol/l, $[Ca^{2+}]_{SM}$ was found to change similarly to global $[Ca^{2+}]_c$ measured by conventional techniques: an initial transient drop was followed by a large biphasic increase, with well synchronized oscillations during the second phase. Simultaneously, insulin secretion was stimulated. This shows that β-cell function was not altered by expression of the fluorescent Ca^{2+}-probe. The amplifying pathway was studied in the presence of diazoxide, to hold K_{ATP} channels open, and of 30mmol/l KCl to depolarize β-cells, induce Ca^{2+} influx and stimulate insulin secretion. Under these conditions, increasing [G] from 3 to 15mmol/l caused a transient decrease in $[Ca^{2+}]_{SM}$ in most cells (22/24), followed by a return to pre-stimulatory values. Importantly, no cell showed an increase in $[Ca^{2+}]_{SM}$ while insulin secretion augmented about 2-fold. Pretreatment of the clusters with thapsigargin, a blocker of Ca^{2+} uptake by the endoplasmic reticulum, attenuated the initial decrease in $[Ca^{2+}]_{SM}$ produced by high glucose, but did not unmask any $[Ca^{2+}]_{SM}$ increase (n=25 cells), while insulin secretion remained stimulated. In control experiments, small $[Ca^{2+}]_{SM}$ fluctuations could be detected during repetitive variations of KCl between 30 and 32mmol/l (9/10 cells), which attests that our system has the required sensitivity to observe potential G effects. Finally, selective expression of LynD3-cpv in β-cells within intact islets did not reveal any $[Ca^{2+}]_{SM}$ increase upon glucose stimulation in the presence of 30mmol/l KCl and diazoxide, whereas the small changes imposed by variations of [KCl] between 30 and 32 mmol/l could be detected.
Conclusion: Amplification of insulin secretion by glucose does not involve an increase in β-cell subplasmalemmal $[Ca^{2+}]$ and can thus be ascribed to an increase in the efficacy of the triggering Ca^{2+} signal.

161

Hormonal inhibition of endocytosis: novel roles for norepinephrine, prostaglandin E_1 and Gz

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Background and aims: Increased $[Ca^{2+}]i$ stimulates both exocytosis and endocytosis. As norepinephrine and other physiological inhibitors of insulin release block exocytosis even in the face of increased $[Ca^{2+}]i$, an increase in endocytosis under such circumstances would be undesirable. Therefore, we tested the hypothesis that inhibitors of exocytosis would inhibit endocytosis when $[Ca^{2+}]i$ is increased.
Materials and methods: Whole cell and cell attached patch clamp capacitance measurements were applied using INS 832/13 cells to investigate the action of inhibitors of insulin exocytosis on endocytosis. Blocking peptides and pharmacological agents were employed in the study.
Results: When the cells were stimulated by depolarizing pulses of -70 mV to +10 mV under conditions of very high Ca^{2+} influx, e.g. 100 msec with 10 mM $[Ca^{2+}]o$ or 500 msec with 2.6 mM $[Ca^{2+}]o$, exocytosis was strongly increased and was followed immediately by endocytosis. Importantly, with these high levels of Ca^{2+} influx the effects of norepinephrine and prostaglandin E_1 (PGE_1) to block the stimulated exocytosis were abolished. This allowed us to study the effects of the inhibitors on endocytosis. Norepinephrine and PGE_1 inhibited endocytosis to the same extent as deltamethrin and cyclosporine, two well-known inhibitors of calcineurin and endocytosis. Inhibition of endocytosis by norepinephrine was blocked by yohimbine indicating that the effect was due to the α_2-adrenergic receptor but, surprisingly, it was not blocked by pre-treating the cells for 48 hours with pertussis toxin indicating the involvement of a pertussis toxin-insensitive G protein. Synthetic peptides mimicking the C-termini of G protein α subunits block the interaction of the G proteins with their receptors and can be used to determine which G proteins are involved in a specific effect. Dialyzing a synthetic peptide mimicking the C-terminus of the α-subunit of Gz into the cells blocked the inhibition of endocytosis by NE whereas a peptide consisting of the same amino acids but in random order was without effect. Inhibition of endocytosis by PGE_1 was also blocked by the Gz peptide. Cell attached capacitance measurements of single vesicles indicated that inhibition by norepinephrine was due to a decrease in the number of endocytotic events without a change in vesicle size. Analysis of fission pore kinetics revealed two distinct fission modes with norepinephrine selectively inhibiting the rapid fission events. As expected, deltamethrin, which is a calcineurin inhibitor and acts to inhibit the earliest stage in the sequence of events leading to endocytosis, i.e. to prevent the activation of calcineurin by increased $[Ca^{2+}]i$, decreased the number of endocytic events without affecting the fission pore kinetics.
Conclusion: These findings determine a novel action of norepinephrine and PGE_1 on endocytosis in insulin secreting cells and a novel action for Gz in the control of endocytosis. Similar mechanisms are likely operating in other cell types also, e.g. where inhibition of synaptic transmission occurs in the CNS.
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162

A novel "distal" inhibitory effect of norepinephrine on the beta cell - retardation of the refilling of the readily releasable granule pool

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Background and aims: The aim of this study was to understand the mechanisms responsible for the "distal" effect by which physiological inhibitors of insulin secretion block exocytosis downstream of increased$[Ca^{2+}]i$
Materials and methods: Whole cell and cell-attached patch clamp capacitance measurements were applied using INS 832/13 cells to investigate the mechanisms governing the distal effects of norepinephrine, and to identify the heterotrimeric G proteins involved. Antibodies against the α and β subunits of the heterotrimeric G proteins, blocking peptides and pharmacological agents were used in the study.
Results: The exocytosis elicited by a depolarizing pulse of 100ms from -70mV to +10mV with 2.6mM $[Ca^{2+}]o$ was inhibited by norepinephrine. This inhibition was blocked by the addition of antibodies against G protein β subunits while antibodies against Gαcommon alone were without effect. In accord with these data, the action of norpinephrine was mimicked by the βγ-activating peptide mSIRK. Moreover, inhibition of exocytosis by norepinephrine was significantly attenuated by botulinum toxin A (BoNT/A), which cleaves off the C-terminal 9 amino acids of SNAP-25, and also by a synthesized 14 amino acid peptide containing the BoNT/A cleavage site on SNAP-25. Thus the interaction between βγ and the C-terminus of SNAP-25 is responsible for the action of norepinephrine to inhibit exocytosis. However, with a train of 15 stimulating pulses each of 100 ms duration, the antibody against Gβ only blocked the inhibitory effect of norepinephrine on the exocytosis triggered by the 1st pulse and an "apparent" inhibitory effect of norepinephrine persisted for the remaining duration of the pulse chain. This persistent effect was abolished by the simultaneous addition of antibodies against Gβ and Gαcommon. These data combined suggested that the antibody against Gβ was blocking all of the inhibitory effect of norepinephrine on exocytosis but that an effect of norepinephrine via Gα to retard the filling of the RRP reduced the availability of granules for release - hence the apparent inhibition of exocytosis by norepinephrine in the presence of antibody against Gβ. A double pulse study to directly determine the time-course of refilling of the RRP was carried out under conditions of high Ca^{2+} influx (500ms stimulations from -70mV to

+10mV with 2.6mM [Ca^{2+}]o. This high level of Ca^{2+} influx blocks the effect of norepinephrine on exocytosis and empties the RRP. The rate of refilling was determined by applying 2nd pulses at intervals of from 1 to 40 seconds. Norepinephrine markedly retarded the refilling rate. To determine which G protein(s) were responsible, peptides mimicking the C-terminal amino acids of G protein α subunits, that block G protein interaction with their receptors, were added intracellularly. The Gαi-1/2 peptide blocked the effect of norepinephrine on refilling. A peptide with the amino acids in random order, and peptides for, Gαi-3, Gαo-1, Gαo-2, and Gαz were without effect.
Conclusion: The distal inhibition of insulin secretion by norepinephrine is due to two effects, 1. Inhibition of exocytosis by a Gβγ interaction with SNAP-25 on the SNARE complex and 2. Retardation of the refilling of the readily releasable pool by Giα-1/2.
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OP 28 Inflammation, obesity and metabolism

163

Complement factor H is expressed in adipose tissue in association with insulin resistance
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Background and aims: Activation of the alternative pathway of the complement system, in which factor H (fH; *CFH*) is a key regulatory component, has been suggested as a link between obesity and metabolic disorders. We aimed to study the associations between circulating and adipose tissue gene expressions of *CFH* and complement factor B (fB; *CFB*) with obesity and insulin resistance.
Materials and methods: Circulating fH and fB were determined by ELISA in 398 subjects (140 with normal glucose tolerance and 258 with altered glucose tolerance). Insulin sensitivity was measured using the frequently sampled intravenous glucose tolerance test (FSIVGTT). *CFH* and *CFB* gene expressions were evaluated in 76 adipose tissue samples, in isolated adipocytes and stromo-vascular cells (SVC) (n=13) by real time PCR using TaqMan® technology. The effects of weight loss were investigated in an independent cohort of 42 subjects.
Results: Circulating fH and fB were significantly increased in subjects with AGT (195.4 ± 63.5 *vs.* 175.2 ± 53.4 μg/ml, p=0.01 and 285.9 ± 90.5 *vs.* 231.95 ± 58.8 μg/ml, p<0.0001, respectively). Both circulating fH and fB were positively associated with BMI, waist-to-hip ratio, systolic and diastolic blood pressure, fasting glucose, glycated hemoglobin, log fasting triglycerides, LBP and sTNFR2; and negatively with insulin sensitivity and HDL-cholesterol. Age (p=0.03) and insulin sensitivity (p=0.01) contributed independently to circulating fB variance after controlling for the effects of waist-to-hip ratio, log fasting triglycerides and LBP in multiple lineal regression models. Only insulin sensitivity (p=0.02) contributed independently to circulating fH variance in the same model.
For the first time, *CFH* gene expression was detected in the adipose tissue (significantly increased in subcutaneous compared with visceral fat). *CFH* gene expression in visceral fat was significantly associated with insulin resistance. In contrast, *CFB* gene expression was significantly increased in omental fat but also in association with fasting glucose and triglycerides. The SVC fraction was the responsible of these differences, although isolated adipocytes also expressed fB and fH at low levels. Weight loss led to significantly decreased circulating fB (p=0.025) and fH (p=0.009) levels.
Conclusion: Increased circulating fH and fB concentrations in subjects with altered glucose tolerance could reflect increased SVC-induced activation of the alternative pathway of complement in omental adipose tissue linked to insulin resistance and metabolic disturbances.
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164

TIMP-3 expression is regulated by SirT1
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Background and aims: We recently identified Tissue Inhibitor of Metalloproteinase 3 (TIMP3), the endogenous inhibitor of A Disintegrin and Metalloprotease Domain 17 (ADAM17) and other Metalloproteinases (MMPs), as a gene modifier for insulin resistance and vascular inflammation in mice. We also described that in human carotid atherosclerotic plaques TIMP3 was significantly reduced in subjects with Type 2 diabetes leading to ADAM17 and MMP9 overactivity.
The aim of this study was to identify metabolic factors reducing TIMP3 expression and activity in Coronary Artery Smooth Muscle Cells (CASMC).
Materials and methods: CASMC (Lonza) were cultured and treated for 24 hours respectively with glucose (20 mM, HG), mannitol (20 mM, Man), insulin (10-7M, INS), 100μg/ml native LDL, 100μg/ml oxidized LDL (oxLDL),

100µg/ml Glycated-LDL (glyLDL). Since previous data suggested that LXR regulates TIMP3 expression, we used LXR agonists such as T0901317, 22-s and 22-r hydroxycholesterol and GW3965. Since LXR is regulated by SirT1 deacetylase, we also used SirT1 inhibitor Sirtinol. CASMC were therefore treated with 10µM 22-R-hydroxycholesterol (RH, Sigma-Aldrich), 10µM 22-Shydroxycholesterol (SH, Sigma-Aldrich), 5µM T0901317 (T09, Sigma-Aldrich), 3µM GW3965 (GW, Sigma-Aldrich), and 50µM Sirtinol (Calbiochem). Proteins and total RNA were extracted and TIMP3 levels and ADAM17 activity were assayed respectively by RT-PCR and fluorimetric assay. Knockdown of SirT1 in CASMC was performed trasfecting 1 µg of SIRT1 siRNA (sc-40986, Santa Cruz Biotechnology) and 0.8 µg of control siRNA-A (sc-40986, Santa Cruz Biotechnology) by Dharmafect1 (Dharmacon). For Timp3 promoter regulation assay we transfected cells with 2 µg of Human SirT1 cDNA clone (RG218134, Origene), 10 ng of renilla and Timp3 Gene Promoter Reporter Vector (LR1034,Panomics) or 2 µg of TransLucent Control Vector using Nucleofector II (program A-033,AMAXA). The Luciferase assay was performed with Dual-Luciferase Reporter Assay System (Promega) according to manufacturer's instructions.

Results: We found that among the various treatments only high glucose and Sirtinol significantly reduced TIMP3 expression in CASMC ($p<0.01$). Treatment of CASMC with Sirtinol determined an increased metalloprotease activity as measured by ADAM17 activity ($p<0.01$). To confirm a direct role of SirT1 in regulation of TIMP3 expression, we used a small interference RNA (siRNA) approach. Knockdown of SirT1 in CASMC resulted in marked reduction of TIMP3 expression but not Timp1/2/4 and ADAM10/17/MMP9. To substantiate SirT1 effects on TIMP3, CASMC were cotransfected with human SirT1 cDNA and Timp3 promoter luciferase reporter vector, confirming that SirT1 positively modulates TIMP3 expression. Human Endothelial Vein Umbilical Cells and Monocyte-like THP-1 cells were treated with Sirtinol resulting in a significant decrease in TIMP3 expression in both cell types ($p<0.01$). Finally, we analyzed TIMP3 and SirT1 expression in human atherosclerotic plaques (n=60) from diabetic and non diabetic subjects with symptomatic cerebrovascular disease. We observed that TIMP3 expression is positively correlated to SirT1 expression in human disease ($R=0.4$, $p<0,03$).

Conclusion: Our data suggest that TIMP3 expression is regulated by SirT1 deacetylase activity in human vascular cells and in human atherosclerotic plaques. A defective control of SirT1 on TIMP3 expression may lead to increased metalloprotease activation and favour the progression of atherosclerosis.

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165

Experimental weight gain in humans induces insulin resistance in absence of systemic or adipose tissue inflammation

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Background and aims: Low grade inflammation is often reported in obesity and is postulated to be causal in the development of insulin resistance and progression to type 2 diabetes. However, supportive evidence in humans is less conclusive, and there is increasing data suggesting that insulin resistance may precede accumulation of macrophages in adipose tissue and the local and systemic inflammation process. Our objective was to assess whether high-fat overfeeding in humans induces insulin resistance, and whether this is associated with an increase in markers of inflammation.

Materials and methods: We performed a prospective experimental 28 day high-fat overfeeding study in 28 healthy individuals. This was achieved by increasing food intake by +1000kcal/day from baseline, and increasing their fat intake from 34.3% to 45.8%. Changes in body weight, body composition (dual X-ray absorptiometry), insulin sensitivity (hyperinsulinaemic-euglycaemic clamp), serum inflammation markers, immune cell activation and macrophage numbers in abdominal subcutaneous adipose tissue (flow cytometry of stroma-vascular fraction) were recorded.

Results: Subjects with a starting BMI of 25.4±0.7 kg/m2 gained in average 2.8±0.2 kg ($p<0.001$) in bodyweight and increased fat mass by 1.8±0.2kg ($p<0.0001$). Insulin sensitivity (glucose infusion rate/fat free mass) decreased by 9.5% from 51.6±3.2 to 46.6±2.9 µmol/kg/min ($p=0.01$). There was a significant increase in circulating C-reactive protein (1.1±0.2 to 1.5±0.3 ng/ml, $p<0.01$) and monocyte chemoattractant protein-1 (MCP-1)(166±14 to 208±22 pg/ml, $p<0.01$), but no change in IL-6 (1.4±0.1 to 1.3±0.1 pg/ml, $p=0.44$) and soluble intercellular adhesion molecule-1 (207±10 to 203±9 ng/ml, $p=0.49$). We observed no change in macrophage numbers in adipose tissue, circulating immune cell numbers or expression of their surface activation markers. Importantly, there was no association between changes in insulin sensitivity and changes in inflammation markers.

Conclusion: Weight gain induced insulin resistance was observed in the absence of development of a significant inflammatory state, suggesting that inflammation is unlikely to be a substantial contributing factor to insulin resistance at this early stage of fat accumulation. The early increase in circulating MCP-1 may play a role in future recruitment of macrophages into adipose tissue.

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166

The role of IL-1R1 mediated macrophage accumulation in adipose tissue - insights into the development of obesity-induced insulin resistance

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Background and aims: Macrophages are a heterogeneous population of cells that play a role in the innate immune response to infection. Recent studies have shown that macrophages are key cells in the development of obesity, wherein there is progressive infiltration of macrophages into the adipose tissue. These adipose tissue macrophages are referred to as classically-activated, or M1, macrophages. They release pro-inflammatory cytokines such as IL-1, IL-6 and TNFα, creating an inflammatory response that can contribute to insulin resistance and type 2 diabetes mellitus (T2DM). In lean individuals macrophages are in an M2-polarization or alternatively-activated state and are thought to protect against inflammation. The IL-1 receptor type 1 (IL-1R1) is responsible for transmitting the pro-inflammatory effects of IL-1. The aim of the present study was to determine the number of adipose tissue macrophages and their activation state using flow cytometry in wildtype (WT) mice and in IL-1R1$^{-/-}$ mice at certain time points over a period of 16 weeks.

Materials and methods: C57BL/6 WT and IL-1R1$^{-/-}$ mice with a C57BL/6 background were fed a high-fat diet (45 % energy from fat) for 16 weeks. At weeks 0, 6 and 16 epididymal adipose tissue (EAT) and visceral adipose tissue (VAT) samples were taken from the C57BL/6 wildtype (WT) and IL-1R1$^{-/-}$ mice. Adipocytes and stromal vascular cells (SVC) were isolated from the adipose tissue by collagenase treatment. SVC were labelled with antibodies for macrophage markers F4/80, CD11B and CD11C and analysed by flow cytometry to determine the number of macrophages and activation status of the macrophages present. Triple-positive cells (F4/80$^+$CD11B$^+$CD11C$^+$) are associated with the M1-polarization state, while double-positive cells (F4/80$^+$CD11B$^+$CD11C$^-$) indicate M2 macrophages.

Results: At week 0 there is a significantly larger population of F4/80$^+$CD11B$^+$CD11C$^-$ (M2) cells in the EAT and VAT of the IL-1R1$^{-/-}$ group compared to the WT group ($p<0.05$) but no difference between groups in the number of F4/80$^+$CD11B$^+$CD11C$^+$ (M1) cells. However after 6 weeks on a high-fat diet there is a larger population of the more active F4/80$^+$CD11B$^+$CD11C$^+$ (M1) cells in the EAT of the WT group compared with the IL-1R1$^{-/-}$ group ($p<0.05$) while there is a reduced number of F4/80$^+$CD11B$^+$CD11C$^-$ (M2) cells in the IL-1R1$^{-/-}$ group compared to week 0 . After 16 weeks on a high-fat diet there is no significant difference in the number of F4/80$^+$CD11B$^+$CD11C$^+$ (M1) cells between the two groups of mice. Interestingly by week 16 there is a higher population of F4/80$^+$CD11B$^+$CD11C$^-$ (M2) cells in the EAT and VAT of the WT group compared to the IL-1R1$^{-/-}$ group ($p<0.05$).

Conclusion: These results imply that impairing IL-1 signalling decreases the macrophages ability to switch from an M2-polarization state to the pro-inflammatory M1-polarization state. This outcome delays the infiltration of M1 macrophages into the adipose tissue, thereby lowering the pro-inflammatory response and attenuating the progression of insulin resistance. Further studies are continuing in order to determine the molecular mechanisms by which this process occurs.

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167

Exercise and weight loss both improve factors in the metabolic syndrome but only weight loss improves adiponectin and inflammatory markers

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Background: Both exercise and weight loss are associated with improvements in the metabolic syndrome but the mechanisms involved may be different for the two interventions. It is suggested that changes in the fat mass may affect other tissues through release of so-called adipokines, and in analogy to that muscle activity may induce more systemic effects through release of myokines. Here we investigated the individual and the combined effect of exercise and diet-induced weight loss on visceral fat (VAT) accumulation, adiponectin (total and HMW), inflammatory markers, and insulin sensitivity.
Material and methods: 80 obese women and men were divided in three group and treated for 12 weeks with 1) exercise only (EXO), 2) Diet-induced weight loss (DIO) - 8 weeks treatment with VLED followed by 4 weeks weight loss maintenance diet, and 3) the combined group with both exercise and diet-induced weight loss (DEX). VAT and fat mass were determined by MRI. Fat and muscle biopsies were taken (PCR determinations). Circulating levels of insulin, glucose (HOMA), lipids, adiponectin and inflammatory markers (Luminex) were determined before and at the end of the intervention.
Results: Weight loss was after the 12 weeks 3.5 kg (EXO) and 12 kg in both DIO and DEX. Vo2 max was increased with 18-22% (P<0.01) in the two exercise groups with no change in DIO. The reduction in VAT was related to the total weight loss and not to the intervention (exercise vs. diet). HOMA decreased in all three groups(p<0.05 in the DIO and DEX groups), however, only to a non-significant level in the EXO group (P=0.09). Circulating total adiponectin and HMW-adiponectin were significantly increased and MIP1α, MCP1, IL-6, IL-15, and IL-18 were significantly reduced (P < 0.05) in the diet-induced weight loss groups (DIO and DEX) without any changes in the EXO group. No changes in muscle mRNA (TNFα, IL-6, IL-15) were observed in any of the groups (neither in the EXO group). Adiponectin mRNA was upregulated in AT in all three groups. There was no gender differences in the response to exercise and diet-induced weight loss.
Conclusion: Both regular exercise and diet-induced weight loss were able to improve some aspects of the metabolic syndrome such as improving insulin sensitivity. In this group of obese subjects we found that larger weight losses improve levels of adiponectin and low grade inflammation whereas exercise had no independent effects on these risk factors. We suggest that the metabolic effects of exercise is mediated primarily in the muscle tissue and the metabolic changes seen in other tissues are mediated by the well-known changes in insulin, adrenalin, GH, FFA etc. associated with muscle work and not by specific myokines. In contrast larger reduction in the fat mass may have systemic effects through changes in the release of adipokines from AT in combination with changes in insulin and FFA.
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168

The effect of weight loss on intra-abdominal fatty acid metabolism in metabolic syndrome

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Background and aims: Abdominal visceral adipose tissue (VAT) is considered to be more metabolically active than abdominal subcutaneous adipose tissue (SAT) and is thought to be more detrimental than SAT. The aim of the study was to compare fatty acid uptake (FU) in VAT and SAT and the effect of weight loss on regional FA metabolism in patients with metabolic syndrome.
Materials and methods: Regional FU was studied in 18 obese non-diabetic subjects (age 43±6 year, BMI 34.0± 3.9 kg/m^2) were studied in fast using PET and a trapping palmitate analogue with [18F]-fluoro-6-thia-heptadecanoic acid (FTHA). Indirect calorimetry and stable FA isotope [U-^{13}C]-palmitate were used to determine FA oxidation. Subjects were studied before and 6 weeks after very-low-calorie-diet (VLCD) for 6 weeks and one recovery week. MRI and MRS were used for anatomical reference and fat content measurements.
Results: The subjects lost 11.2±2.5 kg and body fat 18.5±5.2% (p<0.007). The mass of VAT decreased by -22.7±16.4% and of SAT by 19.5±10.1% (p< 0.001 for both). FU in VAT was 4-fold higher per volume than in abdominal SAT both before and after VLCD (p<0.001). FU into VAT depots (14.2× vs. 12.5×) and SAT depots (7.0× vs.6.5×umol/min) remained unchanged. In contrast FU decreased in femoral SAT (p<0.006), liver and muscle (p<0.01). A decrease in liver adiposity (p<0.001) and muscle fat content was found. EE decreased by 11.5±4.7% (p<0.0001). In whole-body level total fat oxidation was increased (from 4.07±1.29 to 5.82±1.11 umol/(FFMkg×min), (p=0.002) while total CHO oxidation was decreased 14.4±3.4 to 5.3±3.6 umol /(FFMkg×min), (p<0.0001). The non-plasma oxidation (plasma TGs and intraorgan TGs in muscle and adipose tissue) increased from -1.03±1.78 to 1.73±1.69 umol/(FFM×min) (p=0.0002) and the whole-body re-esterification decreased from 15.05±5.18 to 11.56±3.72 umol/(FFM×min) (p=0.04). Neither plasma FFA, palmitate, glycerol nor 3-OHB were changed after VLCD, but cholesterol, LDL-cholesterol and TGs decreased (p<0.002).
Conclusion: We conclude that fatty acids are taken up several folds more in VAT than in SAT in obese subjects. Rapid weight loss does not change the FA retention into VAT, although it reduces FA uptake into liver and muscles and shifts and enhances fat oxidation from non-plasma storages enhanced. The missing adaptation of VAT fatty acid metabolism during dieting might partly explain the problems to maintain the lower body weight.

OP 29 Novel therapies for type 2 diabetes mellitus

169

Dapagliflozin as an add-on to metformin lowers hyperglycaemia in type 2 diabetes patients inadequately controlled with metformin alone

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Background and aims: Dapagliflozin (DAPA), a novel oral antidiabetic agent with an insulin-independent mechanism, is currently being developed for treating type 2 diabetes mellitus (T2DM). DAPA decreases hyperglycaemia by selectively inhibiting glucose reabsorption by renal sodium-glucose co-transporter 2. This trial (Study 014ST) was conducted to evaluate the efficacy and safety of DAPA as an add-on to metformin (Met) over 24 weeks in T2DM patients inadequately controlled with Met alone.
Materials and methods: This randomized, double-blind, placebo-controlled, multicenter trial in North and South America enrolled patients with T2DM, ages 18-77 years old. Eligible patients had inadequate glycaemic control (HbA1c 7.0-10.0%) on stable dosing with Met ≥1500 mg/d. After a 2-wk lead-in phase, 546 patients were equally randomized to once daily DAPA 2.5 mg, 5 mg, 10 mg, or placebo (PBO), plus open-label Met. The primary endpoint was change in HbA1c at wk 24. Other endpoints included changes in fasting plasma glucose (FPG) and % change in total body weight at wk 24.
Results: Compared to add-on PBO at wk 24, all add-on DAPA groups showed significant mean reductions from baseline in HbA1c (**Table**). Endpoint reductions in FPG were also significant for all DAPA groups vs PBO. Greater proportions of patients in all DAPA groups achieved HbA1c <7.0% at wk 24 than patients on PBO. Weight loss with DAPA was continuous and progressive. More patients treated with DAPA achieved weight decreases ≥5% compared to PBO. Generally, adverse events were balanced across all groups. Compared with PBO, rates of urinary tract infections were similar or lower for DAPA (PBO, 8.0%; DAPA 2.5 mg, 4.4%; DAPA 5 mg, 7.3%; DAPA 10 mg, 8.1%), while rates of genital infections were higher (PBO, 5.1%; DAPA 2.5 mg, 8.0%; DAPA 5 mg, 13.1%; DAPA 10 mg, 8.9%). Laboratory monitoring revealed no clinically meaningful changes in markers for renal impairment or increases in mean serum creatinine. Changes in supine BP at wk 24 ranged from -3.1 to -5.9 systolic/-2.1 to -2.7 diastolic mmHg with DAPA, compared to -0.3 systolic/-0.4 diastolic mmHg with PBO. A similar proportion of patients across all 4 treatment groups, including PBO, had BP measurements suggestive of orthostatic hypotension but without reported symptoms. Reports of hypoglycaemia were similar (PBO, 2.9%; DAPA 2.5 mg, 2.2%; DAPA 5 mg, 3.6%; DAPA 10 mg, 3.7%), and none led to discontinuation of study medication.
Conclusion: In T2DM patients who were inadequately controlled with Met alone, the addition of once daily DAPA appeared safe and was associated with significantly improved glycaemic control and clinically meaningful weight loss over 24 weeks compared to PBO.
Supported by: Bristol-Myers Squibb and AstraZeneca

Efficacy Assessments

	PBO + Met [n=137]	DAPA 2.5 mg + Met [n=137]	DAPA 5 mg + Met [n=137]	DAPA 10 mg + Met [n=135]
HbA1c, baseline (%), mean (SD)	8.11 (0.96)	7.99 (0.90)	8.17 (0.96)	7.92 (0.82)
Wk 24 (LOCF) change from baseline in HbA1c (%), adjusted mean (SE)	-0.30 (0.072)	-0.67 (0.072)[b]	-0.70 (0.072)[b]	-0.84 (0.072)[b]
Wk 24 (LOCF) adjusted proportion of patients with HbA1c <7.0%, % (95% CI)	25.9% (19.1, 32.6)	33.0%[a] (25.4, 40.6)	37.5%[b] (30.0, 45.1)	40.6%[b] (32.9, 48.3)
FPG, baseline (mg/dL), mean (SD)	165.6 (46.4)	161.5 (43.1)	169.2 (49.0)	156.0 (38.7)
Wk 24 (LOCF) change from baseline in FPG (mg/dL), adjusted mean (SE)	-6.0 (2.7)	-17.8 (2.7)[b]	-21.5 (2.7)[b]	-23.5 (2.7)[b]
Weight, baseline (kg), mean (SD)	87.7 (19.2)	84.9 (17.8)	84.7 (16.3)	86.3 (17.5)
Wk 24 (LOCF) percent change from baseline in weight, adjusted mean (SE)	-1.02 (0.29)	-2.66 (0.28)	-3.66 (0.28)	-3.43 (0.28)
Wk 24 (LOCF) adjusted proportion of patients with ≥ 5% decrease from baseline in weight, % (95% CI)	5.9% (1.9, 9.8)	24.0% (16.8, 31.2)	25.4% (18.1, 32.7)	28.0% (20.4, 35.5)

LOCF, last observation carried forward.[a]Not statistically significant compared to PBO; [b]Statistically significant compared to PBO

170

Dapagliflozin improves glycaemic control in insulin-resistant patients with type 2 diabetes

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Background and aims: For many T2DM patients who require insulin plus oral antidiabetics (OADs), the effectiveness of treatment is often limited by weight gain and insulin-resistance. Dapagliflozin (DAPA) is an insulin-independent oral agent that blocks renal glucose reabsorption by selectively inhibiting sodium-glucose co-transporter 2. This 12-week pilot study (Study 009) was designed to determine the efficacy and safety of DAPA in T2DM patients who were poorly controlled with insulin (at least 50 units/day) plus OADs (metformin and/or thiazolidinediones).
Materials and methods: The trial involved 26 study centers in the United States and Canada. In order to initially monitor glycaemic parameters with reduced insulin dosing, a small preliminary cohort (n = 4) received 20 mg single-blind DAPA, baseline OADs and 50% of baseline insulin dose. Afterward, the larger primary cohort (n = 71) was randomized to double-blind placebo (PBO), 10 mg DAPA or 20 mg DAPA once daily, plus baseline OADs and 50% of baseline insulin.
Results: At week 12, DAPA lowered HbA1c, postprandial glucose (PPG), and weight more than PBO (**Table**). In both DAPA groups, 65.2% of patients showed decreases in HbA1c ≥0.5% versus 15.8% of patients in the PBO group. Dapagliflozin produced dose-dependent responses in both fasting plasma glucose (FPG) and PPG. There were mean decreases in standing systolic/diastolic blood pressure in both DAPA groups (-7.2/-1.2 mmHg change from baseline of 130.7/78.9 mmHg [10 mg DAPA], -6.1/-3.9 mmHg change from baseline of 126.9/76.5 mmHg [20 mg DAPA]), compared to an increase in the PBO group (+2.8/+0.3 mmHg change from baseline of 128.9/76.9 mmHg). Adverse events (AEs) were balanced across all groups. Most frequently reported AEs (>5% in any group) included pollakiuria (urinary frequency), back pain, nasopharyngitis, nausea, headache, and upper respiratory tract infection. Three patients in the PBO group, 7 patients in the 10 mg DAPA group, and 6 patients in the 20 mg DAPA group reported episodes of hypoglycaemia. Of these, 1 patient in the PBO group reported major hypoglycaemia.
Conclusion: In insulin-resistant patients who had insulin reduced by 50%, DAPA was well tolerated, and improved glycaemic control and lowered weight more than PBO.

Mean Change from Baseline (95%CI) at Week 12 (LOCF)

	PBO [n=23]	10 mg DAPA [n=24]	20 mg DAPA [n=24]
HbA1c, *%*	0.09 (-0.2, 0.4)	-0.61 (-0.9, -0.4)	-0.69 (-0.9, -0.4)
FPG, *mg/dL*	17.8 (1.4, 34.2)	2.4 (-13.6, 18.3)	-9.6 (-25.6, 6.3)
PPG at 120 min, *mg/dL*	18.7 (-13.5, 50.9)	-34.3 (-67.5, -1.1)	-41.9 (-74.8, -8.9)
Weight, *kg*	-1.9 (-2.9, -0.9)	-4.5 (-5.5, -3.5)	-4.3 (-5.3, -3.3)

LOCF, last observation carried forward

Supported by: Bristol-Myers Squibb and AstraZeneca

171

Colesevelam HCl improves glucose metabolism and increases plasma glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide concentrations in subjects with type 2 diabetes

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Background and aims: Colesevelam HCl is a bile-acid sequestrant indicated to improve glycemic control in subjects with type 2 diabetes (T2DM). The aim of this multi-center, randomized, double-blind study was to investigate potential glucose-lowering mechanism(s) of colesevelam.
Materials and methods: Subjects with T2DM (HbA_{1C}, 6.7-10.0%) were randomized to either 3.75g/d colesevelam HCl (n=27) or placebo (n=28) for 12 weeks. Fasting endogenous glucose production (EGP), gluconeogenesis, glycogenolysis and plasma glucose clearance were measured in each subject at baseline and post-treatment using stable isotope infusion studies of [U-$^{13}C_6$]glucose and [2-$^{13}C_1$]glycerol. Plasma glucose, insulin, total glucagon-like peptide 1 (GLP-1), total glucose-dependent insulinotropic polypeptide (GIP) and glucagon concentrations were also measured during fasting state and following a meal tolerance test.
Results: Significant treatment differences in least square (LS) mean (±SE) HbA_{1C} (-0.62 ± 0.23 %, P<0.01) and fasting plasma glucose (-23 ± 11 mg/dl, P<0.05) were observed at 12 weeks with colesevelam vs. placebo. Colesevelam increased fasting total GLP-1 (+10 ± 4 pmol/l, P=0.01), postprandial total GLP-1 AUC (+2,267 ± 750 pmol/l/300 min, P<0.01) and postprandial GIP AUC (+3.859 ± 857 pmol/l/300 min, P<0.0001) compared to placebo. Plasma glucose clearance increased significantly with colesevelam but was unchanged with placebo (+0.26 ± 0.42 and -0.06 ± 0.39 ml/kg FFM/min, respectively, P<0.01 between groups). EGP and glycogenolysis were unchanged with colesevelam and increased significantly with placebo (P<0.05), although the changes from baseline were not different between groups. Fasting and postprandial insulin and glucagon, fasting GIP and absolute gluconeogenesis were not different between the colesevelam and the placebo groups.
Conclusion: Colesevelam reduced HbA_{1C}, fasting plasma glucose and increased glucose clearance in the fasting state, compared to placebo, without the increase in EGP that occurred in placebo. The improvement in glucose homeostasis in the colesevelam group corresponded with an increase in fasting GLP-1 and postprandial GLP-1 and GIP concentrations.
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172

Efficacy and safety of the 11-beta-HSD1 inhibitor, INCB13739, added to metformin therapy in patients with type 2 diabetes

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Background and aims: INCB13739 (13739) is a selective inhibitor of 11-beta-hydroxysteroid dehydrogenase type 1 (11-β-HSD1) being developed for the treatment of type 2 diabetes (T2DM).
Materials and methods: This randomized, double-blind, placebo (PBO)-controlled trial assessed the efficacy and safety of 13739 in combination with metformin (MET) in patients with T2DM inadequately controlled by MET monotherapy. The primary endpoint was the change from baseline in HbA1c at week 12. After a 2 week PBO run-in period, patients (n=159; mean BMI=32.7. kg/m^2; mean HbA1c=8.3%; mean duration of T2DM=6.8 yr) were assigned to receive 13739 at doses of 5 mg (n=27), 15 mg (26), 50 mg (24), 100 mg (27), 200 mg (27) or PBO (28) once-daily in addition to a stable MET regimen for 12 weeks.
Results: Mean changes from baseline in HbA1c were significantly (p<0.01) greater for 13739 100 mg (-0.6%) and 200 mg (-0.5%) vs. PBO (0.0%) at week 12. Although plasma lipids were well controlled at baseline, both the 100 mg and 200 mg doses trended downward with a significant (p=0.03) treatment effect observed for the 100 mg dose in LDL-cholesterol (LDL-C) (-16 and +3 mg/dL for 100 mg vs. PBO, respectively). The frequency and severity of adverse events following 13739 treatment were similar to those observed in subjects randomized to PBO. 13739 did not affect sex hormone levels or the aldosterone-renin axis. A dose dependent increase in morning plasma ACTH was reached by week 4 and then plateaued (mean = 48 pg/mL in the 200 mg group at week 12; normal range <63.3 pg/mL). Plasma DHEA-s increased in a dose-dependent manner in concert with ACTH, reaching a maximum at week 4 in the 200 mg group (mean = 198 ug/dL) which was within age and gender-based normal ranges. Plasma morning cortisol, late-night salivary cortisol, and response to Cortrosyn challenge were unchanged from baseline, indicating that the changes in ACTH likely reflect compensatory HPA axis activity to maintain normal circulating cortisol homeostasis.
Conclusion: 13739 was very well tolerated and is the first 11-β-HSD1 inhibitor to demonstrate improvements in HbA1c and LDL-C in patients with T2DM.

173

Treatment with VI-0521 (phentermine and topiramate) leads to one year durable glycaemic benefit and weight loss in subjects with type 2 diabetes

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Background and aims: VI-0521, a low-dose, once daily combination of phentermine (PHEN) and topiramate (TPM) has been shown to produce significant weight loss, improvements in blood pressure and lipids, and in diabetic subjects, statistically and clinically significant reductions in HbA1c and blood glucose. This study demonstrates that in subjects with type 2 diabetes, previously reported metabolic and cardiovascular benefits persist through one year of treatment, with a favorable tolerability and safety profile.
Materials and methods: This was a 56-week double blind, placebo-controlled, multi-center study. Subjects were type 2 diabetics who had completed a previous (28-week) study evaluating VI-0521, and elected to continue with their randomized treatment [PHEN/TPM 15/92 (n=75) or Placebo (n=55)] for an additional 28 weeks. The primary endpoint for the study was the change in HbA1c from baseline (week 0) to Week 56. Subjects underwent dietary and lifestyle counseling throughout the 1-year period. All subjects were actively managed, in that investigators were required to adjust concomitant anti-diabetic and anti-hypertensive medications in response to blood glucose or blood pressure values that were outside of protocol-specified thresholds.
Results: The 1-year treatment cohort consisted of 130 type 2 diabetic subjects (69% female, mean age 49 years, 86% Caucasian, 59% Hispanic/Latino, 11% Black; mean baseline weight 96kg, BMI 35 kg/m^2, HbA1c 8.7%, mean fasting glucose 9.6 mmol/L). At baseline, 11 % of subjects were drug naïve, 28% were on monotherapy, and the remainder (>60%) were treated with 2 or more oral medications for their diabetes. At week 56, the VI-0521 group showed a reduction from baseline in HbA1c of 1.6% compared to 1.1% in the placebo group (p=0.038). 53% of VI-0521 treated subjects achieved the HbA1c benchmark of less than 7%, with 32% achieving a HbA1c of <6.5%, compared to 40% and 16%, respectively, in subjects managed without VI-0521. In the VI-0521 group, fasting glucose dropped 2.42 mmol/L at 56 weeks, compared to 1.42 mmol/L in placebo (p=0.02), and statistically significant improvements were also seen in postprandial glucose control as measured by 1,5-anhydroglucitol (GlycoMark, p=0.01). These glycemic targets were met in VI-0521 treated subjects despite a net reduction in their anti-diabetic medication, in contrast to the placebo treated subjects who required increased anti-diabetic medications. VI-0521 treated subjects lost 9.4% of their baseline body weight by 56 weeks, compared to 2.7% in the placebo group (p<0.001). VI-0521 was well tolerated, with no treatment related SAEs, and one discontinuation due to an AE in VI-0521, versus none in placebo. Adverse events were generally mild to moderate, and occurred at lower rates in the second six months of the study. Severe events occurred in 6 subjects on VI-0521, and 4 in placebo treated subjects. Greater than 90% of participants in both groups completed 56 weeks of treatment.
Conclusion: At one year, treatment of type 2 diabetic patients with VI-0521 produced sustained and clinically meaningful reductions in HbA1c, fasting and postprandial glucose, and body weight compared to placebo, in a well-tolerated once daily formulation. This was achieved on a background of mandated treatment to standard of care glycemic targets in both placebo and VI-0521 treatment groups.
Supported by: VIVUS, Inc.

174

Farnesoid-X receptor agonists - a new therapeutic class for diabetes and NAFLD - first clinical data

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Background and aims: 6-ethyl chenodeoxycholic acid (CDCA), INT-747, is a novel derivative of the primary human bile acid CDCA, (the natural ligand for the farnesoid-X receptor (FXR), a nuclear hormone receptor). INT-747 is ~100x more potent an FXR agonist than CDCA and *in vitro* increases insulin secretion by human pancreatic islets, enhances adipocyte lipid storage and secretion of adiponectin and leptin.
Materials and methods: This double blind, placebo (Pbo) controlled, study evaluated the effects of INT-747 on insulin sensitivity by means of a two stage euglycemic insulin clamp. Patients with Type 2 diabetes and non-alcoholic fatty liver disease (NAFLD), diagnosed by elevated aminotransferases (20%), imaging (84%) and/or histology (3%), were randomized to receive placebo, INT-747 25mg or 50mg once daily for 6 weeks. 64 patients enrolled (Pbo: n=23; INT-747 25mg: n=20, INT-747 50mg: n=21). Glucose disposal rate (GDR) (mg . kg^{-1} . min^{-1}) was determined (pre and post treatment) after steady state was achieved with low and high dose insulin (Ins) infusions (60 and 120mU x m^2 body surface area/min).
Results: Fasting plasma insulin concentrations tended to be higher after treatment with 25mg vs. Pbo. The greatest responses appeared to occur in patients with BMI <40kg/m^2 and HbA_{1c} >7%. Adverse experiences (AEs) were generally mild/moderate and not clearly different across groups (placebo 61%, 25mg 45%, 50mg 76%). Constipation (19% in 50mg group) was the most common. Minor increases in LDL (25 and 50mg) and decreases in HDL and triglycerides (50mg) were seen. ALT (25mg) and GGT (both doses) decreased by ~25% and 50% (p<0.001), respectively.
Conclusion: In this study INT-747 was well tolerated and improved GDR and weight loss in Type 2 diabetics with NAFLD.
*: p<0.05; †: 0.05 < p < 0.10 compared to placebo

Mean ± s.d.	Δ GDR Low dose Ins	Δ GDR High dose Ins	% Δ GDR Low dose Ins	% Δ GDR High dose Ins	Δ % Weight Change
Pbo (n=17)	- 0.51 ±1.88	- 0.61± 1.88	- 5.5 ± 35.9	- 5.4 ± 24.3	- 0.03 ± 1.96
INT-747 25mg (n=15)	0.69 ± 1.12*	0.73 ± 1.53*	28.0 ± 40.2*	18.3 ± 36.3*	- 1.22 ± 1.75†
INT-747 50mg (n=12)	0.24 ± 1.62	0.42 ± 1.42	20.1 ± 32.6†	10.8 ± 21.8†	- 1.70 ± 2.37*
Both doses (n=27)	0.49 ± 1.36*	0.59 ± 1.46*			

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OP 30 Neuropathy

175

The association between cardiac autonomic neuropathy with metabolic and other factors in subjects with type 1 and type 2 diabetes mellitus

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Background and aims: Cardiac autonomic neuropathy (CAN) is a common complication of diabetes and is associated with poor prognosis. However, little is known about the risk factors for the development of CAN, especially in subjects with type 2 diabetes (T2DM). The aim of this cross-sectional study was to examine the relationship between CAN and clinical and metabolic parameters as well as microvascular and other complications in patients with either type 1 (T1DM) or T2DM.
Materials and methods: A total of 600 patients (T1DM: *n*=200; T2DM: *n*=400) were recruited. Participants with overt nephropathy or macrovascular complications were excluded. CAN was diagnosed when two out of the four classical autonomic function tests were abnormal.
Results: In T1DM, multivariate logistic regression analysis, after adjustment for gender, age, central fat distribution, duration of diabetes and lipids demonstrated that the odds (OR, 95% confidence intervals) of CAN increased with factors such as onset of diabetes in the puberty [2.64 (1.02-6.30), *P*=0.02], microalbuminuria [2.48 (1.32-4.64), *P*=0.005], retinopathy [1.13 (1.04-1.41), *P*<0.001], smoking [1.14 (1.05-1.40), *P*=0.002] and measures such as higher HbA_{1c} values [1.54 (1.05-2.25), *P*=0.02], LDL-cholesterol [1.01 (1.00-1.24), *P*=0.02], triglycerides [1.01 (1.00-1.02), *P*=0.003] and systolic blood pressure [1.03 (1.00-1.05), *P*=0.01]. The same analysis in patients with T2DM, after adjustment for gender, age, blood pressure, lipids, and antidiabetic treatment demonstrated that central fat distribution [1.03 (1.00-1.03), *P*=0.05], longer duration of diabetes [1.04 (1.01-1.08), *P*=0.006], higher HbA_{1c} values [1.19 (1.02-1.38), *P*=0.02], LDL-cholesterol [1.01 (1.00-1.01), *P*=0.004], triglycerides [1.00 (1.00-1.00), *P*=0.05], retinopathy [1.34 (1.17-1.66), *P*=0.001], microalbuminuria [1.21 (1.12-1.35), *P*<0.001], smoking [1.25 (1.11-1.59), *P*=0.002] and severity of smoking (>20 cigarettes/day) [1.34, (1.15-1.71), *P*=0.004] were independently associated with higher odds of CAN.
Conclusion: In patients with T1DM, the risk of CAN is associated with onset of diabetes in the puberty, glycemic control, blood pressure, dyslipidemia, microalbuminuria, retinopathy and smoking, while in patients with T2DM with duration of diabetes, glycemic control, dyslipidemia, microvascular complications, central fat distribution and smoking.

176

Indices of cardiac autonomic function correlate with arterial stiffness in young normotensive and normoalbuminuric patients with type 1 diabetes

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Background and aims: Arterial stiffness is increased in type 1 diabetes (T1D), even before any clinical complications from the disease are evident. The diminished vascular compliance found in diabetes is believed to be multifactorial, with hyperglycemia playing a mandatory pathogenetic role. Conflicting data exist, however, regarding the relationship between cardiac autonomic function and arterial stiffness in T1D. The aim of this study was to investigate the association between cardiac autonomic function and large artery elasticity in young normotensive and normoalbuminuric T1D patients.
Materials and methods: Normotensive T1D patients, aged <40 years, without clinical evidence of nephropathy were consecutively selected from the outpatient diabetes clinic of our institution. Large artery stiffness was assessed by measurement of carotid-femoral pulse wave velocity (PWV). Cardiac autonomic function was assessed by the battery of the cardiovascular tests proposed by Ewing and Clarke. The heart rate response to slow, deep breathing, to Valsalva manoeuvre and to standing up were assessed from ECG recordings of RR intervals.
Results: Data from 66 individuals were analyzed (age 27.1±6.0 years, 31 [47%] men, diabetes duration 12.3±7.7 years, HbA_{1C} 7.4±1.5%). Mean PWV value was 5.6±0.9 m/sec, whereas mean expiration/inspiration (E/I) index

(an index of heart rate variability during deep breathing) was 1.33±0.16, mean 30:15 ratio (an index of heart rate change after taking the upright position) was 1.33±0.18 and mean Valsalva ratio (the ratio of the longest RR interval after the manoeuvre to the shortest interval during it) was 1.94±0.40. There was a strong negative correlation of PWV with E/I index, (r=-0.533 p<0.001) and a weaker one with 30:15 ratio (r=-0.275, p=0.025). In multivariate linear regression analysis, after taking account for confounding factors (i.e. age, sex, diabetes duration, arterial blood pressure, smoking, body mass index and waist to hip ratio), E/I index was the strongest predictor of PWV (standardized β-regression coefficient=-0.348, p=0.002). E/I index, age and waist circumference accounted for the 43.7% of PWV variability.
Conclusion: Cardiac autonomic function expressed as E/I index is a strong predictor of large arterial stiffness, in young normotensive and normoalbuminuric T1D patients.

177

Association between the abnormal test of sudomotor function (Neuropad®) and markers of increased cardiovascular risk and the chronic complications of diabetes

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Background and aims: We have explored the possible role of using a commercially available test of distal autonomic function (Neuropad®) in routine clinical practice by examining clinical and metabolic factors associated with an abnormal response to a test in a population of 499 patients with diabetes.
Materials and methods: 499 patients were recruited from those attending a routine specialist diabetes clinic. All were assessed for the quality of glycaemic control, cardiovascular risk factors and the presence of complications. Somatic sensory neuropathy was documented using the Neuropathy Disability Score, and autonomic neuropathy (sudomotor neuropathy) was determined on the sole of the foot using the Neuropad® response including time to colour change. Patients were divided into two groups: those with normal (pad pink at ten minutes) and abnormal (pad wholly or patchily blue).
Results: There were 151 patients with type 1 (72 male, mean age 41.2±13.7y; mean disease duration 15.4±10.3y) and 348 with type 2 (159 male, mean age 62.0±8.8y; mean disease duration 14.8±8.6y). NDS was >5 (abnormal) in 130 (26.1%) and no change in Neuropad colour by 10 minutes was observed in 121 (24.2%). An abnormal Neuropad test was observed in 70 (19.0% of 369) patients with a normal NDS. Mean time to complete Neuropad colour change was 10.8±7.3 mins in patients with an abnormal NDS, and 7.0±5.5 mins in those without neuropathy (p<0.001). Significant direct correlations were observed between an abnormal Neuropad test and age (r_s=0.31, p<0.001), waist circumference (r_s=0.17, p=0.003), BMI (r_s=0.25, p<0.001); significant negative correlations were observed with height (r_s=-0.17, p<0.001) and serum HDL cholesterol (r_s=-0.13, p=0.003). Patients with abnormal sudomotor function were older (60.8±10.5y) than those who were normal (54.0±14.9y, p<0.001), as well as having a longer disease duration (16.8±9.4 vs. 14.8±9.7, p=0.045), high BMI (29.4±5.8 vs. 27.4±2.5) and a higher prevalence of hypertension (54.6% vs. 42.1%, p=0.018). Patients with an abnormal sudomotor response also had a higher prevalence of maculopathy (29.8% vs 16.7%, p=0.008), symptomatic neuropathy (62.8% vs 48.5%, p=0.04), history of myocardial infarction and/or stent and/or bypass surgery (20.7% vs. 13.2%, p=0.05), peripheral arterial disease (9.1% vs 4.5%, p=0.04), history of foot ulcers (5.8% vs 0.8%, p=0.003) and major amputations (2.5% vs 0.0%, p<0.001).
Conclusion: These findings illustrate the close association between the finding of an abnormal test of sudomotor function, markers of increased cardiovascular risk and the presence of chronic complications of diabetes. There may be a place for inclusion of routine testing of sudomotor function in the routine assessment of people with diabetes. These preliminary data suggest that it may be more sensitive than the NDS in detecting distal neuropathy.

178

Role of oxidative stress in predicting the progression of somatosensory and cardiac autonomic nerve dysfunction in diabetic patients. A 6-year prospective study

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Background and aims: Oxidative stress is implicated in the pathogenesis of experimental diabetic neuropathy, but prospective studies in diabetic patients are lacking. We aimed to evaluate whether the plasma levels of various biomarkers of oxidative stress predict the progression of diabetic neuropathy and mortality over 6 years.
Materials and methods: We followed 89 diabetic patients (mean±SD; age: 54±14 years, diabetes duration: 12±10 years, HbA1c: 9.4±1.7%, 54% male, 69% type 2, 59% with polyneuropathy), 72 of whom underwent nerve function reassessment after 6.2±0.8 years, whereas 17 died after 4.2±1.0 years. Markers of oxidative stress in plasma at baseline included superoxide, hypochlorous acid, peroxynitrite (all ABEL assay), 8-iso-prostaglandin F2α, vitamin E/lipid ratio, and vitamin C. Neuropathy was assessed by symptoms and deficits, motor and sensory nerve conduction velocity (MNCV, SNCV), vibration perception thresholds (VPT), thermal detection thresholds (TDT), and heart rate variability (HRV).
Results: Despite a reduction in HbA1c by -1.4±1.6% (p<0.001), median SNCV, sural SNCV, peroneal MNCV, malleolar VPT, and warm TDT deteriorated from baseline to follow-up (all p<0.05). Multivariate regression analyses demonstrated that increased superoxide generation was associated with a decline in median SNCV (β=-0.997; p=0.036) and deterioration in HRV at rest (OR: 1.63 [95% CI: 1.09-2.44]; p=0.017) from baseline to follow-up. Low vitamin E/lipid ratio predicted a decrease in peroneal MNCV (β=0.760; p=0.036) and tended to relate to the change in malleolar VPT (β=-0.725; p=0.077), while vitamin C was associated with the change in cold TDT (β=-0.094; p=0.032), but after full adjustment, these effects did not persist. Superoxide (highest quartile) was associated with an increased risk of mortality (HR: 5.75 [95% CI: 1.59-20.88]; p=0.047).
Conclusion: Increased plasma superoxide generation predicted the decline in sensory and cardiac autonomic nerve function and mortality over 6 years in diabetic patients, whereas the predictive value of other oxidative stress markers was either less consistent or lacking.

179

The effect of glucose variability on the risk of peripheral and autonomic neuropathy in type 1 diabetes

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Background and aims: While the presence of an effect of glycemic variability on microvascular complications as retinopathy and nephropathy has been negated, it is unknown whether glycemic variability may influence neuropathy. In literature, it is suggested that the nervous system may be particularly susceptible to glucose variability. We analyzed data from the Diabetes Control and Complications Trial (DCCT) to assess whether glycemic variability is a risk factor for the development of peripheral or autonomic diabetic neuropathy.
Materials and methods: Seven-point glucose profiles were collected quarterly during the DCCT in 1441 individuals with type 1 diabetes. Peripheral neuropathy was assessed at baseline and at 5 years follow-up, autonomic neuropathy at baseline and 4 years follow-up. The effect of the glycemic variability, expressed as standard deviation (SD) and mean amplitude of glycemic excursions (MAGE) on the development of peripheral and autonomic neuropathy was assessed using a logistic regression model, with adjustment for age, sex, disease duration, treatment group and prevention cohort. Finally, the additional effect of glucose variability over the effect of HbA1c and mean glucose on neuropathy was computed using the same technique.
Results: Glucose variability, quantified by mean SD and mean MAGE, had no effect on the incidence of clinical neuropathy confirmed by autonomic

or electromyography (EMG) abnormalities (SD Odds Ratio [OR] 1.07, 95% Confidence Interval [CI] I 0.83-1.35, p=0.67; MAGE OR 1.06, CI 0.96-1.20, p=0.23) or clinical neuropathy alone (SD OR 0.95, CI 0.77-1.18, p=0.66; MAGE OR 1.01, CI 0.91-1.11, p=0.90). Glucose variability appeared to have a possible effect on overall autonomic dysfunction but not when adjusting for HbA1c or mean glucose (SD OR 1.08 and 1.09, CI 0.82-1.44 and 0.80-1.48, p=0.58 and 0.57 respectively; MAGE OR 1.09 and 1.10, CI 0.96-1.23 and 0.97-1.25, p=0.18 and 0.13 respectively). Only for one specific autonomic function parameter, beat-to-beat heart rate variation < 20 plus a Valsalva ratio > 1.5, the effect remained significant when adjusting for mean glucose (SD OR 2.64, CI 1.17-5.94, p=0.02; MAGE OR 1.42, CI 1.07-1.90, p=0.02), but not when adjusting for HbA1c (SD OR 1.84, CI 0.90-3.76, p=0.09; MAGE OR 1.30, CI 0.98-1.72, p=0.072).
Conclusion: Glucose variability does not appear to be an additional risk factor in the development of diabetic peripheral or autonomic neuropathy over and above HbA1c or mean glucose.

180

IGT-associated neuropathy and its possible involvement with metabolic syndrome
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Background and aims: Increasing epidemiological evidence implicates the prediabetic state of impaired glucose tolerance (IGT) as a risk factor for macrovascular complications. Recent studies also suggest a causal relationship between IGT and peripheral neuropathy. The clinical characteristics of IGT-associated neuropathy are not well understood. The aim of this study is to determine the prevalence of IGT-associated neuropathy, and to assess associations between peripheral sensory neuropathy (PSN) and each component of metabolic syndrome.
Materials and methods: We examined 150 patients with IGT (age: 54.7±10.0 years, BMI: 23.7±2.8, FBS: 102.4±10.3 mg/dl, HbA1c:5.3±0.3%, CVr-r at rest: 3.61±1.66%; M±SD) and 176 NGT (normal glucose tolerance) control subjects (age: 50.4±11.7, BMI: 22.5±2.8, FBS: 92.5±7.4, HbA1c: 5.1±0.3, CVr-r: 3.88±1.89). We determined the prevalence of PSN in each group. PSN was defined as the existence of neuropathic symptoms and tendon reflex abnormality and impaired vibratory sensation. Two abnormalities out of three were considered pathological. We also investigated associations between PSN and each component of metabolic syndrome (hyperglycemia: IGT & IFG, hypertension, dyslipidemia, waist circumference) in total subjects (N=326) using multivariate statistical analysis for significance testing.
Results: Significant differences were not found between IGT and NGT control subjects regarding the following parameters: age, BMI, FBS, HbA1c, CVr-r. Prevalence of PSN in IGT subjects assessed by our criteria was 18.0%, while that of PSN in NGT control subjects was 3.9%. The prevalence of PSN in IGT was significantly higher than that in control NGT subjects (p<0.001). In univariate analysis, PSN was significantly associated with age (p=0.0008), IGT (p=0.0002) and IFG (p=0.001) and tended to be significantly associated with hypertension (p=0.052) but not with sex, waist circumference and dyslipidemia. In multivariate analysis, PSN was independently associated with IGT (odds ratio 4.34) and IFG (4.47) and tended to be independently associated with hypertension (2.01), age (2.29), waist circumference (1.74) and sex (1.75).
Conclusion: The result that neuropathy is more common in patients with IGT than in normoglycemic controls strongly supports an association between prediabetes and neuropathy. Our data indicates that metabolic syndrome may be involved in the pathogenesis of IGT-associated neuropathy. Longitudinal studies are needed to establish the temporal relationship between IGT-associated neuropathy and diabetic neuropathy.

OP 31 Insulin sensitivity, adiposity and risk assessment

181

Independent effects of weight gain and fetal programming on metabolic complications in SGA adults
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Introduction: The relationship between a low birth weight and the development of insulin resistance (IR) and the Metabolic Syndrome (MS) has been well documented at different periods of life. But little is known about the progression of MS in young adults born small for gestational age (SGA), in particular on the respective roles of SGA and weight gain.
Aim: We have previously reported an increased gain of fat mass with ageing in young adults born SGA beyond the simple effect of time. We hypothesized that being born SGA would promote a marked progression of IR and MS not only reflecting this gain of fat mass but reflecting also fetal programming.
Subjects and methods: Subjects were selected from a community-based cohort where subjects were all born full-term either SGA (Birth Weight< 10th percentile) or appropriate for gestational age (AGA) (25th <BW<75th percentile). 1273 subjects were prospectively followed over 7.5 yr, representing 78 % of the initial cohort. Metabolic syndrome was assessed using the WHO definition.
Results:

	AGA (n=697)	SGA (n=576)	P value
Baseline observation			
Age (years)	22.1 ± 3.8	22.0 ± 3.8	0.78
Sex ratio % (M/F)	47/53	45/55	0.68
BMI (kg/m^2)	22.6 ± 3.7	22.4 ± 4.2	0.39
MS n (%)	1 (0.14)	15 (2.60)	*0.008*
HOMA-IR	1.02 ± 0.5	1.18 ± 0.9	*0.001*
Observation at follow-up			
Follow-up duration	7.5 ± 2.6	7.4 ± 2.5	0.49
BMI (kg/m^2)	24.1 ± 4.3	24.2 ± 5.3	0.65
Delta BMI (kg/m^2)	+ 1.5 ± 2.4	+ 1.8 ± 2.7	0.02
Fat mass (%)	22.1 ± 8.2	23.1 ± 8.9	0.04
HOMA-IR	1.27 ± 0.6	1.41 ± 0.8	*0.004*
Delta HOMA-IR	0.25 ± 0.6	0.24 ± 0.9	0.61/0.28*
MS n (%)	34 (4.9)	51 (8.9)	*0.05/0.08**

All comparisons are adjusted on gender, age, BMI, socio-economical status and family history of metabolic disorders. * Same model with delta BMI instead of BMI
Conclusion: At both visits, subjects born SGA are more insulin resistant and have a significantly higher prevalence of MS in comparison to those born AGA, despite a very steep progression of MS in the AGA group. SGA subjects remain more affected at 30 years of age following a higher gain in body size, which tends to overcast the effect of being born SGA. Our data suggest that being born SGA induces early metabolic disorders, further amplified by the weight gain with time when adults, both probably resulting from fetal programming. Moreover, the modest increase in IR contrasts with the constant and much higher prevalence of MS, suggesting that the metabolic complications are not related only to the degree of fat mass but that fetal programming strongly affects the physiology of the adipose tissue.

182

Fatty Liver Index (FLI) as a new cardiometabolic risk marker: validation in the RISC study

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Background and aims: FLI is a new index recently proposed as a predictor of the presence of fatty liver (FL) which includes in its formula waist, BMI, triglyceride and γGT levels (FLI>60 =likelihood >78% presence FL; FLI<20=likelihood >91% absence of FL). However, these parameters are also implicated in the development of diabetes, CHD and atherosclerosis. Aim of this study was to validate FLI *vs* MR spectroscopy and to evaluate in the RISC population if a high FLI score could predict metabolic alterations and presence of atherosclerosis.
Materials and methods: The RISC study recruited 1308 European subjects (age 30-60 years, BMI 20-45 kg/m^2) free of diabetes, hypertension and clinically manifest cardiovascular diseases (CVD). We evaluated FLI, metabolic profile, glucose tolerance (OGTT), peripheral insulin sensitivity (M/I, by euglycaemic-hyperinsulinaemic clamp), hepatic insulin resistance (HIR=Insulin*EGP measured with glucose tracer, n=400), beta cell function (by mathematical modelling of the C-peptide response to oral glucose), physical activity (PA, by accelerometry), coronary heart disease risk (CHD, by Framingham score), early carotid atherosclerosis (as intima-media thickness, IMT, by ultrasound). In a separate group of subjects (n=37), hepatic fat content (HF) was evaluated by MR spectroscopy and compared with FLI.
Results: Subjects with FLI<20 had no HF (range 0.4-4.2%, n=6), while those with FLI>60 had steatosis (*ie*, HF>5%, range 8.6-24.0%, n=10). FLI was well correlated with HF (r=0.61, p=0.0001). RISC subjects with FLI>60 - *vs* those with FLI<20 - were more insulin resistant at the whole-body level (M/I=88±3 *vs* 167±3 μmol·min^{-1}·kg$_{ffm}^{-1}$·pM^{-1}) and in the liver (HIR=737±51 *vs* 361±16 μmol·min^{-1}·kg$_{ffm}^{-1}$·pM) and beta cell glucose sensitivity was impaired (114±5 *vs* 142±4 pmol·min^{-1}m^{-2}·mM^{-1} , all p<0.0001). The RISC population was at low risk for CHD (only 9% of the subjects had medium-to-high risk, while 83% were below average). By multivariate analysis, 10-year CHD score was positively associated with BMI and waist, and negatively with PA and M/I (AdjR^2=0.24, all p<0.01). FLI was strongly related to CHD in univariate analysis (R^2=0.23) and remained so independently of PA (AdjR^2=0.26, p<0.0001). IMT was low on average (0.602±0.002 mm, range 0.38-1.02) and positively associated with age and established atherosclerotic risk factors (BMI, waist, systolic blood pressure (SBP), LDL, fasting plasma glucose) and negatively with HDL, physical activity, FLI and M/I (all p<0.0001). In a multivariate model, independent determinants of IMT were M/I (p=0.01), age, SBP, LDL and gender (all p<0.0001, AdjR^2=0.27). When FLI was added, it replaced M/I as an independent predictor of IMT (AdjR^2=0.27, p=0.0001).
Conclusion: In middle-aged non-diabetic subjects, high values of FLI were associated with increased insulin resistance, IMT and CHD risk; calculation of FLI from simple metabolic and anthropometric data can be a useful cardiometabolic risk marker.
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183

Relationships between hepatic stearoyl-CoA desaturase 1 activity and mRNA expression with liver fat content in humans

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Background and aims: The enzyme stearoyl-CoA desaturase (SCD) 1 has gained much interest as a future drug target to treat fatty liver and its metabolic consequences. However, there is little and inconsistent human data about expression and activity of this important enzyme. We, therefore, investigated the hepatic activity and expression of SCD1 and their relationships with liver fat content in human liver samples.
Methods: Fifteen subjects undergoing liver surgery were studied. SCD1 activity was estimated from the ratio of oleate (C18:1) to stearate (C18:0) and palmitoleate (C16:1) to palmitate (C16:0) within five lipid subfractions that were separated by thin layer chromatography. Furthermore, SCD1 mRNA expression and liver fat content were measured.
Results: Similar to previous studies, we observed a strong positive correlation between liver fat content and the C18:1/C18:0-ratio in the combined fatty acid (FA) fractions (r=0.98, p<0.0001), which could be interpreted as higher SCD1 activity with increasing liver fat content. However, the hepatic SCD1 mRNA expression did not correlate with liver fat content (r=0.4, p=0.13). To solve this conflicting data, we further analyzed the FA composition of five hepatic lipid subfractions and their contribution to total hepatic FA. With increasing liver fat content the amount of FA from the TG fraction increased (r=0.96, p<0.0001), whereas the FA from the phospholipid (PL) fraction remained unchanged (r=-0.13, p=0.28). These two lipid fractions contain 87±6% of the total hepatic FA and show a distinct FA composition. This results in a 18-fold higher C18:1/C18:0-ratio in TG than in the PL fraction. Supporting the SCD1 mRNA expression data, when the C18:1/C18:0-ratio of the TG fraction was analyzed, the strong correlation with liver fat content, observed in total hepatic FA, was absent (r=0.43, p=0.06; r=0.17, p=0.5 for C16:1/C16:0). This paradox may be due to the fact that with increasing liver fat content the proportion of FA from the TG fraction that has a high C18:1/C18:0-ratio increased. The analysis of total hepatic FA could therefore mislead to the interpretation that SCD1 activity is strongly associated with liver fat content.
Conclusion: We provide important novel information that SCD1 activity and mRNA expression are not elevated in subjects with high liver fat content and that the FA composition of lipid subclasses, rather than of mixed lipids should be interpreted to estimate SCD1 activity.
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184

Hepatic steatosis does not result in insulin resistance in subjects with familial hypobetalipoproteinaemia

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Background and aims: Hepatic steatosis is strongly associated with hepatic and whole body insulin resistance. Yet the causal relationship between hepatic steatosis and insulin resistance remains uncertain. Subjects with familial hypobetalipoproteinemia (FHBL) are characterized by low levels of apolipoprotein B and an increased risk of hepatic steatosis. However FHBL-patients are not characterized by disturbances in glucose metabolism. To investigate the role of liver fat in the development of insulin resistance we studied glucose metabolism in subjects with FHBL and matched controls.
Materials and methods: We measured endogenous glucose production (EGP) and hepatic and peripheral insulin sensitivity (Rd) in the basal state and during a two-step hyperinsulinemic euglycemic clamp using stable isotopes. We included 8 male subjects with established FHBL and 8 healthy controls, matched for sex, age, BMI, waist to hip ratio and physical activity. Hepatic triglyceride content (IHTG) was measured using magnetic resonance spectroscopy (STEAM, TE/TR=20/3000 ms, 6 acquisitions, voxel size 27 cm3). Intramyocellular lipid content (IMCL) was measured by magnetic resonance spectroscopic imaging (TE/TR=30/1100 ms, matrix size 32x32). Voxels inside the soleus muscle were selected for analysis.
Data are presented as median [minimum-maximum].
Results: IHTG was significantly higher in patients compared to controls (27 % [16-38] vs. 5 % [0-31] respectively, p= 0.045), while IMCL was similar in subjects with FHBL and controls (9.6 % [5.7-12.8] vs. 8.8 % [6.7-22.8] respectively).
Basal EGP and basal insulin levels did not differ. Insulin-mediated suppression of EGP as well as Rd were not different between patients and controls (61.2% [17.7-86.9] vs. 75.6% [43.6-82.8] respectively (p=0.48) and 40.6 μmol/kg.min [35.0-42.6] vs. 48.7 μmol/kg.min [24.9-71.8] respectively (p= 0.18)). Glucoregulatory hormones during the clamp were similar.
Conclusion: Subjects with FHBL and severe hepatic steatosis show no hepatic or peripheral insulin resistance. Our results indicate that hepatic steatosis *per se* is not sufficient to cause insulin resistance.

185

Rates of lipid fluxes in the adipose tissue *in vivo* after a mixed meal in morbid obesity

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Background and aims: Although insulin resistance in obesity is established, information on insulin action on lipid fluxes, in morbid obesity, is limited. This study was undertaken in morbidly obese subjects to investigate insulin action on triacylglycerol fluxes and lipolysis across adipose tissue (AD).
Materials and methods: A meal was given to 30 obese [age 34±1yrs, BMI 47±1kg/m^2] and 10 non-obese subjects (age 39±4yrs, BMI 23±1kg/m^2). Plasma samples for glucose, insulin, triglycerides and non-esterified-fatty-acids (NEFA) were taken for 360min from a vein draining the abdominal subcutaneous AD and from the radial artery. AD blood flow (BF) was measured with ^{133}Xe.
Results: In obese vs non-obese: (1) Arterial glucose was similar ($AUC_{0\text{-}360min}$ 2173±73 vs 2040±40mMmin), but insulin was increased (23578±2372 vs 10331±608mU/Lmin, p=0.0004) (2) AD BF was decreased (657±48 vs 1345±102ml/100mltissue, p=0001) (3) Arterial triglycerides (501±37 vs 260±32nmol/Lmin, p=0.0007) and NEFA (166±17 vs 97±11nmol/Lmin, p=0.017) were increased (4) Lipoprotein lipase (LPL) was decreased (5±2 vs 80±3pmol/100mlAD, p=0.009) (5) Arteriovenous triglyceride differences were similar (10±4 vs 19±6mmol/Lmin) (6) AD lipolysis (24±10 vs 10±4pmol$_{glycerol}$/Lmin), NEFA fluxes (34±11 vs 74±32μmol/100mltissue), veno-arterial NEFA differences (20.7±5 vs 21.3±10mmol/100mltissue) and total AD NEFA release (27±9 vs 11±6mmol) were similar.
Conclusion: in morbid obesity: (a) hypertriglycerinemia could be attributed to a defect in the postprandial dynamic adjustment of LPL, partly caused by blunted BF and (b) postprandialy there is a relative impairment of AD to buffer NEFA excess, despite normal NEFA output.

186

Evidence against the involvement of the mTOR/S6-kinase signalling pathway in obesity-associated insulin resistance of skeletal muscle

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Background and aims: The nutrient-sensitive kinase mammalian target of rapamycin (mTOR) and its downstream targets S6-kinase (S6K) and 4E-Binding Protein1 (4E-BP1) are known to mediate amino acid induced insulin desensitisation. Activation of this signalling cascade enhances serine phosphorylation of insulin receptor substrate-1, resulting in impairments of insulin signalling and glucose utilisation. As it is still unknown, whether the mTOR/S6K pathway is involved in obesity induced insulin resistance of human skeletal muscle, this study aimed to provide evidence for or against a link between obesity, the mTOR/S6K pathway and modulation of insulin signalling in man.
Materials and Methods: Morbidly obese insulin resistant patients (n=10) and healthy volunteers (n=7) were studied (age: 37.4±3.6 vs 35.7±5.2 years; BMI: 49.4±1.9 vs 23.8±0.7 kg/m^2; glucose infusion rate during euglycemic-hyperinsulinemic clamp: 1.7±0.3 vs 8.8±1.2 mg*kg^{-1}*h^{-1}; p<0.002). Blood was sampled and needle biopsies were collected from skeletal muscle in the basal state. Muscle samples were blotted, prepared free of blood, fat and connective tissue and snap frozen within 30 seconds in liquid nitrogen. The activity of S6K (relative amount of protein in phosphorylated state), the expression and serine phosphorylation of IRS-1 (Ser307; Ser636/639), the expression of 4E-BP1, p85-PI3-kinase (PI3K) and protein-kinase-B (PKB) as well as the expression and phosphorylation of AMP-kinase (AMPK), which also modulates mTOR/S6K activity, were determined by Western blot analysis. Results are given as means±SEM.
Results: Muscle biopsies from morbidly obese patients exhibited significantly increased serine-phosphorylation of IRS-1 on Ser307 (% increase vs controls: +602±223%, p<0.03), while phosphorylation on Ser636/639 showed a parallel, but not significant trend (+384±263%, ns). Total IRS-1 expression was not different between controls and obese patients (% of controls: 72.7±33.7%, ns) and the expression of downstream insulin signalling proteins PI3K and PKB did not vary between the two groups (% of controls: PI3K, 101.9±16.8%, ns; PKB, 85.6±14.5%, ns). Furthermore, S6K activity was similar in muscle from obese patients and healthy controls (S6K in phosphorylated state: 33±6% vs 31±8%; ns), suggesting that enhanced mTOR signalling is not the cause of obesity-induced IRS-1 serine phosphorylation. In line with this conclusion, a trend towards reduction rather than an increase of 4E-BP1 expression was observed in muscle from obese patients (% decrease vs controls: -35±17%, p=0.061, ns) and the amount of activated (phosphorylated) AMPK was unchanged (phosphorylated AMPK, % of controls, 73.8±29.6, ns).
Conclusions: The results show that obesity induced insulin resistance is associated with an increase of IRS-1 serine phosphorylation in skeletal muscle. Expression of total IRS-1 and downstream insulin signalling proteins PI3K and PKB is not altered in obesity, indicating that reduced insulin signalling is more likely due to phosphorylation/activation deficiencies than due to decreased total protein expression. No evidence was obtained for an involvement of the nutrient-sensitive mTOR/S6K signalling cascade in obesity associated muscle insulin resistance.

OP 32 Clinical immunology

187

Insulin administration may trigger type 1 diabetes in patients with type 2 diabetes having high-risk HLA class II

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Background and aims: Insulin administration causes various types of immune response to insulin, but there had been no reports that insulin administration triggers pancreatic β cell destruction, namely type 1 diabetes (T1DM) in patients with type 2 diabetes (T2DM). Recently, we first reported on 3 cases of T1DM developed after insulin administration. The objective of this study was to further collect such cases, and characterize these clinical, immunological and genetic background.

Material and methods: A total of 6 patients (4 men and 2 women) with T1DM developed after insulin administration were included in this study from diabetic clinics in Japan. Serum or urinary C-peptide, autoantibodies to glutamic acid decarboxylase (GAD65) and IA-2, insulin antibody, and HLA class II haplotypes were analyzed. Using ELISPOT according to the protocol by Moriyama et al., the secretion of Th1-associated interferon-γ and Th2-associated interleukin-4 from peripheral mononuclear cells upon stimulation with diabetes-related antigens, GAD65 protein and several important peptide fragments of insulin, were investigated in 4 cases. Pancreatic biopsy with laparoscope was performed by the specialist of pancreatic surgery in one case. The study was approved by the ethics committee of the Ehime University Hospital.

Results: T2DM was diagnosed at the average age of 42.6 (34-52) years in these cases. Mean maximum BMI was 30.2 (24.2-40.9) kg/m2. They all developed insulin deficiency 8.2 (2-17) months after insulin administration at the age of 59 (50-70) years, but they had neither received insulin therapy nor had autoantibody to GAD65 before insulin administration. Fasting serum C-peptide levels were 1.56 ± 0.68 ng/ml, and urinary C-peptide levels were 83 ± 13 μg/day before insulin administration. After the initiation of insulin administration, serum and urinary C-peptide levels were rapidly decreased to 0.09 ± 0.01ng/ml, and 5.3 ± 6.8 μg/day, respectively. Insulin allergy was developed in 3 cases, and high titer of insulin antibody was observed in 3 cases after insulin administration. Autoantibodies to GAD65 and IA-2 were not detected in 3 cases through their entire clinical course, but were transiently and weakly positive in 2 cases. In one case, high titer of GAD antibody was observed immediately after the development of T1DM. They all had T1DM high-risk HLA class II haplotypes in Japanese. GAD-reactive and insulin-peptides-reactive Th1 cells were identified in 2 of 4 cases. In a histological examination of pancreatic tissues obtained by a pancreatic biopsy in one case, typical insulitis was observed.

Conclusion: The present study suggests that insulin administration may trigger TIDM in patients with T2DM having T1DM high-risk HLA class II haplotypes, and that autoreactive T cells may contribute to the development of T1DM.

188

Interim results of a phase I/II clinical trial of a DNA plasmid vaccine (BHT-3021) for type 1 diabetes

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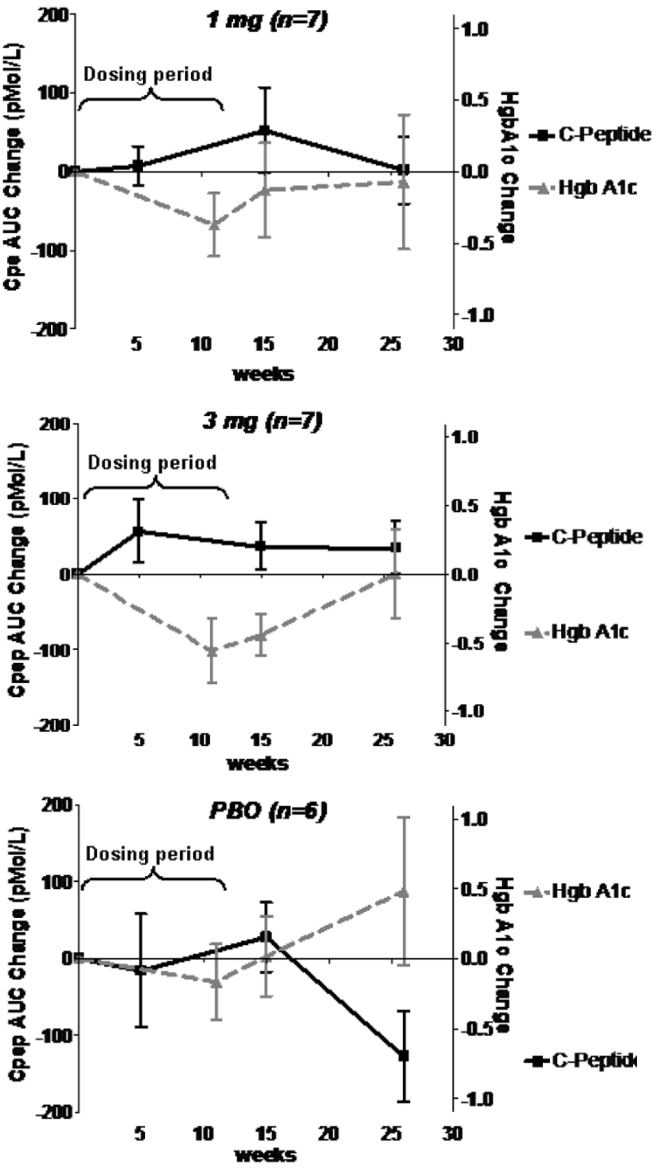
Background and aims: DNA plasmid vaccines are a highly effective antigen-specific treatment of autoimmune disease in various animal models and in a multiple sclerosis clinical trial.

Materials and methods: BHT-3021 is a DNA plasmid that encodes full-length human proinsulin, currently in a phase I/II, randomized, blinded, placebo-controlled, dose escalation trial in T1D patients (0.3 mg, 1 mg, 3 mg or 6 mg), in which BHT-3021 or saline placebo is injected intramuscularly on a weekly basis for 12 weeks.

Results: Complete safety and pancreatic function data to 6 months after randomization are available for the first two dose cohorts (1 mg and 3 mg). Nine C-peptide positive patients were enrolled in the first cohort and eleven in the second cohort, randomized 2:1 active to placebo. Adverse event (AE) and laboratory data on these patients suggest that BHT-3021 is safe and well-tolerated. No serious adverse events have been reported in patients receiving BHT-3021. Most AEs were mild or moderate in severity.

Treatment with BHT-3021 may preserve ß-cell function as measured by C-peptide levels. C-peptide levels were maintained at six months after randomization in patients treated with 1 mg (mean increase of 2 pmol/L from baseline) and with 3 mg of BHT-3021 (mean increase of 34 pmol/L from baseline). In contrast, C-peptide levels declined in placebo treated patients (mean decrease of -127 pmol/L from baseline). At fifteen weeks after randomization, there was a corresponding decline in mean HbA1c levels of 0.13% Hb with 1 mg and of 0.44% Hb with 3 mg of BHT-3021. Patients treated with placebo had no substantial change in HbA1c levels at this time point.

Conclusion: These preliminary data indicate that 1mg and 3mg of BHT-3021 is safe and may preserve ß-cell function as well as improve glycemic control. Complete 6 month data on 0.3mg and 6mg of BHT-3021 are expected by June 2009.



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189

Autoimmunity and obesity in children and young adolescents

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Background and aims: A few obese youths with diagnosis of type 2 diabetes have evidence of islet-cell autoimmunity. The increasingly 'obesogenic' environment which favours insulin resistance could account for the development of islet cell autoimmunity through different mechanisms. Blood glucose concentrations are controlled by a loop incorporating two components, the beta cells which secrete insulin and the insulin sensitive tissues (liver, muscle, adipose) which respond to it. The accelerator hypothesis identifies three processes which variably accelerate beta cell loss: constitution, insulin resistance and the immune response to it. None of the accelerators leads to diabetes in the absence of weight gain, a condition which may explain the rising incidence of all diabetes in the developed and developing world. Raised blood glucose accelerates beta cell apoptosis (glucotoxicity) and, by increasing beta cell immunogenicity, further accelerates apoptosis in subjects genetically predisposed to intense immune response. Accordingly, body mass is central to the development and rising incidence of all types of diabetes including type 1 diabetes (T1D).
Materials and methods: In order to throw new light on this hypothesis, we have investigated pancreas, thyroid and gastric autoimmunity in a population of obese children/young adolescents with normal glucose tolerance (n=251; 109 males and 142 females), impaired glucose tolerance (IGT) (n=113; 53 males and 60 females) and type 2 diabetes (T2D) (n=15; 4 males and 11females). These subjects had a BMI ≥ 30 and their mean age was 13.6, 13.4 and 13.7 years, respectively.
Results: Mean basal/2 hours post glucose insulin levels were 32/168, 41/320 and 58/454 uU/ml, in normal, IGT and T2D subjects, respectively. GAD autoantibodies were detected in 6/251 (2.4%) obese subjects but in none of IGT or T2D subjects. Insulin autoantibodies were detected in 1 obese subject whereas IA-2 autoantibodies were negative in all subjects tested. Furthermore, we detected anti-TPO (thyroperoxidase) autoantibodies in 3/6 GAD positive subjects. Finally anti gastric parietal cells and anti 21-hydroxylase autoantibodies were negative in all subjects tested.
Conclusion: Considering that GAD autoantibodies are detectable in 0.5% of control school children with normal body weight, we conclude that obesity may lead to a significant increase of islet cell autoimmunity in normal youths, a finding in line with the accelerator hypothesis.

190

Zinc transporter 8 autoantibody profiles in patients with recently diagnosed type 1 diabetes

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Background and aims: Zinc transporter 8 (ZnT8) has been identified as a new target-antigen for autoantibodies in type 1 diabetes. The specificity of ZnT8 autoantibodies (ZnT8A) can vary with respect to binding of two ZnT8 allelic variants carrying at amino acid 325 either arginine (R325) or tryptophan (W325). This study was to determine ZnT8A profiles in patients with recent-onset type 1 diabetes.
Materials and methods: ZnT8A were measured in sera of 202 young patients from Germany with recently diagnosed diabetes (median time from diagnosis 1 day, IQR 0-7; median age 8.8 years, IQR 5.2-12.8, range 1.2-18.9) using Protein A-based radiobinding assays and COOH-terminal constructs of the R325 or W325 variants of human ZnT8. Autoantibodies to glutamate decarboxylase (GADA) and insulinoma-associated protein 2 (IA-2A) were also measured in all samples, as were insulin autoantibodies (IAA) in samples from patients obtained within the first two weeks of diagnosis.
Results: ZnT8A were detected in 122 (60%) patients, among them 121 of 176 (69%) with positive other islet autoantibodies (GADA, IA-2A, IAA) and 1 of 26 patients who were negative for other autoantibodies (P<0.0001). Age at diagnosis did not differ between autoantibody-positive and negative patients (P=0.9). Among the 122 ZnT8A positive patients, 41 (34%) had antibodies that only bound the ZnT8 R325 variant, 11 (9%) had antibodies that only bound with the ZnT8 W325 variant, and 70 (57%) had antibodies that bound with both ZnT8 R325W variants. Prevalence of ZnT8A restricted to one of the ZnT8 variants was not associated with age at diagnosis (P=0.9). ZnT8A were more often found in patients who already had autoantibodies against two or more islet autoantigens other than ZnT8: 25 of 45 (56%) patients previously classified as single autoantibody-positive were ZnT8A-positive, as compared to 96 of 131 (73%) with multiple autoantibodies (P=0.03).
Conclusion: Autoimmunity against the COOH-terminal region of ZnT8 is a common feature in young patients with recent-onset type 1 diabetes. ZnT8A are more often found in patients with a more widespread antibody-response to multiple islet autoantigens. Testing for ZnT8 antibodies should be performed against the two major polymorphic variants.
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191

Human mesenchymal stem cells modulate cellular immune response to islet antigen GAD in type 1 diabetes

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Background and aims: MSCs can exert an immunoregulatory activity, modulating several T cell functions, and exerting a profound immunosuppressive effect on virtually any component of the immune system, in a MHC-independent way. Studies on the immunomodulatory potential of MSC in type 1 diabetes, a Th1-mediated autoimmune disease, are lacking. We aimed to evaluate whether human bone marrow-derived MSCs may inhibit islet antigen specific T cell activation in type-1 diabetes. To elucidate the MSC immunoregulatory activity, *in vitro* co-culture experiments with GAD-challenged PBMCs obtained from type-1 diabetic patients were performed and the number of IFN-gamma producing T cells and the release of IFN-gamma were evaluated. To investigate the underlining mechanisms, changes in T cell cytokine profile, to dissect a pro-inflammatory or anti-inflammatory phenotype, and the role of PGE2 were explored.
Materials and methods: IFN-gamma, IL-4 and IL-10 ELISPOT responses to GAD in 9 new-onset type 1 diabetic patients were evaluated in PBMC cocultures and parallel PBMC-hMCSs cocultures. A SI (ratio of mean spot number in the presence of GAD to number in the presence of diluent) ≥ 3 was chosen as positive in ELISPOT assay. Levels of prostaglandin E2 (PGE2), IFN-gamma, IL-4 and IL-10 in supernatants were measured by ELISA. PGE2 inhibition experiments with NS-398 and indomethacin were also performed.
Results: By IFN-gamma ELISPOT, 5 diabetic patients showed a positive response to GAD. The presence of MSCs (1:1 ratio) resulted in a decrease in the number of spots in the IFN-gamma ELISPOT in the presence of GAD (mean spots without MSCs 53.8 ± 32, with MSCs 11.8 ± 8, $p < 0.05$). Coculture transwell experiments, in which MSCs were separated from PBMCs, failed to inhibit the response, indicating that the MSC effect required a physical interaction. Further, supernatants from MSCs did not inhibit the response to GAD. MSCs incubation with the PGE2 synthesis inhibitors abrogated the immune suppressive effect on GAD induced IFN-gamma T cell response. In cocultures, IL-10 secreting cells were not detectable by ELISPOT in response to GAD whereas IL-4 secreting cells were detected. This was confirmed by ELISA that showed that hMCSs significantly inhibited the IFN-gamma production, and increased the IL4 and PGE2 production. Inhibitors of PGE2 abrogated MSCs-mediated immune modulation.
Conclusion: In the present study we found that allogenic human MSCs can abrogate *in vitro* a pro-inflammatory Th1 response to an islet antigenic stimulus, in new onset diabetic patients. The contact between T cells and MSCs in all patients responding to GAD consistently abrogated the T cell production of IFN-gamma, assessed *in vitro* at the single cell level by ELISPOT and inhibited IFN-gamma secretion. MSCs induced in PBMC of responder patients IL-4 producing cells and IL-4 secretion, suggesting a possible switch to an anti-inflammatory Th2 signalling of T cells. Moreover, we found that the contact between MSCs and PBMC is required and enhanced PGE2 production.
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192

Adipose tissue development in non-obese diabetic mice is controlled by the Toll-like receptor 4

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Background and aims: Recent studies point to an effect of body weight development on the progression of type 1 diabetes (T1D). The body weight is largely determined by the adipose tissue mass, which mainly consists of adipocytes, a cell type sharing basic features with innate immune cells. Activation and differentiation of innate immune cells are under strict control of Toll-like receptors (TLR) which mediate the recognition of antigenic microbial structures but may also be involved in the induction of autoimmunity. In a recent study we observed that TLR4, a prominent member of the TLR family, has a significant impact on the pathogenesis of autoimmune diabetes in non-obese diabetic (NOD) mice. Based on these considerations the current study was designed to investigate whether TLR4 is able to modulate the progression of insulin deficiency diabetes by controlling adipose tissue development in the NOD mouse.
Materials and methods: Our studies were performed in NOD mice carrying the spontaneous TLR4 defect of the C57BL/10ScCr ($TLR4^{-/-}$) mouse and in the non diabetes-prone mouse strain C57BL/10ScSn ($TLR4^{+/+}$) and its related, TLR4-deficient strain C57BL/10ScCr. The development of diabetes and body weight was monitored and thin sections of visceral adipose tissue were evaluated morphometrically. Isolated adipocytes were differentiated in vitro in the presence of the TLR4 ligand lipopolysaccharide (LPS). LPS-responsiveness of the cells was assessed by the release of interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) as determined by ELISA.
Results: Female NOD $TLR4^{-/-}$ mice showed a significant acceleration of diabetes development ($p<0.01$). Until the age of 21 weeks 8.3 % of NOD $TLR4^{+/+}$ mice but 71.4 % of NOD $TLR4^{-/-}$ mice developed diabetes. Accelerated diabetes development was associated with significantly increased body weight ($p<0.0001$). At the age of 21 weeks the body weight of NOD $TLR4^{+/+}$ mice was 25.4 ± 1.4 g and of NOD $TLR4^{-/-}$ 27.5 ± 2.3 g. In female mice of the C57BL/10Sc strain TLR4 had no significant effect on body weight development. At the age of 21 weeks C57BL/10ScSn ($TLR4^{+/+}$) mice weighed 27.5 ± 2.4 g and C57/BL10ScCr ($TLR4^{-/-}$) mice weighed 27.4 ± 1.8 g. Histological analyses of adipose tissue revealed smaller adipocyte sizes (668 ± 413 μm^2) in NOD $TLR4^{-/-}$ mice when compared to the tissue of NOD $TLR4^{+/+}$ mice (963 ± 776 μm^2) ($p<0.01$). Compared to NOD $TLR4^{+/+}$ mice the adipose tissue of NOD $TLR4^{-/-}$ mice contained a 2.9 - 4.1 fold higher proportion of cells with the morphology of immature preadipocytes including multiple lipid droplets. Preadipocytes isolated from NOD $TLR4^{+/+}$ mice express TLR4 and showed a dose- and time-dependent release of the inflammatory mediators IL-6 and MCP-1 in response to LPS. NOD $TLR4^{-/-}$ adipocytes were largely unresponsive to LPS. The potential effect of TLR4 on the development of the inflammatory activity of mature NOD mouse-derived adipocytes was investigated by continuous LPS-exposure of cells during in vitro differentiation. Chronic TLR4 activation during adipocyte differentiation significantly reduced LPS-induced IL-6 release from 1.9 ± 0.1 to 1.1 ± 0.1 ng/ml and MCP-1 release from 57.3 ± 14.0 to 45.4 ± 9.1 ng/ml.
Conclusion: Our results identify TLR4 as a regulator of the differentiation and the inflammatory activities of adipocytes in NOD mice. The findings further implicate that TLR4 affects the progression of autoimmune diabetes in NOD mice by controlling the development of adipose tissue.
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OP 33 Incretins

193

Activation of the CaMKK/CaMKIV cascade by exendin-4 stimulates ABCA1 expression in pancreatic beta cells

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Background and aims: The insulin secretagogue hormone GLP-1 (glucagon-like peptide-1) and its long-acting agonist exendin-4 are new treatment agents for diabetes. GLP-1 stimulates glucose-dependent insulin secretion and lowers blood glucose levels. ATP-binding cassette transporter A1 (ABCA1) is a pivotal regulator of lipid efflux from cells to apolipoproteins and plays an important role in reverse cholesterol transfer. Recent reports indicate that ABCA1 in pancreatic βcells influences insulin secretion and glucose homeostasis. This study investigates whether exendin-4 which medicates stimulatory effects on ABCA1 gene expression, could interfere with the Ca2+/calmodulin (CaM)-dependent protein kinase (CaMK) cascade.
Materials and methods: ABCA1 expression was examined by real-time polymerase chain reaction (PCR), Western blot analysis, and reporter gene assay in rat insulin-secreting INS-1 cells incubated with exending-4. CaMKIV activity was assayed by detection of activation-loop phosphorylation (Thr196) of CaMKIV. We investigated the influence of the constitutively active form (CaMKIVc) or dominant-negative mutant (CaMKIVdn) of CaMKIV on ABCA1 promoter activity.
Results: Increased abundance of ABCA1 protein was noted in response to rising concentrations of exendin-4 with maximum induction at 10 nM. Real-time PCR analysis showed at significant increase in the abundance of ABCA1 mRNA in response to rising concentration of exendin-4. Exendin-4 also stimulated ABCA1 promoter activity, but failed to do so in the presence of STO-609, a CaMKK inhibitor. Next we examined CaMKIV, one of the downstream effecter kinases of CaMKK to determine which downstream effecter kinases of CaMKK is imvolved this mechanism. Upregulation of CaMKIVc phosphorylation (at Thr196) peaked after 10 min of exposure to exendin-4. CaMKIVc enhanced or upregulated ABCA1 promoter activity in INS-1 cells. Furthermore, cotransfection of the CaMKIVdn significantly suppressed the exendin-4-upregulated activity of the ABCA1 promoter.
Conclusion: Activation of the CaMKK/CaMKIV cascade by exendin-4 stimulated ABCA1 gene transcription, indicating that exendin-4 plays an important role in insulin secretion in pancreatic β cells.

194

Effect of GLP-1 and Exendin-4 on GLUT-2 and GLUT-4 expression in insulin-resistant state

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Background and aims: GLP-1 is an incretin with andiabetic properties, that acting through specific receptors directly stimulates glucose transport and metabolism in extrapancreatic tissues -liver, muscle and fat-, where also controls the expression of the main glucotransporter, in normal and diabetic states. Exendin-4 (Ex-4), structurally homologous to GLP-1 but more stable when in plasma circulation, shares with GLP-1 many of its glucoregulatory and other actions. Here we studied the effect of GLP-1 and Ex-4, in prolonged treatment, on GLUT-2 and GLUT-4 expression in liver and muscle, respectively, of insulin-resistant (IR) rats, compared to normal (N).
Materials and methods: IR model was induced in male Wistar rats by chronic feeding -8 weeks- with standard chow combined with D-fructose (20% in the drinking water). IR (n=5-10 rats/group) and N (n=5-10 rats/group) rats were 3-days treated with saline (control), GLP-1 (0.86 nmol/kg/h) or Ex-4 (0.1nmol/kg/h); blood samples were taken before and by the end of the treatment for plasma glucose and insulin (RIA) measurements; GLUT-2 and GLUT-4 expression -protein, by Western blot and mRNA, by RT-PCR- was respectively studied in liver and muscle; liver glycogen content was enzymaticaly measured.
Results: In the muscle, GLUT-4-protein in IR was higher than normal (243±39% N-control, $p<0.05$), and so it was the mRNA (3.8±1.0 times N-control, $p<0.05$); in N, GLP-1 treatment slightly reduced GLUT-4-protein (77±8% N-control, $p<0.05$) and increased the mRNA (12.5±2.9 times N-control, $p<0.01$), while in IR, GLP-1 stimulated both GLUT-4-protein

(383±59% IR-control, $p<0.02$) and mRNA (15.7± 3.5 times IR-control, $p<0.01$); Ex-4 treatment in N stimulated both GLUT-4 protein (122±2% N-control, $p<0.001$) and mRNA (1.6±0.2 times N-control, $p<0.02$), and further increased the protein value in IR (176±27% IR-control, $p<0.05$) as well as the mRNA (30±10 times IR-control, $p<0.01$). In the liver, GLUT-2 protein in IR was lower ($p<0.01$) than normal (66±8% N-control) while the mRNA was higher (8.1±1.4 times N-control, $p<0.001$); in N, GLP-1 treatment decreased the GLUT-2-protein value (81±3% N-control, $p<0.05$) and also the mRNA (0.43±0.13 times N-control, $p<0.05$), confirming previous observations, while Ex-4 induced a stimulation in the protein (174±18% N-control, $p<0.02$) without altering the mRNA; in IR, GLP-1 and Ex-4 both increased GLUT-2 protein (GLP-1: 223±28% IR-control; Ex-4: 187±17%, both $p<0.01$), and in a very high extent, the mRNA (overall mean: 567±42 times IR-control). Liver glycogen content in IR (52±4 µg/mg protein) was much lower ($p<0.001$) than that in N (395±34 µg/mg protein); neither GLP-1 nor Ex-4 treatment modified the value in N, but both similarly increased that in IR (GLP-1: 73±6 µg/mg protein, $p<0.01$ *vs* IR-control; Ex-4: 88±13 µg/mg protein, $p<0.05$ *vs* IR-control).

Conclusion: The increase in the reduced liver glycogen content of IR rats, after GLP-1 or Ex-4 treatment, could be accounted by their stimulating effect upon GLUT-2 gene expression, at the transcriptional and translational level. The previously reported normalizing action of GLP-1 and Ex-4 upon muscle glucose transport could also be due to their increasing effect on GLUT-4 expression.

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195

Oleic acid ingestion increases its concentration in the terminal ileum and directly stimulates glucagon-like peptide-1 secretion in protein kinase Cζ-dependent manner

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Background and aims: Luminal nutrients, especially long-chain fatty acids (e.g. oleic acid; OA) increase secretion of the insulinotrophic hormone, glucagon-like peptide-1 (GLP-1) from the intestinal L cells in the terminal ileum and colon. However, it is currently not known whether OA ingestion causes a sufficient increase in distal luminal OA as required for GLP-1 secretion. Furthermore, our lab recently proposed a protein kinase Cζ (PKCζ)-dependent mechanism for OA-induced GLP-1 secretion in vitro; however, its in vivo relevance remained unknown. Therefore, we determined both luminal and tissue OA concentrations in fasted and OA-fed rats, and examined the effects of direct OA-stimulation on GLP-1 secretion using a novel model of intestinal PKCζ knockdown.

Materials and methods: To assess the effects of OA ingestion, fasted Wistar rats (150-250g) received an oral gavage (OG) with 2.5 ml OA solution (90% pure). The rats were sacrificed at 0-120min, followed by collection of plasma samples, and chyme and tissue samples from the terminal ileum. The lipid fraction was analyzed by flame-ionisation chromatography. For PKCζ knockdown, we developed a PKCζ siRNA- and GFP-expressing adenovirus; the knockdown efficiency was verified in vitro by western blot of GLUTag cells, an L cell line. The feasibility and safety of intracolonic infusion (ICI) were assessed by barium ICI and X-ray. Therefore, ICI of OA was performed in fasted rats to directly stimulate the intestinal L cells, followed by blood collection at 0-60min. Some rats received prior ICI of 10^9 pfu/ml PKCζ siRNA-adenovirus to induce intestinal knockdown, followed by 3 days recovery and then ICI of OA. Plasma glucose, insulin and bioactive GLP-1 levels were detected by glucose analyzer, ELISA and ECL-based bioassay, respectively.

Results: Chyme from the fasted rats contained 0.75±0.39µmol/g OA; this was slightly increased at 20 (0.90±0.15µmol/g) and 40 min (1.03±0.16µmol/g) after OG, and was markedly elevated after 60 (105.4±50.0µmol/g, $p<0.01$) and 120 min (52.6±48.8µmol/g, $p<0.05$). While the tissue OA content did not change during the 2hr, OA plasma levels increased after OG, from 37.0±8.3µM in fasted rats to 80.2±20.5µM after 60 and 81.5±16.7µM after 120 min ($p<0.05$). To evaluate the direct effects of OA on GLP-1 secretion, 125 mM (124 µmol/g) of OA was infused into the colon and terminal ileum of anaesthetized, sitagliptin-treated rats. OA infusion did not affect glucose or insulin levels; however, GLP-1 plasma levels increased from 19.7±6.2pg/ml in fasting rats to 68.6±15.9pg/ml after 30 ($p<0.05$ vs. basal and control) and 102.2±21.3pg/ml after 60 min ($p<0.01$ vs. basal and control). Furthermore, pretreatment of rats with the PKCζ siRNA-adenovirus, induced intestinal GFP expression, and caused up to a 75% reduction in the GLP-1 secretory response to ICI of OA.

Conclusion: Following oral ingestion, OA levels in the terminal rat gut are sufficient to induce GLP-1 secretion. Furthermore, PKCζ is necessary for OA-induced GLP-1 secretion in vivo, thereby supporting the role of PKCζ as a novel therapeutic target to enhance GLP-1 levels in patients with type 2 diabetes. Finally, adenovirus-mediated intestinal knockdown is a promising technique to investigate intestinal physiology when knockout models are not viable or available.

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196

Sugar detection by GLUT2 is involved in GLP-1 secretion and incretin effect

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Background and aims: GLUT2 is a sugar transporter but also a membrane receptor sensitive to variation of extracellular sugar concentrations. It triggers a signalling cascade indicating to the cells how to adapt functions to sugar environment. We created a transgenic mouse with impaired signalling downstream GLUT2 (GLUT2-SDD sugar detection deficient mice) and demonstrated *in vivo* the importance of this signalling pathway in the regulation of glucose homeostasis, renal glucose re-absorption and insulin secretion in response to glucose (Stolarczyk *et al.*, PLOSone 2007). GLP-1 is secreted by intestinal L-cells after nutrient ingestion and increased insulin secretion from pancreatic beta-cells during hyperglycemia contributing to the incretin effect. The aim of the present study was to investigate the impact of sugar sensing triggered by GLUT2 on GLP-1 production by enteroendocrine cells in GLUT2-SDD mice.

Materials and methods: Isoglycaemic oral (3.6 g/Kg) and intraperitoneal (ip) glucose administration were performed on 10-week-old GLUT2-SDD and control mice fasted for 24h. The same mice were used for both types of glucose tolerance test (oral and ip) with an interval of one week. GLP-1 and insulin secretion responses were measured during oral and ip tests using multiplex technology. Intestinal GLP-1 content was determined by conventional ELISA. L-cell morphology was evaluated in intestine of the two groups of mice by GLP-1 immunofluorescence.

Results: Glucose intolerance was observed in GLUT2-SDD mice only in response to oral glucose load. Indeed, ip glucose injection induced a similar response in GLUT2-SDD and control mice. The glycaemia areas under curve were respectively in control and transgenic mice 670±50 and 930±50 $mg.dl^{-1}.min^{-1}$ ($p<0.05$) during the oral load, whereas they were 1410±90 and 1610±100 $mg.dl^{-1}.min^{-1}$ during ip administration. As expected the comparison between oral *versus* ip glucose administration in control mice showed that insulin secretion was higher during the oral test than during the ip test (area under curve respectively 220±20 and 101±16 pM. min^{-1} $p<0.01$). In contrast, no difference in insulin secretion was observed in transgenic mice in response to oral compared to ip glucose administration (area under curve respectively 140±20 and 120±20 pM. min^{-1}), suggesting a loss of incretin effect in GLUT2-SDD mice. In accordance with these results, GLP-1 secretion in control mice was increased 5 minutes after oral glucose load but not after ip administration ($p<0.05$) whereas the oral glucose-induced GLP-1 secretion was absent in transgenic mice. L-cell morphology and intestinal GLP-1 content appeared preserved in GLUT2-SDD mice compared to controls, suggesting that glucose detection by GLUT2 is involved in GLP-1 secretion but not in GLP-1 production since intestinal hormonal content is not impaired.

Conclusion: These findings show that insulin response to oral glucose partly relies on efficient secretion of GLP-1, which depends on adequate sugar detection by GLUT2 in the intestine.

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197

GIP and GLP-1 normalize glucose tolerance by normalizing islet adaptation to insulin resistance without increasing glucose effectiveness in high-fat fed mice

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Background and aims: Insulin resistance is adaptively compensated by an increase of insulin secretion and glucose effectiveness. However, these ad-

aptations are inadequate in mice after high-fat feeding that yields markedly reduced glucose elimination after intravenous glucose, because of insufficient hyperinsulinemia and glucose effectiveness. We investigated whether these defective adaptations can be normalized by the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1).

Materials and methods: C57BL/6J mice were fed a normal chow (ND, 11%) or a diet with 60% fat for 8 weeks (HD). After a baseline blood samples was taken, a bolus injection of glucose at 35 g/kg with or without addition of synthetic GIP or GLP-1 (at 0.03, 0.3 or 3 nmol/kg) was given intravenously followed by six blood samples taken within 50 (normal diet) or 75 min (high-fat diet) for analysis of glucose and insulin. Data were analyzed with minimal model that provides insulin sensitivity index (S_I) and glucose effectiveness (S_G). Insulin secretion was assessed as the increase in insulin levels during the first 5 min after injection (dAIR), and glucose elimination was estimated as the percent reduction of glucose (after logarithmic transformation) during the first 20 min after administration (K_G). Beta-cell adaptation was calculated as S_I×dAIR (disposition index, DI) and beta-cell function as delta peak insulin/delta peak glucose.

Results: K_G was lower in HD fed mice (P<0.001). At 0.03 nmol/kg, neither GIP nor GLP-1 significantly affected K_G in HD; at 0.3 nmol/kg, the incretins significantly increased K_G, but not to normal values. However, at 3 nmol/kg, K_G was completely normalized by both GIP (2.28±0.22, n=11) and GLP-1 (1.96±0.24, n=17). This was associated with markedly increased dAIR and beta-cell function (p<0.001); S_I was not affected, but the resulting DI was completely normalized. In contrast, S_G, which was increased by HD, was slightly reduced, but not normalized, by the incretins.

Conclusion: GIP and GLP-1 completely normalize the glucose elimination in severe insulin resistance in mice after high-fat feeding by normalizing the disposition index; this derives from marked upregulation of the islet adaptation to insulin resistance without any effect on insulin sensitivity and with no improvement of insulin-independent mechanisms of glucose elimination.

Metabolic parameters

	ND (n=48)	HD (n=49)	HD with incretins (n=28)
Body weight (g)	22.1±0.2	35.6±0.9	36.6±1.2
K_G (% min^{-1})	1.96±0.12	1.35±0.10	2.10±0.20
S_I (min^{-1}/(pmol/l))	1.14±0.09	0.51±0.06	0.56±0.04
dAIR (pmol/l)	1096±69	594±64	2207±151
DI	12.8±1.3	2.8±0.4	12.7±1.2
S_G (min^{-1})	0.040±0.002	0.096±0.010	0.074±0.006
beta-cell sensitivity	134±9	69±7	273±26

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198

Metformin activates the AMPK pathway and improves survival of murine and human L-cells but does not directly increase GLP-1 secretion

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Background and aims: Intestinal L-cells secrete the hormone glucagon-like peptide-1 (GLP-1) in response to nutrients and other secretagogues; GLP-1 then increases both insulin secretion and satiety. GLP-1 analogs and degradation inhibitors are therefore now in use as therapy for type 2 diabetes. Metformin, a well-established anti-diabetic drug, is known to increase circulating GLP-1 levels in humans through a mechanism that does not involve decreased GLP-1 degradation but may, possibly, include increased release of GLP-1, enhanced L cell proliferation and decreased L cell apoptosis.

Materials and methods: Human (NCI-H716) and mouse (GLUTag) L-cell lines were incubated for 2 hr with medium alone, oleoylethanolamide (OEA: 5-20μM; a novel GPR119-dependent GLP-1 secretagogue, positive control), metformin (5-150μM) or the AMPK-activator AICAR (0.1-1mM; positive 'AMPK' control) prior to RIA for GLP-1 secretion. Activation of the AMPK pathway was verified in both cell lines by presence of phospho-AMPK (normalized to total AMPK), as detected by western blot. To assess L cell survival, GLUTag cells were treated for 24 hr with metformin (150μM), AICAR (1mM) or OEA (10μM), followed by induction of apoptosis with cytokines or staurosporine, and immunoblot for cleaved Caspase-3, an early marker of apoptosis. L cell proliferation following the same treatment (metformin, AICAR or OEA) was assessed by 3H-thymidine incorporation.

Results: Treatment of NCI-H716 or GLUTag cells with different concentrations of metformin or AICAR did not increase GLP-1 secretion (n=6-9); in contrast, treatment with OEA increased GLP-1 release by 2.6±0.2-fold (p<0.001) and 2.1±0.2-fold of basal (p<0.001), in NCI-H716 and GLUTag cells (n=6-9), respectively. Treatment with metformin (150μM) or AICAR (0.1mM) did not affect normalized phospho-AMPK levels after 1 hr of treatment in either cell line; however, metformin increased phospho-AMPK levels in NCI-H716 cells by 1.8- and 1.7-fold, after 6 and 24 hr, respectively (n=3), while AICAR induced a nearly 2-fold increase in phospho-AMPK levels after 24, but not after 6 hr. In contrast, both metformin and AICAR increased the normalized phospho-AMPK in GLUTag cells by 1.5- and 1.8-fold, respectively, after 6, but not after 24 hr of treatment (n=3). Finally, incubation of GLUTag cells with metformin (150μM), AICAR (1mM) and OEA (10μM) decreased L-cell apoptosis by 33±4% (p<0.01), 8±1% (p<0.01) and 33±6% (p<0.01), respectively. While the same concentrations of metformin and OEA did not affect proliferation of the GLUTag cells, AICAR increased proliferation by 74±7% compared to control (p<0.001).

Conclusion: Although metformin is known to increase GLP-1 levels in patients, our study demonstrates that doses of metformin comparable to plasma levels achieved in such patients do not directly stimulate GLP-1 release from intestinal L cell lines; however, metformin improves survival of the L cells by decreasing apoptosis, potentially by modulating the AMPK signaling pathway. Nonetheless, the mechanism of action of metformin to increase GLP-1 levels in humans in vivo needs further exploration. Taken together, these findings suggest that use of metformin, possibly in combination with a direct GLP-1 secretagogue, such as OEA, and a GLP-1 degradation inhibitor, may lead to a favorable GLP-1 profile in patients with type 2 diabetes.

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OP 34 Life and death of beta cells

199

The Fas apoptotic inhibitory protein TOSO induces proliferation in human beta cells

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Background and aims: Decreased β-cell mass reflects a shift from quiescence/proliferation into apoptosis, it plays a crucial role in the pathophysiology of type 1 and type 2 diabetes and limits the outcome of islet transplantation. A major apoptotic pathway which triggers glucose- and autoimmunity- mediated β-cell apoptosis is the activation of the Fas/FasL system. Here we show that switching the Fas pathway using Fas apoptotic inhibitory protein (Faim/TOSO), which regulates apoptosis upstream of caspase 8, is a promising target to block β-cell apoptosis and to increase proliferation.
Materials and methods: TOSO expression in pancreatic sections was analyzed by immunofluorescence in 8 patients with T2DM and 8 healthy controls. Human isolated islets were exposed to increasing glucose (5.5-33.3 mM) and IL-1β (0.1-5 ng/ml) with and without TOSO down-regulation by siRNA (siTOSO) or β-cell specific TOSO over-expression by RIP-TOSO plasmid transfection. Apoptosis, proliferation and TOSO expression were analyzed. To answer the question if there is a sub-population of β-cells in human islets in culture, which is especially replication-active, we incorporated the thymidine analogs 5-chloro-2-deoxyuridine (CldU) and 5-iodo-2-deoxyuridine (IdU) into cultured β-cells after TOSO transfection and measured cycles of proliferation.
Results: Triple immunostaining for TOSO, insulin and glucagon in pancreatic sections of healthy controls and patients with T2DM revealed that TOSO is clearly expressed in the β-cells and down-regulated in diabetes. We observed a 5.5-fold increase in TOSO by short-term incubation of islets with glucose (12 h), a condition when glucose induced proliferation (1.8-fold increase in Ki67 positive β-cells). In contrast, TOSO was down-regulated to almost undetectable levels after long-term incubation for 72 h, a condition where glucose induced β-cell apoptosis (2.5-fold increased β-cell apoptosis at 33.3 mM compared to 5.5 mM glucose). Previously, we have found that the cytokine IL-1β has a dual role on β-cell turnover; it induces proliferation at low dose and apoptosis at high dose. In line with this finding, low dose IL-1β induced and high dose almost depleted TOSO mRNA expression. These studies lead to the hypothesis that TOSO may regulate a switch between proliferation and apoptosis in the β-cell.
To investigate the physiological role of TOSO, we over-expressed and depleted TOSO in the β-cell. Incubation of human islets with siTOSO for 4 days depleted TOSO expression together with 3.5-fold induction of apoptosis. β-cell apoptosis was 4-fold increased by the cytokine mix IL-1β and IFNγ and 3.5-fold by 33.3 mM glucose, which was prevented by RIP-TOSO over-expression. On day 2 and 4 after RIP-TOSO transfection, we incorporated the thymidine analogs 5-chloro-2-deoxyuridine (CldU) and 5-iodo-2-deoxyuridine (IdU), respectively, into cultured islets. RIP-TOSO resulted in a 3.5-fold induction of proliferation. Only a small percentage (~2%) of the proliferating β-cells were co-labeled for CldU and IdU suggesting a very limited capacity of β-cells to undergo multiple rounds of proliferation.
Conclusion: Our data suggest that TOSO is an important regulator of β-cell turnover and could be an important tool to switch β-cell apoptosis into proliferation and to maintain and generate a sufficient β-cell mass.
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200

Protective roles of S-nitrosoglutathione reductase (GSNOR), a negative regulator of protein S-nitrosylation, in obesity- and streptozotocin-induced diabetes

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Background: Nitric oxide (NO) and inducible NO synthase (iNOS) play important roles in dysfunction and destruction of pancreatic β-cells as well as obesity-induced insulin resistance. However, it remains largely unknown how NO and iNOS modulate function and survival of β-cells. We have previously shown that Akt/PKB is reversibly inactivated by protein S-nitrosylation, the covalent attachment of NO moiety to cysteine thiols and the major mediator of diverse actions of NO. S-nitrosoglutathione (GSNO) reductase (GSNOR), which is expressed in various cell types, including β-cells, decomposes GSNO and thereby reduces S-nitrosylated proteins in cells.
Methods and results: GSNOR knockout (-/-) mice exhibited glucose intolerance, insulin resistance, attenuated insulin secretion, increased blood glucose levels and lower plasma insulin concentrations under fasted and fed conditions, as compared with wild-type mice (Fasted BS: KO: 158 ± 4 mg/dl; WT: 137 ± 3, $p<0.0001$, Fasted Insulin: KO: 0.74 ± 0.04 ng/ml; WT: 0.96 ± 0.05, $p<0.01$). Moreover, GSNOR deficiency aggravated high-fat diet-induced diabetes. Multiple low-dose streptozotocin treatment (MLD-STZ, 37.5 mg/kg/day, IP, for 5 consecutive days) induced diabetes (BS ≥ 300 mg/dl) in 14 out of 21 male GSNOR-/- mice and enhanced β-cell apoptosis, although none of wild-type mice (n=22) became diabetic by MLD-STZ (χ^2: $P<0.001$). siRNA-mediated knockdown of GSNOR accelerated both NO donor- and cytokine (IL-1β + IFN-γ)-induced apoptosis in cultured INS-1 cells. Notably, iNOS deficiency almost completely blocked MLD-STZ-induced diabetes in GSNOR-/- mice and reversed the increases in β-cell apoptosis, but did not affect resting hyperglycemia or low insulin levels.
Conclusion: These results indicate that GSNOR plays a protective role against iNOS-mediated apoptosis of β-cells, while under normal condition GSNOR exerts beneficial effects on β-cell function via iNOS-independent mechanisms. Our findings highlight GSNOR as a potential anti-diabetogenic molecular target.
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201

miRNAs posttranscriptionally regulate cytokine-induced Bcl-2 family members

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Background and aims: In β-cells, cytokine-induced pro-apoptotic signaling is dependent on Bcl-2 family proteins. However, the regulation of Bcl-2 proteins at mRNA and protein levels following cytokine exposure is incompletely understood. In other cell systems, the expression of Bcl-2 family members is regulated by miRNAs. The aims of this study were to characterize the effects of cytokines on target Bcl-2 family member mRNA and protein expressions and to investigate a putative regulatory role of miRNA.
Materials and methods: INS-1 cells were exposed to IL-1β (160pg/ml) and IFNγ (5ng/ml) for 1 to 24h, and expressions of Bcl-2, Bak1, Bax, $BclX_L$, Bid were assessed with qRT-PCR and immunoblotting. Bioinformatic tools were used to identify miRNAs with putative target sites in the 3`UTR of the Bcl-2 member RNAs. miR-150, -15b, -27b, -125a, -326, -133a and Let-7c were chosen for further examination. The expressions of miR-150, -326, -133a and Let-7c were characterized by qRT-PCR in the absence or presence of cytokines (12 and 24h). A reporter construct containing the Luciferase gene cloned with the 3'UTR of Bid was used to investigate a regulatory role of miRNA on Luciferase activity and Bid protein expression. A construct containing the Luciferase gene with its native 3'UTR was used as a negative control. Luciferase activity was measured in the presence or absence of 4 miRNAs with putative binding sites in the Bid 3'UTR (miR-326, miR-15b, miR27b and miR125b) and 4 negative control miRNAs.

Results: Cytokines induced expression of Bak, Bax, $BclX_L$ and Bid (fold induction 3.5, 2.7, 2.6 and 8.1, respectively, all $p<0.05$, Student's paired t-test), but not Bcl-2 mRNAs; however this was not correlated with increased protein expressions. Cytokines were further found to increase the expression of miR-150, miR-326 and miR-133a (fold induction 1.5, 4.0 and 1.9, respectively, all $p<0.05$), but not Let-7c. All of the investigated candidate miRNAs, but none of the negative controls inhibited Luciferase activity from the Luciferase-Bid 3'UTR reporter system (49-53%, $p<0.05$). Further, when the Bid 3'UTR containing construct was used to sequester endogenously expressed miRNAs, cytokines induced Bid protein expression 3.4 fold relative to the effect of over-expressing a construct containing the native 3'UTR of Luciferase (n=2-3).
Conclusion: This study has demonstrated mRNA but not protein levels of Bcl-2 family members to be up-regulated following cytokine exposure. We suggest that the posttranscriptional repression of at least Bid is mediated by miRNAs. miRNAs are thereby suggested at novel regulators of cytokine-induced signalling, and modulation of miRNAs may represent a new strategy to prevent cytokine-induced β-cell apoptosis.
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202

Beta cell maturation enhances cytokine sensitivity via iron metabolism
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Background and aims: Pro-inflammatory cytokines and reactive oxygen species (ROS) generation play major roles in the pathogenesis of type 1 diabetes (T1D) and in islet transplantation by causing impairment of β-cell function and apoptosis. β-cell acquisition of sensitivity to IL-1β is associated with β-cell maturation and pancreatic duodenal homeobox factor-1 (Pdx-1) expression, but the molecular mechanisms are unknown. The aim of this study was to compare IL-1β-induced signaling and apoptosis in immature- and mature β-cells and reveal molecular mechanisms based on gene expression profiling.
Materials and methods: INS-1rαβ cells with doxycycline-inducible Pdx-1 or dominant negative (DN)-Pdx-1 expression were a gift from Claes B. Wollheim. IL-1β-induced cell death was determined by TUNEL, cleavage of caspase-3 and ELISA death detection assay. NFκB activation was determined by electric mobility shift assay (EMSA) and Luciferase reporter assay. Affymetrix microarray analysis was used for gene expression profiling and candidate genes verified in newborn rat islets by Western blotting and realtime PCR. Iron uptake was measured by the calcein assay and ^{55}Fe uptake. ROS formation was measured by using the H_2DCF dye. Iron chelator desferrioxamine (DFO) and siRNA-mediated divalent metal transporter 1 (DMT1) knockdown was used to block IL-1β-induced iron uptake.
Results: Pdx-1 over-expressing INS-1rαβ cells were more sensitive to the toxic effect of IL-1β compared to wildtype and DN-Pdx-1 expressing cells (310%, $p<0,05$). IL-1β-induced NFκB transcriptional activity was increased (>200%, $p<0,05$) in Pdx-1 overexpressing cells and bioinformatic analysis of microarray data suggested altered iron homeostasis as an important factor in IL-1β sensitivity of Pdx-1 expressing cells. Observations were verified in IL-1β-exposed rat and human islets, where increased expression (400%, $p<0,01$) of the NFκB-regulated iron transporter DMT1 and several other iron regulated genes were shown. This correlated with increased IL-1β-induced iron uptake and iron-catalysed ROS formation in cell lines and rat islets. Immunostaining of rat pancreatic sections indicate high basal DMT1 expression in β-cells cells compared α-cells. Pharmacological inhibition of iron uptake by treatment with DFO reduced IL-1β-induced ROS formation and cell death dose-dependently. DMT1 knockdown with three different siRNA protected INS-1 cells against IL-1β-induced apoptotic cleavage of caspase3.
Conclusion: Pdx-1-driven β-cell maturation causes IL-1β-sensitivity in insulin secreting cells. Our data suggest that this process depends upon enhanced NFκB activity and increased expression of the NFκB-regulated iron transporter DMT1, leading to increased iron uptake, ROS production and apoptotic β-cell death.
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203

Morphologic changes of the endocrine pancreas in Japanese non-obese type 2 diabetes
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Background and aims: It has been demonstrated that the "beta cell mass" in the pancreas of the patients with type 2 diabetes mellitus (T2DM) is decreased even in the early stage of the disease in the Caucasian population. In Japanese population, the capacity of insulin secretion has been shown to be less robust compared to that of the Caucasian population, and this is thought to be more important than the existence of insulin resistance in view of the pathoetiology in the development of Japanese T2DM. However, little is known on the morphologic change in the endocrine pancreas of the patients with T2DM. Therefore, we tried to clarify the morphologic change of the beta cells of pancreas in patients with Japanese non-obese T2DM.
Materials and methods: Pancreas tissues of patients with non-obese T2DM aged 65.1±10.5 years (DM, n=17) and age-matched non-obese non-diabetic subjects aged 67.3±10.5 years (non-DM, n=15) were obtained by surgical operations and autopsies. BMI of both groups of diabetic patients and non-diabetic subjects were 20.3±3.1 and 20.0±2.4 kg/m^2, respectively. Three sets (more than 500 μm distant from each other) of formalin-fixed, paraffin-embedded sections were made from head, -body and -tail portions of each pancreas. At least 3 pancreatic sections from each set were immunostained with islet hormones (insulin, glucagon and somatostain) by immuno-fluorescent and/or immuno-histochemical method. From these sections, we calculated relative beta cell area (%), relative islet number (/cm^2), relative beta cell number (/cm^2) and beta cell size (μm^2) using digital image analyzer (BZ-H1A) equipped on a fluorescence microscope BZ-8000 (KEYENCE).
Results: Relative beta cell area was significantly decreased in DM group compared to that of non-DM group (0.80 ± 0.78 vs 1.05 ± 0.80%, $p<0.05$), especially in pancreas head (0.48±0.24 vs 0.76±0.44%, $p<0.001$). There was a trend to increase in the relative islet number in DM group. Relative beta cell number was significantly increased only in a pancreas tail of the DM group (28,399.4±16,445.6 vs. 20,166.5±11,771.9/cm^2, $p<0.05$). Beta cell size was significantly decreased in all portion of the pancreas in DM group (45.7±27.1 vs 80.6±33.3 /μm^2, $p<0.001$).
Conclusion: In the pancreas of Japanese patients with non-obese T2DM, the relative beta cell area was decreased, especially in the head portion, but the relative number of beta cells does not appear to be decreased at least in the present study. Our results indicate that the decrease in the relative beta cell area, so-called "beta cell mass", in non-obese type 2 diabetes mellitus was mainly caused by the reduction of the beta cell size.

204

PDX-1 translocates to the cytosol in type 2 diabetes
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Background and aims: The transcription factor PDX-1 plays a critical role during β-cell development and in glucose induced insulin gene transcription in adult β-cells. Glucose exposure up to 24 h leads to translocation of PDX-1 from the nuclear periphery to the nucleoplasm, whereas under conditions of oxidative stress, PDX-1 shuttles from the nucleus to the cytosol. The possible pathophysiological role of such PDX-1 translocation is unknown and disturbances in PDX-1 shuttling may be an important mechanism of β-cell failure in T2DM. In the present study we asked the following questions: where is PDX-1 located in diabetic islets and does normalizing glycemia change PDX-1 localization?
Materials and methods: PDX-1 expression in pancreatic sections was analyzed by immunofluorescence staining in 8 patients with T2DM and 8 healthy controls, and in 2 animal models of T2DM, the *db/db* mouse and the high fat/high sucrose diet fed mouse (HFD). Islets from the mice were isolated and analyzed for PDX-1 localization and apoptosis by Western blotting. Human islets were cultured for 96 h with increasing glucose concentrations, or with 2 ng/ml IL-1β with or without addition of recombinant human IL-1Ra and PDX-1 localization was analyzed in Bouin fixed islet sections.

Results: In all islets from patients with T2DM, PDX-1 protein was localized in the cytosol, whereas in the non-diabetic controls, PDX-1 was in the nucleus. Also, in the hyperglycemic 8 week old db/db mice and in mice fed the HFD for 16 weeks, PDX-1 protein was localized in the cytosol and β-cell apoptosis was increased. In contrast, IL-1Ra overexpression in both mouse models maintained normoglycemia, β-cell survival and PDX-1 nuclear localization.
Importantly, in human islets exposed for 96 h to 22.2 mM glucose or to 2 ng/ml IL-1β, PDX-1 was found in the cytosol, whereas IL-1Ra induced a similar restoration of PDX-1 nuclear translocation in isolated islets. We found the JNK-pathway as a possible link in the downstream signaling from IL-1Ra to PDX-1. In isolated human islets, phosphorylated JNK-levels were increased by 11.1 and 33.3 mM glucose or in the presence of IL-1β, this was inhibited by IL-1Ra treatment.
Previous studies suggested that JNK-activation leads to PDX-1 export. Human islets were treated with 22.2 mM glucose or IL-1β, with or without the addition of a small peptide that inhibits JNK-activity. In the glucose and IL-1β treated cells, we found cells that co-express insulin and PDX-1 in the cytoplasm, as well as cells that showed cytoplasmic PDX-1 with almost no detectable insulin. JNK-inhibition prevented glucose- and IL-1β-induced PDX-1 translocation and kept the transcription factor in the nucleus in most cells, indicating that the JNK-pathway is involved in regulating PDX-1 localization.
Conclusion: Our results suggest that β-cell failure correlates with the cytosolic localization of PDX-1. IL-1Ra improves β-cell survival and function through maintaining PDX-1 expression in the nucleus and suggest a potential role for IL-1Ra in the treatment of diabetes.
Supported by: University of Bremen Doctoral Fellowship to AA, DFG grant to KM

OP 35 Obesity and adipose tissue

205

A dominant mutation in the obesity susceptibility gene *FTO* results in reduced fat mass with enhanced lipid and carbohydrate metabolism
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Background and aims: Human single-nucleotide polymorphisms within intron 1 of the *FTO* gene are associated with increased body mass index and type 2 diabetes. The risk allele is not associated with fetal growth but confers an increased risk of obesity with homozygous risk allele carriers weighing ~3kg more than controls. As the obesity-associated SNPs are intronic it is unclear whether changes in FTO expression or splicing are the cause of obesity or if regulatory elements within intron 1 influence upstream or downstream genes. We tested the idea that a single base pair change in the *Fto* gene may reveal a body weight and metabolic phenotype.
Materials and methods: A novel *N*-ethyl-*N*-nitrosourea (ENU) point mutation in exon 6 of the *Fto* gene was identified (Fto^{I367F}). Phenotyping of heterozygous and homozygous mice fed standard (SD) or high fat diets (HFD) was performed by 10 and 20 week food intake measurement and 18 week metabolic rate assessment. Body composition was determined by dual energy X-ray absorptiometry at 24 weeks. Gene expression analysis by microarray in white adipose tissue (WAT), skeletal muscle and liver was performed at 16 weeks and blood plasma and tissues were collected and analysed at 24 weeks.
Results: We show that homozygous and heterozygous mice carrying the dominant Fto^{I367F} mutation exhibited a maturity-onset reduction in body weight diverging from wildtype at 12 weeks of age ($P < 0.01$) and becoming ~10% less than wildtype by 24 weeks old ($P < 0.01$). Analysis of 24 week old Fto^{I367F} mice and controls revealed a 16-18% reduction in total fat mass per mouse compared to wildtype ($P<0.01$). Fto^{I367F} mice on HFD also exhibit reduced adiposity (37.5% ± 1.0 compared to 42.2% ± 0.9, $P< 0.01$) and increased lean mass (24.5g ± 0.52 compared to 26.4g ± 0.34, $P< 0.01$). Fto^{I367F} mice have increased energy expenditure ($P < 0.01$), and unchanged food intake and locomotor activity. Biochemical *in vitro* studies by non-denaturing mass spectrometry suggest the mutation modifies FTO function likely by altering its dimerisation state. Gene expression profiling in homozygote Fto^{I367F} mice revealed substantial (≥ ± 1.5 fold) mRNA expression changes in WAT (n = 359), skeletal muscle (n= 210) and liver (n = 339) including some fat and carbohydrate metabolism genes and an improved inflammatory profile in the WAT of Fto^{I367F} mice.
Conclusion: The Fto^{I367F} *mouse* provides direct functional evidence that *Fto* is a causal gene underlying obesity. In contrast to the recently published mouse knockout mutation in *Fto* our model is fully viable and is not growth retarded. The Fto^{I367F} mouse provides a physiologically relevant model for studying FTO due to similarities with the effect size of the human association as demonstrated by the dominant maturity-onset reduction in weight and fat mass. This study suggests that a search for human coding mutations may be informative and that inhibition of FTO activity is a possible development for the treatment of morbid obesity.
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206

Adipose tissue overexpression of vascular endothelial growth factor in transgenic mice protects against diet-induced insulin resistance and obesity
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Background and aims: Obesity is a metabolic disease whose frequency has increased dramatically during the last few years. During development of obesity there is an expansion of fat mass which may lead to hypoxia in certain

parts of adipose tissue because angiogenesis becomes insufficient to maintain normoxia in the entire adipose depot. This reduction in adipose tissue blood flow may trigger insulin resistance via effects on adipokine expression and/or adipocyte differentiation. One of the main factors involved in adipose tissue angiogenesis is the vascular endothelial growth factor (VEGF), which can be secreted by adipose tissue. To examine whether an increase in adipose tissue angiogenesis could protect against obesity-induced hypoxia and, in consequence, decrease inflammation and insulin resistance in this tissue, transgenic mice overexpressing VEGF in adipose tissue were generated.
Materials and methods: Transgenic animals were generated by microinjection into oocytes of a chimeric gene in which the murine VEGF cDNA was under control of the aP2 promoter and were then characterized.
Results: In aP2VEGF transgenic mice, adipose tissue VEGF expression and VEGF serum levels were increased compared with control animals. Moreover, adipose tissue vessel density was higher in transgenic mice than in controls. However, no differences in body weight, food intake or serum glucose levels were observed between groups. Similarly, serum levels of free-fatty acid, triglyceride, glycerol and hormones like adiponectin, leptin and insulin were unchanged. When fed a high fat diet, body weight gain was lower in transgenic mice than in controls. This was parallel to lower WAT weight and to different adipocyte size distribution with decreased adipocyte mean area. Energy expenditure was increased in transgenic mice related to controls. However, no differences in food intake were observed between control and transgenic mice. Moreover, trangenic mice under a HFD also showed a decrease in insulin serum levels in parallel with an increase in whole body insulin sensitivity. In these transgenic mice, fed either a high-fat diet or a chow diet, the overexpression of VEGF in adipose tissue led to an increase in macrophage infiltration. However, flow cytometry analysis of white adipose tissue of these animals showed that the infiltrate population of transgenic mice was different from the population of control mice. White adipose tissue infiltrate of transgenic mice had increased MGL-1 and GR-1 levels and decreased MHC class II and neuropilin, all these indicating that whereas adipose tissue from control mice on a HFD have M1 proinflammatory macrophages, adipose tissue from aP2VEGF transgenic animals both in chow and in HFD had increased M2 "alternatively activated" macrophages, which may protect adipocytes from inflammation.
Conclusion: Thus, these results suggest that the increase in energy expenditure and the M2 inflitrate in white adipose tissue of aP2VEGF transgenic mice protects them against high fat diet-induced insulin resistance and obesity.
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207

The inflammasome-mediated caspase-1 activation controls adipocyte differentiation and insulin sensitivity
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Background and aims: In recent years it has become evident that obesity is associated with chronic low grade inflammation that contributes to the development of insulin resistance. It is believed that obesity-induced inflammation mainly originates from expanding adipose tissue. Recently, important metabolic effects have been attributed to Interleukin (IL)-1β. Processing of IL-1β requires cleavage by caspase-1, a cysteine protease regulated by a complex of proteins including NALP3 and ASC that are clustered into the inflammasome. The aim of our study was to test whether caspase-1 and the inflammasome are involved in the development of obesity and insulin resistance.
Materials and methods: White adipose tissue (WAT) from humans, diet- or genetically (db/db and ob/ob) induced obese animals and caspase-1- or NALP3-deficient animals were studied using qPCR and western blot analysis. In addition, WAT and insulin sensitivity of ob/ob animals that were treated for two weeks with an inhibitor of caspase-1 were examined.
Results: qPCR and Western blot analysis revealed that caspase-1 is well expressed in both human and mouse WAT. Interestingly, caspase-1 activity in WAT was increased during both diet- and genetically-induced obesity, resulting in elevated levels of mature IL-1β in WAT of obese animals. Differentiation of pre-adipocytes isolated from caspase-1- and NALP3- deficient mice resulted in more metabolically active fat cells that were more insulin sensitive and produced more adiponectin compared to cells originating from wild-type animals. WAT of caspase-1-deficient animals displayed a change in adipose tissue morphology towards smaller adipocytes. Caspase-1- and NALP3-deficient animals had a significant improvement of insulin sensitivity compared to wild-type mice. Finally, treatment of Ob/Ob animals with a chemical inhibitor of caspase-1 for two weeks led to a robust improvement of insulin sensitivity and reduction in weight gain.
Conclusion: Caspase-1 is present in human and mouse adipocytes and affects adipose tissue functioning and insulin sensitivity during the development of obesity. Our data reveal a physiological role of caspase-1 and suggests that caspase-1 inhibition represents a novel therapeutic target in clinical conditions associated with obesity and insulin resistance.
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208

Gender differences in 11β-hydroxysteroid dehydrogenase activity in relation to markers of obesity and insulin resistance
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Background and aims: Inhibition of 11β-hydroxysteroid dehydrogenase 1 (11βHSD1), an enzyme that regulates local tissue cortisol levels, is a potential treatment of obesity and type 2 diabetes. Increased 11βHSD1 activity and expression in adipose tissue has previously been shown to be linked to obesity. The main purpose of this study was to assess the relationship between 11βHSD1 activity in human subcutaneous (sc) adipocytes and different indices of adiposity (including body fat, adipocyte size, BMI and waist circumference) as well as plasma markers of insulin resistance and, in addition, to address the impact of gender.
Materials and methods: Non-diabetic male (M, n=23) and female (F, n=31) volunteers were recruited; age 47.8±1.9 (mean±SEM), BMI 26.8±0.5 (range 21-38 kg/m^2). Blood sampling, sc abdominal adipose tissue biopsies and anthropometric measurements were performed following overnight fast and body fat was measured by bioimpedance. Sc and omental (om) adipose biopsies (n=4) were obtained during elective abdominal surgery. Mature adipocytes were isolated by collagenase digestion and cell number and size determined. 11βHSD1 activity was measured in the presence or absence of a novel specific and potent 11βHSD1 inhibitor during 5-6 h incubation with 100 nmol/l cortisone including ^{3}H-cortisone tracer. ^{3}H-cortisone and ^{3}H-cortisol in the incubation media were measured using radiometric HPLC and activity was expressed as % conversion of cortisone to cortisol per h per 10^6 cells.
Results: There was no significant difference in sc 11βHSD1 activity between M (3.8±0.6%/h/10^6 cells) and F (3.4±0.7%/h/10^6 cells). In the subjects where both sc and om cells were assessed, 11βHSD1 activity did not differ between the depots; sc 2.4±1.2%/h/10^6 cells; om 2.4±1.2%/h/10^6 cells. A near complete inhibition of 11βHSD1 activity was achieved with the novel inhibitor in both sc and om adipose tissue, and potency was similar (IC50 1-2 nM) for both fat depots. 11βHSD1 activity in sc adipocytes positively correlated with body fat (M, r=0.57, p=0.009; F, r=0.43, p=0.04) and cell size (F, r=0.59, p=0.001; M, r=0.40, p=0.06). A positive correlation between 11βHSD1 activity and BMI (M, r=0.47, p=0.025; F, r=0.31, p=0.08) was statistically significant only in men, and this was also true for waist circumference (M, r=0.63, p=0.001; F r=0.26, p=0.18). A positive correlation was also observed with fasting glucose in both groups (M, r=0.41, p=0.049; F, r=0.46, p=0.016), although only men displayed a positive correlation to fasting insulin (r=0.58, p=0.005). Multiple regression analyses, including the above variables, showed that only adipocyte size predicted 11βHSD1 activity (M, r^2=0.27, p=0.028, F, r^2=0.47, p=0.001).
Conclusion: The data demonstrates increased 11βHSD1 activity in human subcutaneous adipocytes with obesity, and adipocyte size is an important

and independent marker in this relationship. In men, but not women, there was also an association between markers of insulin resistance, i.e. waist and serum insulin, and 11βHSD1 activity. The data suggest that there are differences between men and women in the link between 11βHSD1 activity and metabolic phenotypes.

209

Adipose tissue is directly targeted and initiated by bacterial LPS at the early onset of HFD-induced metabolic diseases

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Background and aims: We recently showed, in the mouse and in human that a fat-enriched diet (HFD) increased plasma Lipopolysaccharide (LPS) concentration in the blood and was responsible for the low tone inflammation observed during metabolic diseases. This mechanism depended on the LPS receptor CD14 and targeted the adipose tissue since CD14 knockout mice did not developed obesity in response to HFD. Conversely, in response to a one month LPS infusion the adipose cell number was increased and the cells were smaller suggesting and increased precursor proliferation.
Materials and methods: We here aimed to determine whether LPS could trigger directly the adipose depot and initiate the proliferation of adipose tissue precursors without recruiting bone marrow cells. To this aim we grafted adipose depots from wild type and CD14 KO mice on the back of CD14 KO mice. The CD14 KO recipient mice are unable to generate inflammation in response to a LPS infusion. The grafted mice underwent a continuous one month infusion of LPS by the mean of osmotic minipumps.
Results: At completion of the infusion we showed that HKII, GLUT4, FAS, and ACC mRNA concentrations and the insulin-stimulated glucose utilization rates (clamp experiments) were reduced in the grafted wild type fat pad when compared to the CD14 KO fat pad. This suggested a state of insulin resistance induced by the LPS infusion, and hence inflammation, which was directly affecting the wild type fat pad. This was confirmed by the increased quantification of the mRNA coding for the cytokines IL1, PAI-1, TNFa and the chemokines MCP-1, MIF, osteopontin and by the high amount of F4/80 positive cells as determined by FACS analysis and adipose tissue histology. Importantly, this set of data was similar to that obtained in mice treated with HFD for one month. In a second set of experiments we determined that the number of cells from the vascular fraction was dramatically increased only in the grafted wild type fat pad. We determined by FACS analysis that all precursor populations were similarly increased including preadipocytes, macrophages, and preendothelial cells. In vitro LPS stimulation increased the proliferation of cell types isolated from the stroma vascular fraction of wild type but not CD14 adipose depot. Conversely, in vitro the LPS treatment reduced the adipose cell differentiation process as assessed by lipid accumulation and the expression of adipogenic markers from wild type but not from CD14KO precursors.
Conclusion: We showed that LPS, which originates from intestinal microflora, can trigger directly the adipose depot by a CD14 dependent mechanism to initiate the proliferation of adipose tissue precursors. Hence intestinal microflora could be a regulatory factor controlling adipose tissue plasticity and insulin resistance.
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210

The life-span determinant p66shcA mediates fat-induced insulin resistance

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Background and aims: Emerging evidence indicates in fatty acid and cytokine- induced oxidative stress a major cause of peripheral insulin resistance. The life-span determinant p66shcA promotes organismal ageing in part by eliciting the generation of Reactive Oxygen Species in mitochondria; additionally, structural features of p66shc clearly indicate that this adapter molecule may directly participate in signal transduction downstream of the Insulin RTK receptor. Thus far, however, a potential role role for p66shcA in the establishment of adipose-induced insulin resistance has not been investigated.
Materials and methods: Sex and age-matched lean Wild Type (WT) and p66-deficient (p66KO) C57Bl6 mice were subdued to glucose (1g/Kg) and Insulin (1 U/Kg) tolerance tests, and early signaling events elicited by Insulin in the white fat analysed biochemically. Obese mice were obtained by genetic crossbreeding with C57Bl6 Lep^{Ob}, leptin-deficient mice. $WT^{Ob/Ob}$ and $p66KO^{Ob/Ob}$ hybrid mice were monitored for 80 weeks for food consumption and weight gain, and glucose homeostasis and Insulin responses assessed as described for lean mice. Committed adipocytic precursor cells were prepared from WT and p66KO adipose tissue, serum starved for 16 hours and stimulated *in vitro* with insulin (100 ng/ml for 10'). Phosphorylation of Insulin Receptor Substrate-1 AKT/PKB kinase, and activation of the mTOR/S6K pathway were assessed by phospho-specific immunoblotting of total protein extracts. Molecular interactions between p66shc and component of the insulin signaling cascade were further investigated in 3T3-L1 preadipocytes and in HEK 293-T cell upon overexpression or siRNA-mediated knock-down of proteins of interest.
Results: Glucose and Insulin tolerance tests revealed no significant differences in lean p66KO mice compared to WT controls except for a slight reduction in blood glucose response to acute insulin. However, lack of p66 reduced fat accumulation and ameliorated fasting hyperglycaemia and glucose intolerance of Ob/Ob animals. In keeping with this finding, biochemical analysis of white fat from p66KO animals revealed higher expression of the master Insulin receptor transducer IRS-1 and of enhanced interaction of IRS-1 with PI3Kinase. Importantly,also isolated adipocyte precursors from p66KO obese mice appeared to be resistant to insulin desentization induced by in vitro exposure to TNF alpha or excess free fatty acid, when compared to p66-WT control cells. Mechanistic studies performed in 3T3L1 and 293-T cells revealed that p66shc form a complex with IRS-1 in insulin stimulated cells, and facilitates IRS recruitment of the S6K kinase, a putative mediator of insulin receptor desensitization by nutrient overload. Additionally, changes in p66shc levels, achieved by genetic manipulation, positively correlated with phosphorylation of both S6 Kinase and IRS-1. Finally, in 3T3L1 cells, FFA-induced insulin resistance was accompanied by Ser 36 phosphorylation of p66shc, and both responses were prevented by the ROS scavenger Catalase.
Conclusion: Taken together the above observations
a) Demonstrate the involvement of p66shc in the establishment of adipose-associated insulin resistance in vivo and in vitro, providing a link between oxidative stress, ageing and Type II Diabetes.
b) Outline a potential molecular mechanism in the p66-mediated association between IRS-1 and S6K, as components of a signal-inhibitory loop responsible for insulin resistance.
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OP 36 Insulin therapy in type 2 diabetes mellitus

211

Metformin, sulphonylurea and insulin therapies maintain glycaemic control over five years in 4900 people with type 2 diabetes

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Background and aims: Type 2 diabetes is characterised by insulin resistance and impaired beta cell function that deteriorates over time. It is generally accepted that glycaemic control worsens over time, even with the intensification of standard diabetes care (metformin, sulphonylureas and insulin). In the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study we investigated the hypothesis that a PPARα agonist, fenofibrate, could reduce the risk of cardiovascular disease in patients with type 2 diabetes. Because the FIELD study was not directed at improving glycaemic control or modifying other aspects of diabetes care, it also provided the opportunity to observe standard diabetes care, predominantly in the primary care setting, in 63 different regions within Australia, New Zealand and Finland.
Materials and methods: Glycaemic control and diabetes management in the placebo-treated cohort of 4900 patients (age 62 ± 7 years, 37% women, median duration of diabetes 5 years) were monitored for a median follow-up period of 5 years.
Results: Median HbA1c was 6.9% at baseline (2 measurements) and 7.0% at study end (p<0.0001); median fasting glucose was 8.5 mmol/L and fell to 7.9 mmol/L respectively (p<0.0001). Median weight was 86.3 kg at baseline and fell to 85.0 kg at study end (p<0.0001). Baseline therapy for diabetes was lifestyle measures only in 27%, oral agents in 67% and insulin in 14% (8% also taking oral agents). During the study insulin use increased to 28% (19% also taking oral agents) and use of oral agents to 77%. Only 2% of patients at baseline and 4% at study end were taking any oral agents other than metformin or sulphonylureas. Insulin therapy was initiated in 855 of the 4900 patients (20% of those not already taking insulin) at a median HbA1c of 8.2% and resulted in a sustained reduction to 7.6%. Median weight at insulin initiation was 86.5 kg and increased by 4.6 kg over the subsequent 5 years. Progression to insulin treatment in those on oral hypoglycaemic medication at baseline was associated with worse insulin secretory function. Both HOMA2-%S and HOMA2-%B predicted treatment progression in logistic models.
Conclusion: The FIELD study showed that glycaemic control in type 2 diabetes can be maintained in a large cohort with standard diabetes therapy (metformin, sulphonylureas and insulin) over a period of 5 years. The glycaemic benefits of newer therapies for type 2 diabetes should be judged against what is achievable with standard diabetes care. These findings are relevant to the delivery of diabetes care in many other countries, including those with more limited healthcare resources.
FIELD study supported by Laboratoires Fournier, France (now Solvay Pharmaceuticals) and Medical Research Council, Australia

212

Is there an association between frequency of self adjustment of insulin dose and metabolic control in patients with diabetes mellitus type 2?

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Background and aims: Intensified insulin therapy is widely used in patients with diabetes type 2 (DM2). In patients with type 1 diabetes frequent self monitoring and adaptation of insulin dose improved metabolic control, quality of life and reduced hypoglycaemia. Recent randomised controlled studies did not reveal an advantage of intensified insulin therapy in DM2. However, no study mentioned the frequency of insulin dose adjustment by the patients themselves. We studied the association of insulin dose adjustment in a large group of well educated patients with DM2 in Germany.
Materials and methods: We assessed the number of self adjustment of the insulin dose in 300 insulin treated patients with DM2 (age 66.8y; time since diagnosis 15.8y; BMI 32.9; HbA1c 7.4%) in an university outpatient department. The number of self adjustments of insulin dose was obtained from the last 14 days of the patient diary. All patients had a structured education on conventional or flexible insulin therapy. Self adjustment of insulin dose was defined as each change of the insulin dose compared to the same time of the previous day. Skipped and additional injections were also counted as dose adjustment. The patients were grouped to: now adjustments, 1-13 adjustments and >=14 adjustments per 14 days.
Results: 193 patients (64%) adapted their insulin dose at least once in 14 days. 107 patients used a fixed schedule without self adjustment. Patients with frequent adjustments (>=14) compared to the group with no self adjustment were younger (64.5 vs. 68.9y; p=0.001), had a higher insulin dose (77.3U vs. 53.6U/d; p=0,001), more injections (3.7 vs. 2.4/d; p=0,001), more blood glucose self controls (26.3 vs. 18.6/wk; p<0,001), a higher social status (11.5 vs. 9.8; p=0,001; max. score 21) and a longer follow up (8.2 vs 4.8y; p<0,001). No significant differences were observed regarding last BMI (32.8 vs. 33.4 kg/m²), last HbA1c (7.29 vs. 7.46%) and mean HbA1c (7.73 vs. 7.82%). In the regression analysis with mean individual HbA1c as dependent variable, time since diagnosis, number of self adjustments of insulin doses, number of insulin injections, number of blood glucose self monitoring, BMI and social status score as independent variables, only the number of blood glucose self monitoring (R^2 0,41; p<0.001) and BMI (R^2 0.65; p<0.001) had a significant association with HbA1c.
Conclusion: In an university outpatient department two thirds of well educated patients with diabetes type 2 on insulin therapy practice insulin dose self adjustments at least once per 14 days. The hypothesis that frequent self adjustments of the insulin dose is associated with better metabolic control was not confirmed in this retrospective cohort analysis. A randomised controlled trial is needed for the definitive proof of evidence regarding the value of self adjustment of insulin dose in DM2.

213

Once daily initiation with biphasic insulin aspart 30 (BIAsp 30) versus insulin glargine in patients with type 2 diabetes inadequately controlled with oral drugs - a randomized controlled trial

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Background and aims: The aim of the trial was to compare the efficacy and safety of biphasic insulin aspart 30 (BIAsp 30) with insulin glargine, both administered once daily to insulin-naive subjects with type 2 diabetes inadequately controlled with two oral anti-diabetic drugs
Materials and methods: In this 26-week, open-label, randomized, parallel-group, multinational, treat-to-target trial, 480 insulin-naïve subjects (mean age 56 years, mean HbA1c 8.5% and duration of diabetes 9.3 years) from 15 countries were randomized to receive either BIAsp 30 before dinner or insulin glargine at bedtime, both in combination with metformin and glimepiride. In all, 433 subjects completed the trial.
Results: After 26 weeks of treatment, the estimated mean reduction in HbA_{1c} (from baseline) was −1.41% with BIAsp 30 and −1.25% with insulin glargine, with a statistcally significant difference of −0.16%, 95%CI [−0.30;−0.02], p=0.029. The plasma glucose levels at end of treatment were significantly lower with BIAsp 30 both after dinner (BIAsp 30-glargine = -0.52, 95%CI [−1.02; −0.03], p=0.04) and at bedtime (BIAsp 30-glargine = −0.78, 95%CI [−1.25;−0.31], p<0.01) than with insulin glargine. The rate of hypoglycaemia was low in both treatment arms, and major hypoglycaemic episodes were few (three episodes with BIAsp 30 and two with insulin glargine). The relative risk of experiencing a nocturnal (0:00-6:00 a.m.) hypoglycaemic episode, however, was significantly higher with BIAsp 30 than with insulin glargine (1.1 vs 0.5 episodes/year, RR = 2.41, 95%CI [1.34;4.34], p=0.003). At initiation, the mean insulin daily dose was 0.18 U/kg in both treatment groups but after 26 weeks of treatment the dose had increased to 0.32 U/kg with BIAsp 30 vs 0.29 U/Kg with insulin glargine. Increase in body weight was similar in both arms: 1.74 kg with BIAsp 30 and 1.67kg with insulin glargine. The safety profile, as judged from reports of adverse events and clinical laboratory data, was comparable for the two treatments, and treatment satisfaction was not significantly different.
Conclusion: Findings from once daily BIAsp 30 versus once daily insulin glargine both in combination with metformin and glimipiride:
1: HbA_{1c} and postprandial hyperglycaemia was reduced significantly more with BIAsp 30
2: Low rate of hypoglycaemia was observed with both regimens, but significantly higher rate of nocturnal hypoglycaemia with BIAsp 30
3: Weight gain and treatment satisfaction were similar with the two regimens

214

Examining patient outcomes following different regimens of biphasic insulin aspart 30/70 (BIAsp 30, NovoMix® 30) in the large, multinational, IMPROVE™ observational study

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Background and aims: The IMPROVE observational study has shown that biphasic insulin aspart 30/70 (BIAsp 30) improves glycaemic control in patients with type 2 diabetes, regardless of pre-study therapy. Here we present a sub-analysis of effectiveness and safety of BIAsp 30 in patients grouped according to their starting and final injection frequency to determine which regimens resulted in the best patient outcomes.
Materials and methods: From the total of 52,419 patients from China, India, Japan, Canada, Russia, Italy, Poland and Greece enrolled in the IMPROVE study, n= 41,436 were included in this analysis as they had injection frequency data at baseline and final visit. Patients were divided into five treatment regimens based on their starting and final injection frequency of BIAsp 30: once-daily (OD) to OD (n=2943), OD to twice-daily (BID) (n=2613), BID to BID (n=33,880), OD to thrice-daily (TID) (n=141), and BID to TID (n=1859). Effectiveness and safety parameters and dose were analysed in these groups.
Results: Baseline patient demographics are given in the Table. Patients who started on BIAsp 30 BID had shorter duration of diabetes than those who started on BIAsp 30 OD (Table). Patients in all five dosing regimens showed significant reductions from baseline in HbA_{1c}, fasting blood glucose (FBG) and postprandial blood glucose (PPBG) at the end of the study (all $p<0.0001$; the Table below shows PPBG for breakfast across dosing regimens, and a similar pattern was seen for PPBG after lunch and dinner, also all $p<0.0001$ for change from baseline). Those patients who started BIAsp 30 therapy with BID injections consistently achieved greater reductions in glycaemic parameters than did those who started with an OD injection (Table). Consequently, over 50% of patients who started on BIAsp 30 BID achieved HbA_{1c} <7.0%, compared with approximately 40% for those who started on BIAsp 30 OD (Table). The final injection frequency did not appear to influence glycaemic outcome. All groups showed a significant reduction in major hypoglycaemia. Minor hypoglycaemia decreased in those who finished on OD or BID BIAsp 30 but increased in those who finished on TID BIAsp 30 (Table). BIAsp 30 daily doses from baseline to final visit increased with the number of daily insulin injections (Table).
Conclusion: BIAsp 30 is an effective therapy for patients with type 2 diabetes, regardless of initial or final injection regimen. Starting BIAsp 30 therapy with BID injections was more effective at improving glycaemic control than starting with an OD injection. Higher final injection frequency (TID) and hence dose was associated with increased minor hypoglycaemia compared with pre-study therapy, highlighting the importance of careful titration when BIAsp 30 is used in a TID regimen.
Supported by: Novo Nordisk

*$p<0.05$,**$p<0.001$,***$p<0.0001$

New therapy (BIAsp 30) to final dosing regimen					
Parameter (SD)	OD to OD n=2943	OD to BID n=2613	BID to BID n=33880	OD to TID n=141	BID to TID n=1859
Sex ,% (male)	55	53	60	49	55
Age, years	56.3 (12.0)	55.9 (11.1)	54.7 (12.0)	57.5 (11.7)	55.5 (11.6)
Diabetes duration, years	8.0 (6.3)	7.6 (5.8)	6.7 (5.9)	7.7 (6.1)	6.5 (6.0)
BMI, kg/m^2	26.6 (5.1)	27.4 (5.3)	25.7 (4.4)	31.1 (7.9)	26.7 (5.0)
Mean HbA_{1c}, %. Baseline; Change	8.9 (1.7); -1.6 (1.5)***	9.2 (1.7); -1.8 (1.7)***	9.3 (1.9); -2.3 (1.8)***	9.0 (1.8); -1.6 (1.9)***	9.6 (1.9); -2.4 (2.0)***
Patients reaching HbA_{1c}<7%, %	42.6	40.3	54.5	39.7	52.8
Mean FBG, mmol/L. Baseline; Change	9.8 (2.8); -3.3 (2.8)***	10.5 (2.8); -3.4 (2.9)***	10.9 (3.2); -4.3 (3.2)***	10.4 (3.2); -3.2 (3.0)***	11.2 (3.3); -4.5 (3.5)***
Mean PPBG breakfast, mmol/L. Baseline; Change	13.6 (4.1); -4.9 (4.0)***	14.2 (4.1); -4.6 (4.2)***	15.1 (4.5); -6.6 (4.5)***	14.1 (5.2); -4.9 (4.5)***	14.8 (4.6); -6.5 (4.6)***
Mean daily BIAsp 30 dose, U/kg. Baseline;Final; Change	0.18 (0.08); 0.20 (0.09); 0.02 (0.06)	0.17 (0.09); 0.37 (0.16); 0.20 (0.15)	0.40 (0.17); 0.44(0.17); 0.04 (0.16)	0.16 (0.07); 0.52 (0.21); 0.36 (0.21)	0.39 (0.17); 0.57 (0.21); 0.18 (0.19)
Major hypoglycaemia, events/patient year. Baseline; Change	0.127; -0.124***	0.078; -0.073***	0.091; -0.084***	0.028; -0.028***	0.084; -0.082***
Minor hypoglycaemia, events/patient year. Baseline; Change	2.433; -1.049**	2.010; -0.139^{NS}	2.825; -0.202^{NS}	5.993; 0.277^{NS}	3.238; 0.951*

215

Long-term sustained safety and efficacy of continued use of Technosphere insulin in subjects with type 2 diabetes

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Background and aims: Technosphere Insulin (TI) is a rapid-acting inhaled insulin with an action profile that mimics early meal-related insulin release. Safety and efficacy of TI has been previously demonstrated in controlled clinical trials of up to 2 years duration. The goal of this study was to examine the changes in lung function and glycemic control in subjects with type 2 diabetes over 4 years of continued treatment with TI.
Materials and methods: Subjects with diabetes who had completed any of the two 3-month, controlled, randomized phase II clinical trials were offered continued open-label TI as their exclusive prandial insulin regimen. Spirometry and lung carbon monoxide diffusing capacity (DL_{CO}) were measured every 6 and 12 months, respectively. HbA_{1c} was measured every 3 months. Hypoglycemia for this study was defined as blood glucose levels less than 3.5 mmol/l or symptoms of hypoglycemia that resolved after appropriate caloric intake.
Results: A total of 229 subjects (59% male, 41% female) were enrolled in the trial. In all, 199 subjects were exposed to treatment for >1 year, 175 for >2 years, 60 for >3 years, and 31 for >42 months. Annualized change in forced expiratory volume in 1 second was -0.048±0.0006 l/year and in DL_{CO} was -0.332±0.085 ml/min/mm Hg after 4 years of continued treatment with TI. Mean HbA_{1c} was 7.97% at baseline, 7.88% at month 3, 7.79% at month 6, 7.97% at month 12, 7.87% at month 18, 8.04% at month 24, 8.06% at month 30, 7.81% at month 36, 7.40% at month 42, and 6.45% at month 48. Overall, hypoglycemia rates remained stable at 0.31 events/subject-month during the first 6 months and 0.42 events/subject-month after 3 years, as measured over the final 12 months of TI therapy.
Conclusion: Changes in the lung functions after 4 years of TI therapy were small and similar to the changes expected in adults with type 2 diabetes. TI therapy maintained sustainable glycemic control for at least 4 years.

216

Healthcare costs of long-acting insulin analogues compared with NPH insulin in patients using a basal regimen: a Danish perspective

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Background and aims: Insulin analogues may be able to offer health benefits to patients with diabetes, but also carry a higher price compared with conventional human insulins. The objective of this study was to compare the healthcare cost outcome of a basal insulin regimen of long-acting insulin analogues (LAIA) with neutral protamine Hagedorn (NPH) insulin, in patients with type 2 diabetes (T2D). Analysis was based on Danish healthcare costs.
Materials and methods: Data were extracted from national registers to involve the entire Danish population. Included in the analysis were prescription, hospital in- and out-patient care costs and socioeconomic variables. Suitable patients were identified during a 1 year index period (2005) and allocated to treatment with either LAIA or NPH insulin. Inclusion criteria were at least two prescriptions in the index period of LAIA (insulin detemir or insulin glargine) or NPH respectively and no switch to the other product in the follow-up period. Patients with at least one oral antidiabetic drug prescription between 1995 and 2007 and fewer than two prescriptions of fast-

acting insulin were identified. The patients from the LAIA group (n=303) were matched with patients from the NPH group (n=8523) with respect to observable variables using propensity scores. Healthcare costs were analysed for a follow-up period of 2 years, maximum. Figure 1 illustrates the distribution of costs for each of the treatment populations.
Results: The highest proportion of healthcare costs in both treatment groups could be attributed to inpatient care, accounting for 46% and 38% in the NPH and LAIA groups, respectively. Analysed in isolation the cost of basal insulin based on prescription data was significantly lower for the NPH treatment group compared to the LAIA group (p<0.0001). Overall annual healthcare costs however, which included all prescription medicine, amounted to €6230 in the NPH group and €5524 in the LAIA group. This difference in total costs between treatments groups was not statistically significant (p=0.302).
Conclusion: Costs for patients with T2D on a basal insulin regimen using LAIA do not seem to be higher in terms of healthcare resource than costs for patients using NPH insulin. This national study is ongoing and is expected to be revised with longer follow up and increasingly robust hospital data.

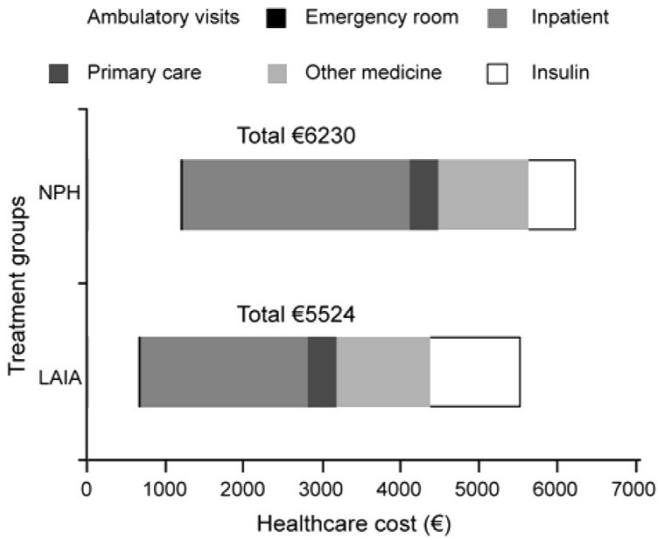

Figure. Follow-up annual healthcare costs 2005/6. NPH, neutral protamine Hagedorn insulin; LAIA, long-acting insulin analogues. Exchange rate: 2nd January 2009

Supported by: Novo Nordisk

OP 37 To amputate or not to amputate

217

To amputate or not to amputate? Healing rates following minor amputation vs conservative treatment of diabetic foot ulcers
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Background and aims: Minor amputation is performed frequently as part of the management of diabetic foot ulcers. The decision to amputate is made by the professional in charge and is guided by factors such as the extent of tissue loss and bone involvement. The extent to which healing rates following minor amputation or conservative treatment differ is unclear. Therefore we have examined this in a large European cohort of diabetic foot ulcer patients.
Materials and methods: We examined 1088 patients from the Eurodiale study, a prospective cohort study of patients with new diabetic foot ulcers who were followed, on a monthly basis, until healing, death, major amputation or up to a maximum of 1 year. Ulcers were treated according to international guidelines. Patient, foot and ulcer characteristics were obtained at baseline, and data on minor amputations were collected on a monthly basis. Multiple logistic regression models were used to compare the probabilities of healing between those subjects who underwent vs. those who did not underwent a minor amputation. These analyses were first adjusted for the differences between patients in time at which minor amputations took place (model 1) and then for characteristics previously identify to be independently associated with minor amputation (i.e. male sex, depth of the ulcer, peripheral artery disease and infection, model 2) and further for additional characteristics also previously identified as independent predictors of healing in this cohort (model 3).
Results: Within the follow-up period 194 patients (22%) underwent at least one minor amputation; overall healing rate was 77%. Patients who underwent a minor amputation were more likely to heal than those who did not: OR=1.69 (95%CI: 0.98-2.92, model 1). After taking into account the baseline differences, between these 2 groups, in independent correlates of minor amputation, the odds of healing were 2.63 times greater (95%CI: 1.47-4.70) among subjects who underwent minor amputation than those who did not (model 2). Further adjustments for other characteristics known to independently determine healing only slightly attenuated these odds: OR=2.53 (95%CI: 1.39-4.59).
Conclusion: Performing a minor amputation is associated with a higher probability of healing as compared to conservative treatment of diabetic foot ulcers. Nevertheless, for every individual diabetic patient with a foot ulcer the choice for conservative treatment or minor amputation should be made taking into account the potential short- and long term burden, risks and benefits.
The Eurodiale study was supported by the EC (5 FP Grant QLG4-CT-2002-01524)

218

Diabetic foot disease and reduction of major amputations in Italy: results of an Italian 5-year study (2001-2005)
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Background and aims: Until now in Italy clear data on the incidence of amputation rate in diabetic subjects compared to non diabetic subjects , as well as on the hospitalization rate for diabetic foot problems are not available. Moreover the amount of peripheral arterial disease and relative vascular procedures has not been well evaluated yet. In the present study we present accurate and complete data on hospitalization for diabetic foot (ulcers, gangrene, diabetes-related lower extremity amputations (DRLEAs) and non diabetes-related lower extremity amputations (non-DRLEAs) in Italy over a 5 -year period (2001-2005).the data base for this study was taken from Italian Register of DRG (ICD9-CM ed 2002), containing information regarding all hospital admissions for diabetic patients, including diabetic foot problems occurred during years 2001-2005.

Results: From 2001 to 2005 in Italy the hospitalization rate (HR) (for all causes) for diabetes patients significantly increased from 9.2 (in 2001) (x 1000 inhabitants) to 10.24 (in 2005): in these years the prevalence of diabetes increased from 3.9 (x 100 inhabitants) in 2001 to 4.2 (x 100 inhabitants)(Italian National Health data base). In table 1 is shown the hospitalization rate (per 100,000 inhabitants) of diabetic population; in years 2001 - 2005 hospitalization for ulcers increased by 42%; gangrene by 28% and hospitalization for peripheral arterial occlusive disease by 27%. In the same period of time it was observed that, while minor amputations were increased, major amputations seemed to show a linear trend to reduction. The ratio between DRLEAs and non-DRLEAs was in these years about 75%. Moreover, in 2001 the total number of vascular procedures was 4549 (2285 endovascular peripheral revascularization angioplasty and 2264 surgical procedures peripheral bypass); in 2005 the total number of vascular procedures rose to 10325 with a significant increase of peripheral angioplasty (7735) in comparison with surgical procedures.
Hospitalization rate= patients recovered at least once a year for a specific pathology/ inhabitants
Conclusion: In the study period (2001-2005) in Italy the incidence rate of major DRLEAs was decreased.These results could be possibly explained with the implementation of Diabetic Foot Care occurred in Italy since1999, following the publication of International Consensus on Diabetic Foot. It is notable the increasing amount of peripheral angioplasty as a diagnostic-therapeutic approach in the peripheral arterial occlusive disease. This finding provides support for the critical role of revascularization in diabetic foot disease and we expect this technique has become even more feasible in the last years.

Table1	2001	2002	2003	2004	2005
Peripheral arterial occlusive disease :	42.0	47.8	50.3	52.4	53.5
Diabetic foot Ulcers	22.2	26.3	29.1	30.2	31.6
Gangrena	13.0	15.4	16.0	16.5	16.7
Peripheral amputations*:					
minor	4.8	5.6	6.0	6.7	6.7
major	3.3	3.7	3.8	3.6	3.5
total	8.4	9.8	10.2	10.6	10.6

*Traumatic and neoplastic amputations are not included , minor: ICD9 8411-8412, major: ICD9 8413-8419

Supported by: Italian Diabetic Foot Study Group SID-AMD

219

A multiprofessional project to reduce the incidence of lower-limb amputation in the diabetic population

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Background and aims: Many diabetic patients develop peripheral vascular disease that, when severe, may require lower-limb amputation (LLA). To avoid LLA, educational and therapeutic handling must be proposed. Since 2003, action plan to reduce the incidence of LLA was performed in the country of Bethune (North of France, 250 000 h). This action plan includes: vocational training of podiatrists, nurses, and medical doctors, distribution of nylon filaments for medical doctor, campaigns of detection, educational intervention program...
The purpose of this study was to examine the effects of interprofessional educational and therapeutic handling on the incidence of LLA in the diabetic population.
Materials and methods: This study was conducted in the Central Hospital of Bethune. The inclusion criteria were LLA performed because of peripheral vascular and/or neuropathy disease(s) since 1/01/2000 through 31/12/07. All patients undergoing LLA in the operating room were recorded consecutively. The anthropometric and clinical data were collected, then analyzed from Khi² or paired Student's t-test.
Results: During the 7-year period, LLA was performed on 285 patients. 213 were diabetic (65% men; age: 66.0 yrs; HbA1c: 8.3%). Since 2003, although the number of hospitalized patients in hospital increased, the number of LLA decreased year by year : 29 in 2000 vs 19 in 2007. Since 2005, the incidence of LLA was significantly reduced (1,97 0/00 000 vs 1.34 in 2007). The number of amputated patients because of peripheral vascular disease was similar; whereas the number of amputated patients with neuropathy decreased. LLA especially concerned: men > 60 years, with type 2 diabetes since more than 10 years, not controlled, with diabetic complications (e.g., retinopathy, nephropathy, ischemic cardiomyopathy...). Moreover, the patients have foot wound since more than 8 weeks. The population with peripheral artery disease was older ($p < 0.001$), their incidence of reamputation was higher ($p < 0.05$). Their LLA was often proximal ($p < 0.001$) with a higher complication rate ($p < 0.001$), and a lower prognostic ($p < 0.001$).
Conclusion: These results suggest that the multiprofessional educational and therapeutic handling have beneficial effects on the incidence of LLA in the diabetic patients with neuropathy. Further studies are necessary to verify these results.

220

Can we limit frequency of amputations in diabetic foot ulcer (DFU) patients?

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Background: DFU clinic in Warsaw was established in 2000 and provides specialized DFU care for the whole Mazovian region (over 5 mln inhabitants).
Among 1200 patients of our DFU Outpatient Clinic (DOC) 107 were qualified for high amputations before admission but the limbs were saved. In the region covered by DOC incidence of high amputations decreased from 8.3 per 100,000 in 2006 to 7.1 in 2007.
The aim of the study was to describe the group who underwent plantar or high amputations in order to investigate possibilities of reducing frequency of amputations.
Methods: Analysis comprised 866 patients of DFU clinic in Warsaw with complete data available, including 420 patients with amputations.
Classification of DFU was based on:
• Assessment for diabetes peripheral neuropathy: neurotensimeter, monofilament, camerton
• Assessment for peripheral artery disease: ABI < 0.7 - ischemic type
• Charcot artropathy was distinguished based on clinical presentation and presence of foot bone lesions.
Amputations were classified as plantar of high (above the ankle).
Results:

Characteristics of the patients of the clinic (whole group) and of the patients with amputation

Whole group (N=866)	*Amputation group (N=420)*
Sex: 65.3% males, 34.7.% females	Sex: 74.6% males, 25.4% females
Residence: 70.0% urban, 30% rural	Residence: 80.8% urban, 19.2%rural
-Education: 37.5% primary, 50.0% secondary, 12.5% higher,	-Education: 42.9 primary, 53.0% secondary, 8.1% higher
DFU type: 60.7% neuropathic, 10.3% ischemic, 29% mixed	DFU type: 23.7% neuropathic, 39.3% ischemic, 37.1% mixed

There were 442 amputations, including 86 (19,5%) high amputations.
Frequency of amputation significantly higher:
• in patients from rural areas
• in ischemic DFU (Table 1)
Plantar amputations were significantly more frequent in case of ulcers localized in the foot areas 1 and 2 independently of the type of DFU.
Plantar amputations were more frequent on the left foot.
Localization of lesions preceding plantar amputations
The percentage of high amputations among all amputations decreased significantly from 31,9% in 2000-2001 to 14,1% in 2006-2007.
The percentage of high amputations was higher among persons with elementary education (36.2%) then among persons who completed secondary schools or university degrees (19.9%).
Conclusion: 1. Lesions localized in fields 1 and 2 often lead to plantar amputations and should be closely followed.
2. Less educated people may require reinforced educational messages in order to prevent amputations, especially high amputations.
3. Population of rural areas may have poor access to specialized care and may profit from telemedicine.

221

The mortality of patients with acute Charcot foot compared to patients with neuropathic ulcers and the normal population

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Background and aims: The mortality associated with the acute Charcot foot is not clear, and its assessment is made difficult by the rarity of the condition and the influence of population selection on those managed in specialist centres. Two studies have previously reported that mortality is very low: Armstrong et al reported no deaths in a population of 55 patients, with a mean follow-up of 92.6 weeks while Fabrin et al noted just two deaths in a retrospective study with a total of 115 patients followed for a mean of 48 months. On the other hand, we have previously reported much higher mortality in a series of 47 patients who presented between 1980 and 2000. These patients were compared with an equivalent population, referred with neuropathic ulceration. 44.7% of patients with Charcot died after 3.7 years mean follow-up, compared with 34.0% died of those with neuropathic ulcers, after a similar follow-up period, but these were not significantly different. The purpose of this study was to attempt to confirm our previous findings in a more recent population attending in the same specialist centre. We have therefore reviewed a population presenting with an acute Charcot foot, and compared the outcome in a matched population with neuropathic foot ulcers.
Materials and methods: Details of all patients with an acute Charcot foot and newly referred to our multidisciplinary service between 2000 and 1st October 2007 were extracted from the specialised foot clinic database, and survival checked against the hospital master patient index. The results were compared with those from a population referred with a neuropathic foot ulcer first referred over the same time, and matched for age, gender and diabetes duration and also a matched normal population derived from normative UK data .
Results: A total of 70 (70% Type 2 diabetes, mean age 57.4 (SD ±12) years; 68.8% male) patients presented with an acute Charcot foot to our multidisciplinary service. There were 66 matched patients with neuropathic foot ulcers. By 1 October 2008, 13 (18.6%) patients with a Charcot foot had died (8 men) at a mean age of 61.7±11.5 years and after a median of 2.1 (95% CI 0.5-5.6) years. 22 (33.3%) patients in the control group had also died (20 men) at mean age of 65.1±11.0 years, and after a median of 1.3 (0.2-5.6 years (p=0.13). Kaplan-Meier survival analysis was performed. Log rank tests for the equality of the survivor function for the Charcot and control patients showed no significant difference between groups (p=0.17). When the two cohorts (1980 to 2000 and 2000 to 2007; 117 patients with acute Charcot and 113 controls)were combined, the median time to death in the Charcot patients was 3.7 (95% CI 0.09-14.9) years and there was no different from the control ulcer patients: 2.7 (0.2-15.5) years (p=0.57). Kaplan-Meier survival analysis similarly showed no difference between the 2 groups (p=0.81). The median survival of a matched normal population derived from normative UK data was 22.3 (CI 19.4-25.3) years, compared with 7.9 (5.1-11.8) and 8.4 (6.0-9.8) years in Charcot and neuropathic ulcer groups, respectively (p<0.001).
Conclusion: These data confirm the high mortality in patients with an acute Charcot foot, and also show that mortality is equally high in those with neuropathic foot ulcers. The high mortality in both groups may relate to an increased prevalence of arterial calcification associated with neuropathy, and the effect of arterial calcification on pulse pressure and cardiovascular mortality.

222

Serum levels of osteoprotegerin are raised in diabetic peripheral neuropathy and are significantly correlated with peripheral arterial calcification

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Background and aims: The osteoprotegerin (OPG) signalling pathway has been linked with diabetic neuropathy. The aim was to measure serum OPG in diabetic patients with and without neuropathy and to investigate its possible association with vibration perception threshold as a marker of large fibre neuropathy and with the presence of peripheral arterial calcification on foot and ankle radiographs.
Materials and methods: We studied 45 patients with diabetic neuropathy, 88 patients without neuropathy and 22 healthy controls. Serum levels of osteoprotegerin were measured using ELISA (Biomedica, Austria). All patients had palpable foot pulses. Large fibre neuropathy was assessed by measuring vibration perception threshold (VPT) at the apex of the hallux and the mean of both feet was calculated. A subset of 38 patients was also assessed for the presence of peripheral arterial calcification on foot and ankle x-rays.
Results: Serum OPG was significantly raised in patients with diabetic neuropathy compared with patients without neuropathy (5.1 pmol/l [3.8-6.1] versus 3.9 pmol/l [3.0-4.9], p=0.005; median [25th-75th percentile]) and also compared with healthy controls (5.1 pmol/l [3.8-6.1] versus 2.9 pmol/l [2.6-3.8], p<0.001). Overall, serum OPG levels were positively correlated with the mean VPT (r= 0.262, p=0.009). In the subset of 38 patients, serum OPG was significantly correlated with the presence of peripheral arterial calcification (r= 0.445, p=0.005) as well as the mean VPT (r=0.253, p=0.012). Serum OPG was raised in both type 1 and type 2 diabetic neuropathic patients. In type 1 diabetes, serum OPG was significantly raised in patients with neuropathy compared with patients without neuropathy (5.0 pmol/l [3.9-5.7] versus 3.8 pmol/l [2.9-4.6], p=0.042) and also compared with healthy controls (5.0 pmol/l [3.9-5.7] versus 2.9 pmol/l [2.6-3.8], p<0.001). Furthermore, in type 2 diabetes serum OPG was raised in patients with neuropathy compared with patients without neuropathy (5.5 pmol/l [3.4-6.6] versus 4.0 pmol/l [3.2-4.9], p=0.05) and also compared with healthy controls (5.5 pmol/l [3.4-6.6] versus 2.9 pmol/l [2.6-3.8], p<0.001).
Conclusion: Serum OPG is raised in both type 1 and type 2 diabetic patients with neuropathy. Furthermore, serum OPG is significantly correlated with peripheral neuropathy and peripheral arterial calcification.
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OP 38 In vivo insulin action - mechanisms

223

Serum Fibroblast Growth Factor 21 in human obesity: regulation by hyperinsulinaemia and relationship with energy expenditure and glucose and lipid oxidation

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Background and aims: Recent studies suggested that Fibroblast Growth Factor 21 (FGF21) might increase glucose uptake in adipocytes and reduce plasma glucose and triglycerides in animal models of diabetes. The aim of the present study was to assess serum FGF21 concentration in lean and obese subjects, its regulation by hyperinsulinemia and relationship with insulin sensitivity, resting energy expenditure (REE) and glucose and lipid oxidation.
Materials and methods: We examined 85 subjects with normal glucose tolerance, 42 lean (BMI <25 kg/m^2, 19 males and 23 females) and 43 overweight or obese (BMI between 25 and 40 kg/m^2, 20 males and 23 females). Euglycemic hyperinsulinemic clamp was performed in all subjects. In 28 participants, 14 lean and 14 obese, serum FGF21 was also measured after clamp. In 48 subjects, 25 lean and 23 obese, indirect calorimetry was performed in the baseline state and during last 30 minutes of the clamp. An increase in respiratory quotient (ΔRQ) in response to insulin was used as a measure of metabolic flexibility.
Results: Fasting serum FGF21 did not differ between the lean and obese groups. Hyperinsulinemia resulted in an increase in serum FGF21 (p=0.024), due to an increase in the obese (p=0.022), but not in the lean group (p=0.43). In consequence, post-clamp serum FGF21 was markedly higher in the obese subjects (p=0.029). Fasting FGF21 was related to waist-to-hip ratio (r=0.35, p=0.001), triglycerides (r=0.44, p<0.0001), and REE in the baseline and hyperinsulinemic state (r=0.40, p=0.004 and r=0.34, p=0.018, respectively). Post-clamp FGF21 was associated with BMI (r=0.51, p=0.004), waist circumference (r=0.58, p=0.0007), fasting and post-load insulin (r=0.52, p=0.004 and r=0.41, p=0.029, respectively), baseline and clamp REE (r=0.46, p=0.02 and r=0.40, p=0.049, respectively) and ΔRQ (r=-0.47, p=0.01). In the obese group, additional correlations between fasting FGF21 and an increase in glucose oxidation (r=-0.53, p=0.009) and a decrease in lipid oxidation (r=0.45, p=0.025) in response to insulin was observed.
Conclusion: Our data show that serum FGF21 is associated with insulin resistance-related abnormalities of human obesity.
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224

Angiotensin II increases insulin-stimulated whole body glucose disposal but impairs insulin-induced capillary recruitment in healthy volunteers

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Background and aims: Insulin induces precapillary vasodilatation and capillary recruitment, contributing to glucose uptake (↓) and blood pressure(↓) regulation. Blocking the renin-angiotensin system (RAS) in hypertensive subjects improves insulin sensitivity. In contrast, studies in healthy subjects have demonstrated enhanced insulin-stimulated glucose uptake with Angiotensin II (AII) infusion. Possibly, this effect is mediated via the microcirculation. In the present study we determined the effects of AII on insulin-induced microvascular function in relation to insulin sensitivity and blood pressure regulation in healthy subjects.
Materials and methods: In 18 lean healthy subjects microvascular function was examined at baseline, during insulin infusion (hyperinsulinaemic euglycaemic clamp 50mU·kg^{-1}·hr^{-1}), and during co-infusion of AII (2ng·kg^{-1}·min^{-1}) or phenylephrine (0.5μg·kg^{-1}·min^{-1}) (PE; pharmacological control). Using capillaroscopy we determined skin capillary density (n/mm^2), capillary recruitment (peak n/mm^2 during post-occlusive reactive hyperemia, PRH), and capillary density during venous congestion (n/mm^2). We determined whole body glucose uptake (WBGU, (mg·kg^{-1}·min^{-1}·pmol·L^{-1})*100) and blood pressure. All subjects were tested on a high sodium diet (220 mmol/day). In addition, we studied the pressor responses to AII and PE in seven subjects during normoinsulinaemia.
Results: Compared to the basal state, hyperinsulinaemia increased baseline capillary density (56.4±13.3 vs. 52.8±11.2 n/mm^2, P<0.01), capillary recruitment (71.7±10.3 vs. 68.7±8.7 n/mm^2, P=0.03), and density during venous congestion (82.0±13.1 vs. 80.0±12.7 n/mm^2, P<0.01). Infusion of AII, but not PE, reduced insulin-induced capillary recruitment (65.2±8.0 vs. 71.7±10.3 n/mm^2, P=0.02) and venous congestion (73.9±12.1 vs. 82.0±13.1 n/mm^2, P=0.03). AII and PE had an equal pressor response during normoinsulinemia (ΔMAP AII 14.2; PE 12.9mmHg). During hyperinsulinemia the pressor response to PE, but not to AII, was reduced (p=0.03) (ΔMAP AII 12.7; PE 5.3 mmHg). AII, but not PE infusion, augmented insulin-stimulated WBGU (2.4±0.71 vs. 2.68±0.64; P<0.01).
Conclusion: Our data indicate that insulin increases skin capillary density, skin capillary recruitment (PRH), and density during venous congestion. We found a reduction in insulin-stimulated capillary recruitment and density at venous congestion with AII, and not PE infusion. In addition, our blood pressure data indicate that AII, and not PE, affects insulin-mediated blood pressure reduction. Therefore, our data suggest possible interactions of AII with insulin signaling pathways at different levels in the (micro)circulation (i.e., at capillary level and the level of the resistance arteri(ol)es). However, despite these attenuating effects of AII on insulin-mediated capillary recruitment, AII infusion stimulated whole body glucose uptake. Because skin microcirculation is thought to be representative of that of skeletal muscle, these data suggest that AII influences glucose disposal in tissues other than skeletal muscle, and (or) that AII influences muscle glucose uptake through non-vascular effects.

225

Effect of hyperglycaemia on whole-body glucose metabolism and muscle glycogen synthase activity during physiological hyperinsulinaemia in type 2 diabetes

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Background and aims: In type 2 diabetes, insulin-stimulated glucose disposal (Rd) is reduced, mainly due to decreased glycogen synthesis in skeletal muscle. This has been associated with decreased insulin activation of glycogen synthase (GS). Previous investigations have suggested that hyperglycemia may compensate for these defects, although sufficient control studies were lacking. In this study, we examined the effect of hyperglycemia on insulin action on glucose metabolism and GS activity in patients with type 2 diabetes.
Materials and methods: Whole-body glucose metabolism was studied in 12 patients with type 2 diabetes and 10 lean, healthy and 10 obese non-diabetic control subjects by means of indirect calorimetry and tracers during a euglycemic-hyperinsulinemic clamp. In addition, the diabetic subjects underwent a hyperglycemic-hyperinsulinemic clamp. Muscle biopsies from m. vastus lateralis were obtained for examination of GS fractional velocity and I-form activity, which are believed to reflect the degree of dephosphorylation of GS.
Results: During euglycemia (~5.5 mmol/l), insulin-stimulated Rd, glucose oxidation, and non-oxidative glucose metabolism were reduced in the diabetic group compared to both control groups (all p<0,01). Insulin-stimulated GS activity were also lower in the diabetic group compared with the obese and lean groups (I-form activity: type 2 diabetes 28.5 ± 2.7, obese 36.2 ± 2.4, lean 38.7 ± 2.4 %; p<0.01). When the diabetic subjects were studied under hyperglycemia (9.3 mmol/l), the insulin-stimulated values of glucose metabolism were all normalized compared with the control groups, whereas the insulin activation of GS (I-form activity: type 2 diabetes 28.6 ± 2.0) remained impaired compared to the control groups (p<0.01).
Conclusion: These data confirm that hyperglycemia compensates for decreased whole-body glucose disposal in type 2 diabetes. However, in contrast to previous less well-controlled studies, normalization of insulin action on GS activity does not seem to be the cellular mechanism in skeletal muscle that contributes to the compensatory effect of hyperglycemia in type 2 diabetes.

226

Intramyocellular ATP flux is not dependent on insulin action in type 2 diabetes

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Background and aims: Mitochondrial dysfunction has been implicated in the pathogenesis of type 2 diabetes (T2D). The reduced capacity of mitochondria to produce ATP in muscle of type 2 diabetes could be a consequence of reduced insulin action and hence reduced need for ATP rather than a primary mitochondrial defect. We hypothesised that normalising the rate of glycogen synthesis by the mass action effect of hyperglycaemia would normalise ATP flux in T2D.
Materials and methods: ^{31}P magnetic resonance spectroscopy (MRS) was used for the direct in vivo measurement of the rates of intramyocellular ATP flux and inorganic phosphate (Pi) concentration whilst ^{13}C MRS was used to measure glucose storage as muscle glycogen. ^{13}C-enriched glucose (20%) was infused to clamp blood glucose. Measurements were made before and during standard isoglycaemic hyperinsulinaemic (40mU/m^2/min) clamps and hyperglycaemic hyperinsulinaemic clamps on separate days. Eleven sedentary T2D subjects (age 56 ± 2 years; BMI 29.1 ± 1.2 kg/m^2; HbA_{1c} 6.6 ± 0.2%) and five age-, BMI-, and physical activity-matched normal glucose tolerance subjects were studied.
Results: Insulin stimulated muscle glycogen synthesis was 43% lower in T2D than controls during isoglycaemic hyperinsulinaemia (glycogen increment from basal: 20.6 ± 11.2 vs. 58.8 ± 10.6 units; *p<0.05*). Under conditions of hyperglycaemic hyperinsulinaemia, both T2D and controls had increased muscle glycogen synthesis by ~2.6 fold compared to that observed under isoglycaemic hyperinsulinaemia (glycogen increment from basal: 55.2 ± 18.1 vs. 199.6 ± 43.4 units; *p<0.05*). These observations were consistent with overall glucose disposal rates (T2D: 4.79 ± 0.57 vs. 7.69 ± 0.80 and Controls: 6.73 ± 0.57 vs. 11.81 ± 1.10 mg/kg/min for iso- and hyper-glycaemia respectively; *p<0.05*). In contrast, ATP flux did not change in T2D or controls after 90 min of isoglycaemic hyperinsulinaemia (-4.2 vs. 4.2% from basal respectively). ATP flux during hyperglycaemic hyperinsulinaemia did not change in T2D (2.1% from basal) but increased by 28.5% (*p<0.05* vs. basal) in controls. The calculation of ATP flux depends upon measurement of intramyocellular Pi, and this increased to a similar extent in T2D and controls (to 4.32 ± 0.23 vs. 4.31 ± 0.27 and 4.59 ± 0.55 vs. 4.91 ± 0.47 mmol/l for iso- and hyper-glycaemia respectively).
Conclusion: These data demonstrate that the defect in insulin-stimulated intramyocellular ATP flux in T2D is not directly related to insulin-stimulated muscle glycogen synthesis.
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227

Proteome analysis in muscle biopsies of lean, obese and obese type 2 diabetic patients

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Background and aims: The mechanisms of insulin resistance in obesity and diabetes are largely unknown. Here we analysed human skeletal muscle tissue using a proteomic approach with the goal to identify proteins with different expression between lean, obese and/or obese diabetic subjects. Since biopsies were obtained before and at the end of hyperinsulinemic clamps, the effect of insulin on protein expression could also be analysed.
Materials and methods: Proteome analysis was perfomed using M. quadriceps biopsies obtained before (basal, B) and at the end of 180 min glucose clamps (400mU/m^2/min, CL) from 10 lean (L; BMI 24.2±0.5), 11 obese (O; BMI 33.7±1.4) and 10 obese type 2-diabetic (OD; BMI 33.5±1.1; HbA_{1c} 7.3±0.4) patients. 50 µg protein muscle tissue lysates were labeled with CyDye fluor and expression profiles analysed by 2D-differential gel electrophoresis (2D-DIGE). For this, 31 gels were run resulting in 93 spot maps (62 individual samples and 31 internal standards). Analysis of relevant spots was performed by nano liquid chromatography electrospray ionization-MS/MS analysis (nano-HPLC/ESI-MS/MS).
Results: About 2400 individual spots were detected in all gels. Of these, 548 spots were considered relevant based on the criteria that there was at least one significant difference (t-test) and a mean difference of at least two-fold between the 6 groups of samples (basal and clamp biopsies of L, O, OD), and 95 of these proteins were identified by nano-HPLC/ESI-MS/MS (37% structural, 26% metabolic, 37% unassigned proteins). Using these criteria, between O and L 145 and 74 spots were different in the basal and clamp biopsies, respectively, and 26 in both. Of these, 10 spots have been identified (70% structural, 30% metabolic). Between OD and O 55 and 55 spots were different in the basal and clamp biopsies, respectively, with 9 being different in both. If all 31 basal and 31 clamp biopsies were compared, differences were found in 71 spots of which 8 have been identified (62.5% structural, 25% metabolic, 12.5% unassigned). Since several clinical parameters showed a considerable overlap between groups, it was also analysed in all 31 basal and clamp biopsies, respectively, whether spot volumes were correlated to these parameters. Significant correlations (spearman correlation on ranks) with clamp GDR were found in 209 and 97 spots of basal or clamp biopsies, respectively, and in 58 spots both B and CL biopsies were positively (31) or inversely (27) correlated. 15 spots have been identified (47% structural, 53% metabolic). Other significant correlations were observed with BMI (B 132, CL 43, both B and CL 16 spots), serum adiponectin (B 92, CL 116, both B and CL 31 spots), FFA concentration (B 30, CL 31, both B and CL 2 spots), basal glucose (B 73, CL 52, both B and CL 9 spots) or basal lipid oxidation (B 101, CL 55, both B and CL 19 spots).
Conclusion: Using this approach it was possible to identify proteins whose expression is associated with obesity or type 2-diabetes, respectively and proteins whose expression is influenced by a 180 min in vivo insulin stimulation. Moreover, in this relatively large data set, correlations between the abundance of proteins and clinical parameters could be analysed.
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228

Adding exercise to a 16-week very low calorie diet increases skeletal muscle mitochondrial copy number and peak oxygen consumption in obese, insulin-treated type 2 diabetic patients

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Background and aims: Loss of 50% overweight using a very low calorie diet (VLCD, Modifast®, 450 kcal/day) improves whole-body insulin sensitivity and PKB-regulated skeletal muscle insulin signalling in obese insulin-dependent type 2 diabetic (DM2) patients. This study investigates whether adding exercise to calorie restriction has additional beneficial effects, especially on skeletal muscle.
Materials and methods: Twenty-seven obese (BMI 37.2 ± 0.89 kg/m^2 (mean ± SEM)) insulin-treated DM2 patients underwent a 16-week VLCD. Thirteen of them simultaneously followed an exercise programme consisting of one-hour in-hospital training and four 30-minute training sessions on a home-bicycle weekly. Oral glucose-lowering agents and insulin were discontinued 3 weeks prior to and at the start of the VLCD, respectively. A euglycemic-hyperinsulinemic clamp with stable isotopes (6,6-2H2-glucose) combined with skeletal muscle biopsies, indirect calorimetry and peak oxygen consumption testing (VO2max) were performed before and after the intervention.
Results: Baseline characteristics between study groups were identical. Both groups lost considerable weight (-23.7 ± 1.7 kg VLCD-only vs. -27.2 ± 1.9 kg VLCD+exercise, p=NS) with the exercise-group losing more fat mass. Glycemic control improved considerably and similarly in both groups (HbA1c: VLCD-only 7.9 ± 0.2 % to 6.7 ± 0.3 %, p=0.008; VLCD+exercise 7.5 ± 0.4 % to 5.7 ± 0.2 %, p<0.0001) despite the fact that all glucose-lowering agents including insulin were withdrawn. Insulin-stimulated glucose-disposal increased equally in both study groups (15.0 ± 0.9 to 39.2 ± 4.7 µmol.min^{-1}.kglbm^{-1} VLCD-only vs. 17.0 ± 1,0 to 37.5 ± 3.5 µmol.min^{-1}.kglbm^{-1} in VLCD+exercise), as did PKB-regulated insulin signaling (PRAS40 and AS160). In contrast, skeletal muscle mitochondrial DNA (mtDNA) content increased only in the VLCD+exercise group (1211 ± 185 to 2288 ± 358, p=0.016), not in the VLCD-only group (1397 ± 240 to 1196 ± 179, p=NS). The increase in mtDNA was accompanied by an increased VO_{2max} (+6.6 ± 1.7 ml.min^{-1}.kglbm^{-1} vs. +0.7 ± 1.5 ml.min^{-1}.kglbm^{-1} VLCD-only, p=0.017)

and lower basal glucose oxidation (and hence higher basal lipid oxidation) rates and a greater increase in insulin-stimulated glucose oxidation in the VLCD+exercise group as compared to the VLCD-only group, suggesting enhanced metabolic flexibility.
Conclusion: Adding exercise to a 16-week VLCD does not have additional effects on whole-body insulin sensitivity or PKB-regulated signalling pathways. It does however preserve lean body mass, and increases skeletal muscle mtDNA content. The latter was associated with an increase in VO2max and a greater flexibility to switch between glucose and lipid oxidation.
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OP 39 Healthcare delivery

229

The cost of diabetes complications: registry-based analysis of days absent from work
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Background and aims: As a chronic disease, many of the costs of diabetes stem from the associated complications rather then the disease itself. These add a considerable burden on health care systems across Europe. But patients, employers and society incur an additional, indirect cost trough the absence from work caused by sickness days and early retirement, yet little is known about the size of such costs. The unique Danish person identification number enables researchers to combine data from different administrative registries, including the municipal sickness benefit register that contains data on absence from work and subsidies paid. The aim of this study was to analyse the annual number of days absent from work associated with a range of complications in individuals with diabetes.
Materials and methods: 34882 adults aged 18-70 years with a history of hospital diagnosed diabetes (ICD-10 code: E10-E14) were identified from a large national sample of the Danish population (n=2.2 mio. persons (40% of the Danish population) with 6 years' hospital utilisation data). The occurrence of a complication was defined as a hospital admission with a specified diagnosis or procedure code. Data on sickness episodes with municipal subsidy (i.e. sickness periods longer than 14 calendar days) were retrieved for each individual. Those individuals in early retirement (n=9720), voluntary retirement (n=3320) and receiving social benefits (n=1335) were assumed to be absent from work the whole year. Days absent from work attributable to complications were defined as the difference in mean absence days between individuals with and without the specified complication and were calculated for the first and subsequent years after the initial episode of the recorded complication.
Results: The table shows the 10 most frequent complications in the adult diabetic population and the average attributable days of absence from work. Angina pectoris, ischemic stroke and heart failure were the three most frequent complications. Heart failure, amputation, renal disease and peripheral vascular disease were on average associated with more than three months additional absence from work during the first year and subsequent years. Ulcers and neuropathy were complications that were associated with more days absent from work during the first year than in subsequent years.
Conclusion: Diabetes complications are associated with a substantial number of additional days absent from work. The avoidance of these complications could benefit both patients and society.

Additional days absent from work assoc. with diff. compl. in diabetes patients (n=34882)

Complication	First year of complication		Subsequent years of complication	
	n with compli-cation	Additional days absent from work (95% CI)	n with #compli-cation	Additional days absent from work (95% CI)
Myocardial infarction	839	62.4 (51-71)	1419	64.9 (56-74)
Angina pectoris	1432	56.0 (47-65)	3500	71.6 (66-78)
Peripheral vascular disease	723	86.1 (73-99)	1692	91.6 (83-100)
Ischemic stroke	919	81.6 (70-93)	1905	94.7 (87-103)
Heart failure	896	95.0 (84-106)	1557	102.5 (94-111)
Renal disease	341	93.7 (75-112)	575	93.1 (79-107)
Uninfected ulcer	360	82.6 (65-100)	578	74.7 (61-89)
Infected ulcer	289	39.2 (19-59)	676	16.0 (2-29)
Neuropathy	317	38.7 (20-58)	1083	19.1 (9-30)
Amputation	325	99.2 (80-118)	422	90.2 (74-107)

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230

The English national diabetes audit 2003-2008

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Background and aims: In England standards were established in 2003 by the Diabetes National Service Framework and subsequently by NICE guidelines for the key annual processes and treatment targets of diabetes care. To facilitate improvement towards these standards a national clinical annual audit was established concurrently.
Materials and methods: A dataset was approved by the NHS Information Standards Board and implemented in clinical information systems. Automated and manual annual data extraction systems were established by the NHS Information Centre (IC) to which data is uploaded securely and confidentially. This data is linked via a unique NHS number to hospital discharge data which is also compiled by the IC. Summary reports are produced annually and participants can also use a secure online data analysis tool.
Results: Participating clinical services collaboratively audited 253,000 patient records in 2003-4 rising to 1,420,000 in 2007-8. The audit now includes 67% of the diagnosed population or 57% of the estimated (YHPHO PBS3 model) population of people with diabetes in England. The diagnosed versus estimated ratio has improved (77% 2003-4 increased to 86% 2007-8). Care processes completion rates have improved year on year e.g. HbA1c measurement (76% 2003-4 to 91% 2007-8), retinal screening (47% 2003-4 to 69% 2007-8), while completion of all nine key processes has improved from 7% in 2003-4 to 40% in 2007-8. Treatment target achievement rates have also improved e.g. HbA1c<7.5% 53% in 2003-4 increasing to 63% in 2007-8, Cholesterol<5mmol/l 65% to 78%. However these encouraging average national figures conceal wide variations; thus in 2007-8 some health economies achieved all nine key care processes in only 16% while in others it was 63% while for glucose control in some areas only 40% of people with diabetes achieved HbA1c <7.5% in while others 72% achieved this level; and nationally the HbA1c <7.5% target was achieved in only 18% of children and young people. The pattern in respect of acute and late complications also shows national trends and local variation: thus overall the rates of DKA, MI, CVA & Major amputation have remained stable while renal failure treatment is increasing; but, for example, DKA, MI & CVA rates vary three to four fold, renal failure treatment rates vary six to eight fold and major amputation rates vary more than tenfold between localities.
Conclusion: This national quality monitoring programme is: helping local care providers accurately to benchmark against peer services; facilitating improvements in care and outcomes; and providing commissioners and policymakers with robust information about performance against the national standards. It has documented significant improvement over 5yr and the continuing extent of local variation suggests much more improvement is possible.
Supported by: Healthcare Commission National Clinical Audit Programme

231

Appraisal and comparison of guidelines for the management of people with type 2 diabetes in eight European countries

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Background and aims: A wealth of evidence exists for process and outcome measures for the management of people with type 2 diabetes (T2DM); numerous guidelines have been developed internationally with the aim of transferring this evidence to clinical practice. There have been previous reports of variations between evidence based recommendations in guidelines produced in different countries. The current study is part of a research programme investigating standards of care for people with type 2 diabetes across eight countries in Europe. We aimed to compare the guidelines from these countries, including rigour of development and recommendations for clinical practice.
Materials and methods: The most recent guidelines for T2DM were identified from Belgium, England & Wales, France, Germany, Ireland, Italy, the Netherlands and Sweden. The Appraisal of Guidelines for Research and Evaluation (AGREE) instrument was used to assess the quality of each guideline in six domains: scope and purpose; stakeholder involvement; rigour of development; clarity and presentation; applicability; and editorial independence. In addition, a study-specific data collection form was developed to extract details of each guideline's recommendations for key process and outcome indicators selected for clinical relevance. Appraisal and data extraction were conducted independently by two researchers in the UK or the country of origin of the guideline.
Results: The date of publication of the guidelines ranged from 2000 to 2008. There was considerable variation in AGREE domain scores for different guidelines, including a range of 31% to 95% for rigour of development. The highest mean domain scores (from pooled results for all countries) related to reporting of the scope and purpose of the guidelines and clarity and presentation (mean score 81% for both domains). Only three guidelines (England & Wales, the Netherlands, Sweden) were given the overall assessment 'strongly recommended' by their appraisers, but the others were all rated as 'recommend with provisos or alterations'. Specific recommendations given in the eight guidelines were broadly similar but, at detailed level, there were variations, particularly in relation to the level of information provided. Specified targets ranged from <6.5% to <7% for HbA1c and from <130mmHg to <140mmHg and 80mmHg to <85mmHg for systolic and diastolic blood pressure respectively. Only half of the guidelines provided a target for BMI and only two (Belgium, Germany) included waist measurement targets. Information included in recommendations for self-management education ranged from a complete lack of detail to a comprehensive list of topics to be covered.
Conclusion: Appraisal of the current guidelines for the management of T2DM in eight European countries indicated considerable variation in respect of AGREE quality measures, including rigour of development. Although there was a substantial degree of consensus for specified targets, there were some differences at detailed level and wide variation in the amount of information provided.
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232

Differences in the quality of diabetes care caused by social inequalities disappear after treatment in a tertiary care centre in Germany

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Background and aims: Low social status is associated with an increased number of diabetic late complication and higher mortality. We studied the association of social status and quality of diabetes care at entry and at last observation in a tertiary care centre in Germany.
Materials and methods: Social status and quality of care (HbA1c, blood pressure, BMI) were assessed in 582 patients (128 DM1: age 48.7y; duration of diabetes 19.7y; 51% female, HbA1c 7.4%; BMI 26.8; follow up 8,8J; 454 DM2: age 65.2y; time since diagnosis 13.7y; 45.5% female; HbA1c 7.3%; BMI 32.2, follow up 5.5y; insulin therapy 71.5%) at entry and at last observation in a large university outpatient department for endocrinology and metabolic diseases. Social status (score 3-21) was determined by three components (score 1-7 each): education, highest professional position achieved and household net income.
Results: The mean social score (SS) was 11.65 (min. 3; max. 20) in DM1 and 10.86 (3-21) in DM2. At entry patients with DM1 and the lowest SS-tertile were older (59.5 vs 47.5y; p=0.001), had a higher BMI (25.8 vs 23.0; p=0.001), diabetes duration (18.9 vs. 16.4 n.s.), a higher HbA1c (9.4 vs 8.8; n.s.), higher syst. BP (141.5 vs. 130.3; p=0.002) and higher diast. BP (86.2 vs. 78.2; p=0.001) compared to the highest SS-tertile. After a mean follow up of 8.8y differences in syst. BP (134.8 vs. 140.0; n.s.), diast. BP (85.1 vs. 84.3; n.s.) and HbA1c (7.4 vs. 7.5; n.s.) between the lowest and highest SS tertile were equalized HbA1c decreased by 0.056% per each point of social score after adjustment for diabetes duration and self monitoring (p=0.006). At entry patients with DM2 and the lowest SS tertile had a longer duration of diabetes (15.1 vs 13.1y; p=0.034), higher BMI (31.8 vs 28.7; p=0.001) and higher HbA1c (8.9 vs 8.4; p=0.022). No differences were seen regarding age (65.4 vs 66.3y; n.s.), syst. (144.9 vs. 143.2; n.s) and diast. BP (80.8 vs 82.5; n.s.). After a mean follow up of 5.5y differences in HbA1c (7.2 vs. 7.2%; n.s.) were neutralized or did not change BMI (33.3 vs 29.9; p=0.001), syst. (143.8 vs 141.7; n.s.) and diast. BP (86.2 vs 86.0; n.s.). HbA1c increased by 0.031% per each kg/m² after adjustment for SS, diabetes duration, self monitoring and dose adjustments (p=0.006).

Conclusion: Low social status is associated with worse quality of care at entry in a tertiary care centre. These differences partly disappeared after 5-9 years treatment and education in a tertiary care centre.

233

Relationship between HbA_{1c} and risk of micro/macrovascular complications and healthcare costs among type 2 diabetes in a US managed care cohort

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Background and aims: Poor glycemic control (>7.0%) in patients with type 2 diabetes (T2D) may increase risk of complications, leading to higher healthcare costs. This study examined the relationship between HbA1c level and diabetes-related complications and costs among T2D patients.
Methods: Using a large database of privately-insured individuals in the United States, a retrospective claims analysis was conducted on T2D patients in 2002-2003 with no history of diabetes-related complications or recent T2D medication changes. Total healthcare costs included facility, provider, and pharmacy costs. Costs of diabetes-related complications were derived from claims with an ICD code for a macrovascular (myocardial infarction, CVD, stroke, CAD, peripheral vascular disease) or microvascular (neuropathy, nephropathy, amputation, retinopathy) diabetes-related complications. Cox models assessed the association between HbA1c and risk of macro/microvascular diabetes-related complications, adjusting for patient characteristics. Patients were followed up to 3 years. General linear models (GLM) and 2-part models assessed the association between HbA1c and costs for those with 2+ years follow-up.
Results: Of the 41,182 patients with no history of diabetes-related complications, 26.8% and 44.7% developed macro- and microvascular complications, respectively. Risk of macrovascular diabetes-related complications increased 2% and risk of microvascular diabetes-related complications increased 3% for each one unit increase in HbA1c (HR=1.02, 95% CI 1.01-1.03 and HR=1.03, 95% CI 1.02-1.04, respectively). Among the 11,053 patients with 2+ years follow-up, total mean adjusted costs were 26,167 US dollars (95% CI 25,929-26,406 US dollars) and did not differ by HbA1c strata. Costs related to diabetes-related complications increased with increasing HbA1c (Figure). In the short term there was a decrease in provider costs as HbA1c increased, from 8,384 US dollars in those with HbA1c < 6.5 to 7,648 US dollars in those with HbA1c > 9.0 that was not reflected in facility and pharmacy costs.
Conclusion: Total costs did not significantly differ by HbA1c strata. With increasing HbA1c, diabetic-related complications increased coinciding with related costs, particularly for HbA1c >8.0%. While provider costs are higher for patients with better glycemic control, in the long run they may be offset by lower costs related to diabetes-related complications.

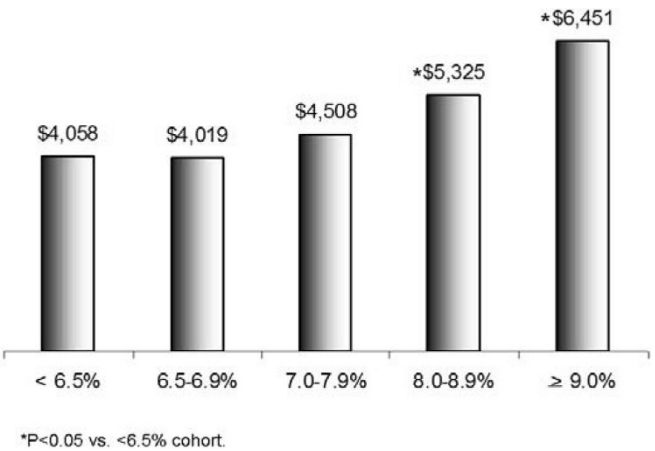

Figure: Adjusted Mean DC Costs by HbA1c Level

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234

Guarding medical prescriptions in patients with renal failure: a new warning system on renal dysfunction

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Background and aims: Many guidelines stress the importance of monitoring medical prescriptions to prevent adverse drug reactions (ADR), especially in patients with polypharmacy or comorbidity, like in diabetes mellitus (DM). From data of the Dutch HARM (Hospital-Admissions-Related-to-Medication) study in 2006 an incidence of 41.000 medication-related admissions in the Netherlands was calculated (of which 19.000 possibly preventable). Renal failure appeared one of the main independent risk factors. Considering the increasing incidence of renal impairment in DM, these patients are more at risk for medication-related complications.
The aim is to optimize monitoring the adequacy of medical prescriptions in DM patients with decreased renal function in primary care, by means of automatic data processing of laboratory estimated glomerular filtration rate (eGFR) values below 40 ml/min/1.73 m^2 towards both pharmacists as well as general practitioners (GPs).
Materials and methods: From June until November 2008 a pilot study was performed in which the regional laboratory selected all patients with an eGFR ≤ 40 ml/min/1.73 m^2 (MDRD4) and biweekly informed the two participating dispensaries and six GPs. Within the study population a subgroup of patients with DM was identified. Pharmacists checked the identified patients' actual drug regimen on current contra-indications. In all these patients an alarm signal warned for low eGFR with every relevant new prescription. All signals and interventions triggered by this warning were registered.
Results: During the study period a total of 121 patients with an eGFR ≤ 40 ml/min/1.73 m^2 were registered, of whom 43 patients were treated by the participating GPs. Current medication lists were evaluated and revealed a contra-indication for 147 maintenance medications, and 204 warnings were registered on new and repeat prescriptions. Six percent (n=22) of the warnings resulted in consultation with the prescribing physician, mainly because of a suspected overdose or an absolute contra-indication in relation to eGFR. Overall, 24% of the study population had DM, with a median eGFR of 28 ml/min/1.73 m^2. In half of this subgroup the prescribed medication was not started and an alternative treatment proposed instead, due to the risks of (nephro)toxicity. This concerned antibiotics, analgetics and statins. Pharmacists advised a dose-reduction of anti-gout medication in 3 patients. One patient (eGFR 23 ml/min/1.73 m^2) on metformin changed to insulin therapy. In the overall study population mainly antibiotics, diuretics and anti-gout therapy were discontinued. Several times GPs were reminded to check on serum digoxin levels or on renal function shortly after start of new medication.
Conclusion: This pilot study shows that the introduction of a relatively simple warning system improves patient care and safety. A considerable amount of patients with a low eGFR had DM, a population vulnerable for adverse drugs reactions. This collaboration of pharmacists, clinical chemists, GPs and nephrologists resulted in a stricter medication monitoring for patients with an impaired renal function and DM.

OP 40 Monogenic diabetes and obesity

235

Maturity-onset diabetes of the young in Danish and Czech families due to mutation in the insulin gene

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Background and aims: Insulin gene (*INS*) mutations have recently been described as a common cause of permanent neonatal diabetes (PNDM) and a rare cause of diabetes diagnosed in childhood or adulthood. We aimed to determine the prevalence, phenotype and treatment of *INS* mutation in MODYX patients, gestational diabetes patients (GDM) and GAD65 negative type 1 diabetic patients.
Materials and methods: *INS* was sequenced in 117 MODYX patients (n = 48 Danish and n = 69 Czech), 85 patients with GDM, 37 GAD negative type 1 diabetic patients and 96 glucose tolerant individuals. All MODYX probands were from families fulfilling the conventional criteria of MODY and they were all screened negative for mutations in the MODY genes *HNF4A*, *GCK* and *HNF1A*. All women with GDM and all GAD negative type 1 diabetic patients had a positive family history of diabetes. The control group were randomly selected from the population based sampled Inter99 study.
Results: One novel heterozygous mutation c.17G>A, R6H, was identified in the pre-proinsulin gene (*INS*) in a Danish MODYX family (se figure 1). The proband was diagnosed at 20 years of age with mild diabetes and treated with diet and oral hypoglycaemic agent (OHA). His son (20 years) carried the mutation but had a normal glucose tolerance. The brother to the proband carried the mutation and was diagnosed with diabetes at 51 years of age; a daughter of the brother carried the mutation and was diagnosed with impaired glucose tolerance at the age of 26. In a Czech MODY proband a previous described R46Q mutation was found (se figure 1). The mutation co-segregates with diabetes in two affected relatives and is not present in any of three non-diabetic family members or 96 control individuals. The proband, a 17 years old girl, was diagnosed with mild diabetes at 13 years of age. The proband was treated with insulin since onset of diabetes. Her mother was diagnosed at 14 years of age with symptoms of polyuria and polydipsia and treated with insulin from the onset of diabetes. The grandmother was diagnosed with diabetes at 35 years of age and treated with OHA and insulin. No diabetogenic mutations were found in GDM women or GAD negative type 1 diabetic patients.
Conclusion: Mutations in *INS* can be a rare cause of MODY and we conclude that screening for mutations in *INS* is recommended in MODYX patients.

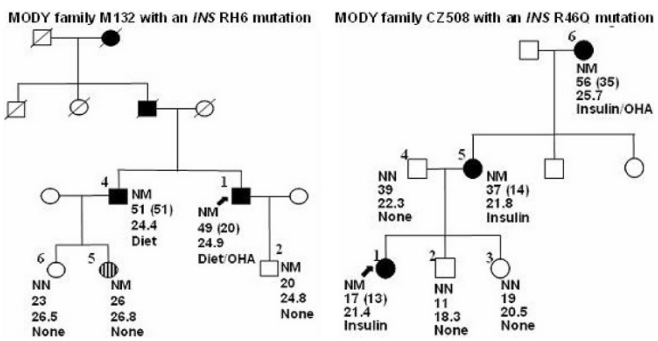

Figure 1: Pedrigrees of the MODY families with the *INS*RH6 and R46Q mutations. The symbols denote the following square: male, circle: female, empty symbol: normal grucose tolerant subject, filled symbol: diabetic subject, striated filled: impaired glucose tolerance status, symbol with arrowhead: proband. The text to each individual represents the following: Genotype, age of examination (age of diagnosis), BMI (kg/m^2) and treatment.

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236

Mutations in the insulin gene resulting in a MODY phenotype: marked differences in clinical presentation, metabolic status and pathogenic effect through ER retention

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Background and aims: Heterozygous mutations in the human insulin (*INS*) gene have recently been identified as a (common) cause of non-syndromic neonatal or early-infancy diabetes. Here, we sought to identify *INS* mutations that are associated with maturity-onset diabetes of the young (MODY) or non autoimmune diabetes in mid-adult life, and to explore the molecular mechanisms involved.
Materials and methods: The *INS* gene was sequenced in 16 French Caucasian probands with MODY, but without known MODY associated mutations, 95 patients with non autoimmune early-onset diabetes (diagnosed <35 years) presented with at least one affected first degree relative, and 292 normoglycemic controls. Three identified insulin mutants were generated by site-directed mutagenesis of cDNA encoding human preproinsulin-GFP (C-peptide) chimera. GFP localisation was assessed by Nipkov spinning disc confocal microscopy and secreted proinsulin was measured by radioimmunoassay. ER stress marker mRNAs (XBP-1, BiP, CHOP) were quantitated by real-time RT-PCR.
Results: A novel mutation in the *INS* gene, L30M affecting the hydrophobic core, was identified in five individuals with early onset of diabetes (onset 17-36 years, range of BMI: 19-25.5 kg/m^2 at last examination) in a single family. In contrast to wild type preproinsulin, L30M preproinsulin-GFP fluorescence was largely colocalised with ER markers in MIN6 β-cells. Correspondingly, secretion of mutant proinsulin from HEK293 cells was significantly (p=0.0043) compromised with only 40.7% for L30M compared to 83.1% for wild type proinsulin being secreted. By contrast, a novel mutation in the signal peptide (R6H) identified in a Danish patient displayed normal targeting to secretory granules and proinsulin secretion. A previously-described mutation at the B/C junction (R55C) was also identified in a MODY proband. Whilst the patient developed diabetes at nine years and required exogenous insulin therapy, this mutation also co-segregated with diabetes in two other diabetic relatives (mother at 37 and sister at 33 years). The underlying molecular pathology of the latter is currently under study.
Conclusion: We describe three novel *INS* mutations co-segregating with early-onset diabetes in mid adult life with a clinical presentation compatible to a MODY pattern. These lead to the production of insulin molecules with markedly different trafficking properties and effects on ER stress, possibly implying distinct molecular pathological mechanisms for each.
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237

Functional characterisation of the glucokinase regulatory protein gene variant P446L shows diminished regulation by fructose-6 phosphate resulting in increased glucokinase activity

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Background and aims: Genome wide association scans and subsequent fine mapping have identified a common SNP rs1260326 (c.1403 c>t, p.P446L) associated with raised triglycerides and lower fasting plasma glucose levels in the general population. Individuals homozygous for the risk allele (L) have on average a 0.15 mmol/L increase in triglyceride levels and a 0.06 mmol/L reduction in fasting glucose levels compared to individuals with 2 copies of the wild-type allele (P). Glucokinase regulatory protein (GKRP) inhibits glu-

cokinase (GCK) activity in liver by binding competitively with respect to its substrate glucose. GKRP is regulated by the phosphate esters fructose 6- and fructose 1-phosphate (F6P and F1P), which enhance or diminish its inhibition of GCK activity, respectively. We hypothesised that variant P446L-GKRP could have a reduced ability to inhibit GCK, thus resulting in increased glycolytic flux and glucose uptake in liver.

Materials and methods: Recombinant human GCK, and both wild-type (WT) and P446L GKRP preparations were made (n = 3 independent protein preparations for both regulatory proteins). GCK kinetic activity was determined spectrophotometrically using an $NADP^+$-coupled assay. P446L-GKRP and WT-GKRP inhibition of GCK activity and regulation by F1P and F6P were determined. Activity/mg was calculated for each GKRP preparation and experiments carried out using either the same activities or equimolar concentrations of protein of WT and P446L-GKRP. One GKRP unit was defined as that causing 50% inhibition of 10 m-units/ml GCK under standard assay conditions in 5mM glucose at 37°C.

Results: Assays matched for protein activity/mg showed no difference in dose-dependent inhibition of GCK activity with WT and P446L-GKRP over a glucose concentration range of 0-100mM. There was also no difference in F1P-mediated regulation at concentrations up to and including 500μM (92.8 ± 1.2% GCK activity for WT vs. 93.6 ± 1.4% for P446L-GKRP; means ± SEM; n = 18; P = 0.24). However, regulation by 25-500μM F6P was significantly attenuated with P446L-GKRP (31.9 ± 2.6 to 23.3 ± 1.0% vs 28.5 ± 2.8 to 21.2 ± 0.8% for WT; n = 18; P = 0.03). Experiments using equimolar concentrations of both WT and P446L-GKRP confirmed that, there was no difference in F1P-mediated inhibition of the 2 regulatory proteins at concentrations up to 500μM (92.5 ± 2.6% GCK activity for WT vs. 90.1 ± 1.8% GCK activity for P446L; n = 9; P = 0.20), and that the response of P446L-GKRP to 0-500μM F6P was significantly diminished compared to WT (71.9 ± 1.4 to 33.8 ± 3.1% vs. 63.7 ± 2.6 to 14.1 ± 1.2% respectively; n = 9; $P = 4x10^{-11}$).

Conclusion: P446L-GKRP has diminished F6P regulation at physiological concentrations of this phosphate ester resulting in increased GCK activity. This is predicted to increase glycolytic flux in the liver, promoting both increased glucose metabolism and elevating concentrations of malonyl-CoA. Since malonyl-CoA is both a substrate for *de novo* lipogenesis and an inhibitor of carnitine-palmitoyl transferase-1, increased concentrations would be predicted to inhibit fatty acid oxidation and therefore channel fatty acyl-CoA into triglyceride synthesis.

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238

Investigating novel mutational mechanisms for glucokinase mutations with near normal or paradoxical kinetics

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Background and aims: Heterozygous inactivating mutations of glucokinase (*GCK*) increase the threshold for glucose stimulated insulin secretion to around 7mmol/L and result in elevated fasting plasma glucose levels (>5.5mmol/L) causing maturity-onset diabetes of the young (MODY) subtype glucokinase (GCK). Functional studies have identified a number of *GCK* mutations (A53S, G72R, H137R, G264S G299R and V367M) where routine kinetics has failed to explain the GCK-MODY phenotype. It is therefore possible that there are alternative mechanisms involved that explain the pathogenic effects of these mutations. We hypothesised that defects in regulation by GCK activators and inhibitors could be a novel mutational mechanism. The aim of our study was to kinetically examine these 6 mutations in the presence of 3 different GCK regulators: a synthetic small molecular activator, human glucokinase regulatory protein (GKRP) and the bi-functional enzyme PFK-2/FBPase-2.

Materials and methods: Recombinant human islet wild-type GCK and the selected mutations were expressed in the form of glutathione S-transferase (GST) fusion proteins. Kinetics in the presence of the 3 different regulators was determined spectrophotometrically using an NADP+ coupled assay with glucose-6-phosphate dehydrogenase.

Results: As expected, all of the mutant GCK enzymes showed near normal kinetics, with relative activity indices ranging from 0.76 to 1.58 relative to wild type. The G72R mutant was the only enzyme that failed to respond to either the GCK activator (fold increased activity of 0.96 vs 16.95 ± 1.1 [WT] at 60μM) or GKRP (decreased activity of 1.03 ± 0.04 vs 3.55 ± 0.43 fold [WT] at 200nM). This is consistent with previous reports on G72R. When using PFK-2/FBPase-2, G72R showed the lowest response of all 4 mutants (G72R activity increased by only 1.6 ± 0.02 fold [n=4] with 200μM, significantly less [p>0.005] than WT 2.0 ± 0.03 [n=16]). This lack of response to the activator may reflect a defect in binding which could have additional consequences for GCK catalytic stability.

Conclusion: This is the first study examining the response of mutant A53S, H137R, G264S G299R, V367M proteins to regulators of GCK. Only the G72R enzyme showed a marked lack of response to all GCK regulators compared to wild type GCK. This study gives further insight into the importance of GCK residue G72 in the activation of GCK by the bi-functional enzyme and suggests that the binding site could lie within this region. Our data supports the existence of alternative mutational mechanisms (e.g. post-translational or transcriptional mechanisms yet to be determined) which are responsible for the hyperglycaemia in patients with these *GCK* mutations.

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239

Loss-of-function mutations in SIM1 cause a specific form of Prader-Willi-like syndrome

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Background and aims: In mice, haploinsufficiency of SIM1 induces hyperphagic obesity, the chromosomal abnormalities in the SIM1 region have been rarely described in children with syndromic obesity. However, the involvement of this transcription factor in syndromic and monogenic obesity has never been conclusively demonstrated in humans.

Materials and methods: We sequenced the SIM1 coding region in 44 children affected by the Prader-Willi syndrome-like (PWSL) and 246 patients with severe familial obesity (BMI ≥ 40 kg/m² for adults and BMI ≥ 97th percentile for age and gender for children). Familial cosegregation studies and functional analysis evaluated whether mutations had a loss-of-function effect causing obesity.

Results: Four non-synonymous mutations (Q152E, E458K, R581G and T714A) were identified in four Prader-Willi syndrome-like cases and the H323Y mutation was found in two obese sisters. The five mutations led to a significant decrease in SIM1 transcriptional activity (range of residual activity: 20.4-53.5%) in reporter assay analysis. None of these mutations were identified in a further 3,896 non-obese individuals and 3,543 obese patients. We then carefully assessed the syndromic phenotypes of the PWSL patients (4 probands and 5 relatives with available data) and observed features that are characteristic of SIM1-deficiency: a round face, a small nose, depressed nasal bridge, prominent philtrum and narrow palpebral fissure. None of the patients was hypotonic at birth but 6/9 were hyperphagic, all displayed mental retardation. Six of them also had behavioural troubles and five had development retardation. In contrast to 15q PWS and to 6q chromosomal abnormalities-related syndrome short hands were not observed in SIM1-deficient subjects and hypogonadism was anecdotal (2/7).

Conclusion: Mutations responsible for SIM1 haplo-insufficiency have been found in 9% of our PWSL children. They are characterized by a specific spectrum of syndromic phenotype that may help for diagnosis. Screening of SIM1 mutations may take part in further diagnosis procedures, complementing cytogenetics.

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240

The use of 1,5 anhydroglucitol level as a clinical test to differentiate subtypes of diabetes

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Background and aims: Assigning the correct molecular diagnosis in Maturity-onset diabetes of the young (MODY) informs prognosis and treatment, but currently requires genetic resequencing. It would be advantageous to identify biochemical tests which aid prioritisation of samples for genetic testing. 1,5 Anhydroglucitol (1,5AG) is a monosaccharide which undergoes renal tubular re-absorption. Glucose competes for re-absorption at the same carrier, so with glycosuria, 1,5AG serum levels decrease. Recently, it was shown that patients with HNF1A-MODY (who have low renal glucose threshold) have lower 1,5AG levels than patients with Type 2 DM (T2DM) matched for glycaemic control. We aimed to investigate 1,5AG levels in a wider range of diabetes subtypes and evaluate its role in clinical diabetes diagnostics.
Materials and methods: Serum 1,5AG was analysed in subjects with: HNF1A-MODY (n=23); GCK-MODY (n=24); Type 1 diabetes (T1DM, n=29); latent autoimmune diabetes of adulthood (LADA, n=42); and T2DM (n=206). Data were log-transformed and ANOVA and pairwise comparisons calculated. ROC curve analysis was performed to assess the discriminative accuracy of 1,5AG with regard to diabetes aetiology.
Results: No difference in 1,5AG levels were seen between patients with HNF1A-MODY and T2DM (p=0.15), T1DM (p=0.97) or LADA (p=0.5), although after correcting for HbA1c 1,5AG levels were lower in patients with HNF1A-MODY compared to T2DM (p=0.001).
1,5AG levels in patients with GCK-MODY were higher than all other groups. Means [SD range] in μg/ml were: GCK-MODY 13.06 [5.74-29.74]; HNF1A-MODY 4.23 [2.12-8.44]; T1DM 3.09 [1.45- 6.57]; LADA 3.46 [1.42- 8.45] and T2DM 5.43 [2.12-13.23], $p<10^{-4}$ for GCK-MODY v.s. each group.
The discriminative accuracy of 1,5AG levels (as per ROC curve analysis) was 0.80 for GCK-MODY v.s. T2DM, 0.86 for GCK-MODY v.s. HNF1A-MODY, but only 0.60 for HNF1A-MODY v.s. T2DM.
Conclusion: Our data suggests that 1,5AG levels are unlikely to be useful in differentiating HNF1A-MODY from T2DM in a clinical setting. In this dataset 1,5AG levels performed well in discriminating GCK-MODY from all other subtypes. The higher 1,5AG levels in GCK-MODY are likely explained by the known modest post-challenge glucose increment. This requires replication in undifferentiated sample sets, but suggests measurement of 1,5AG could help inform decisions regarding diagnostic testing for GCK-MODY.
Supported by: NIHR Programme Grant, MolPAGE, Diabetes UK, Oxford Radcliffe Hospitals Charitable Fund

OP 41 Insulin secretion in vivo

241

Plasma insulin and glucagon responses to a maximal hyperglycaemic stimulus and arginine infusion in subjects with impaired glucose tolerance and type 2 diabetes mellitus

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Objective: Progression from impaired glucose tolerance (IGT) to type 2 diabetes mellitus (T2DM) is determined by beta cell failure to compensate for increased insulin resistance. However, the natural history of these islet abnormalities remain poorly defined. Though, impaired glucagon suppression following a test meal has been reported in T2DM, glucagon response to a maximal hyperglycemic stimulus and non-glucose stimulus Arginine in IGT is not clear.
Methods: We assessed glucagon suppression and release under maximal glycemic and non-glycemic (arginine)-stimulated conditions in subjects with NGT (n=13, age=42±4 y, BMI=28±2, HbA_{1c}=5.1±0.1%), IGT (n=12, age=52±2 y, BMI=33±1, HbA_{1c}=5.8±0.1%) and T2DM (n=15, age=53±3 y, BMI=34±1, HbA_{1c}=8.1±0.2%) individuals. Each subject received OGTT and two (+125 and 400 mg/dl) hyperglycemic clamp followed by IV Arginine (5 grams) bolus. Insulin, C peptide and glucagon were measured q 15 min during OGTT and q 2-10 min during hyperglycemic clamp.
Results: Acute insulin response (AIR_{0-10}) was decreased in IGT vs. NGT (252 ±47 vs. 323±59 μU/ml) and further reduced in T2DM (14±8 vs. 323±59 μU/ml, p<0.001). Second phase insulin response (AUC_{10-80}) was similar in NGT and IGT (2717 ±605 vs. 2921 ± 608 μU/ml) and markedly decreased in T2DM (633±180 μU/ml, p<0.001). During the hyperglycemic second step AUC_{80-160} was similar in IGT and NGT (5261±1300 vs. 6282 ±2300 μU/ml), but reduced in T2DM (1105 ±250 μU/ml, p<0.001). After adjusting for insulin resistance (Matsuda index), the insulin secretion/insulin sensitivity index ($AIR_{0-10,}$ $AUC_{10-80,}$ AUC_{80-160}) was significantly diminished in both IGT and T2DM vs. NGT (all p<0.001). Glucagon suppression during fist step (AUC_{10-80}) was impaired by 69% (-114 pg/ml) in IGT and 71% (-108 pg/ml) in T2DM vs. NGT (-363 pg/ml). Similar defects in glucagon suppression were observed during the second clamp step in IGT and T2DM (both p<0.01). In contrast, arginine elicited a 2.0-and a 2.6-fold greater increase in glucagon secretion in IGT and T2DM (both p<0.001) vs. NGT.
Conclusion: Regulation of glucagon suppression and release is impaired in IGT, as well as T2DM, indicating early dysregulation of alpha cell physiology, in addition to impaired beta cell function in the natural history of T2DM.

242

Effects of variations in duodenal glucose load on glycaemia, insulin and GLP-1 in type 2 diabetes

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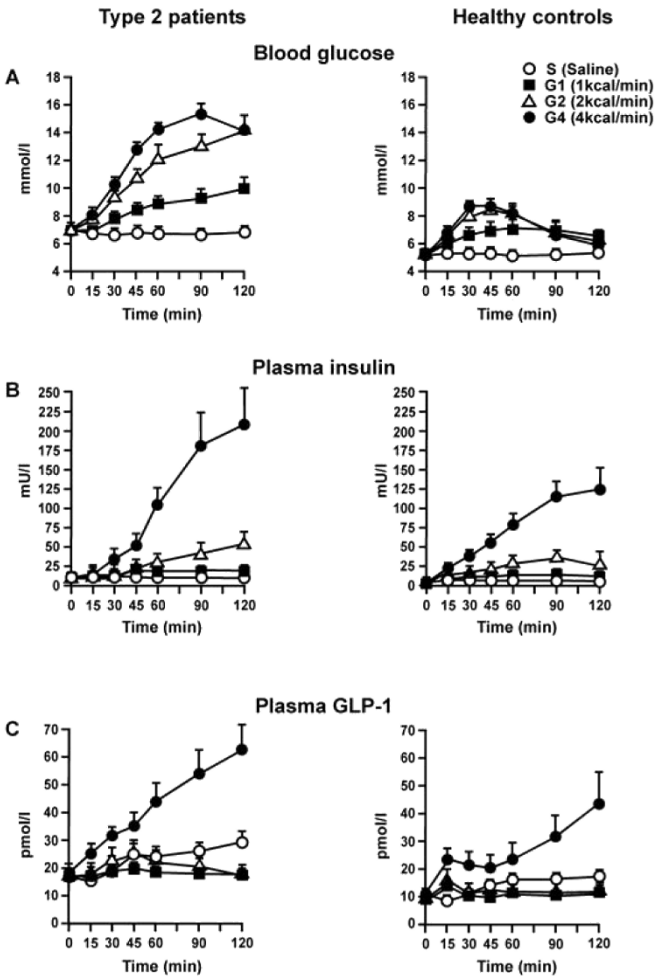
Background and aims: The postprandial incretin response has been reported to be deficient in type 2 diabetes (T2D), but most studies have not controlled for variations in the rate of gastric emptying. We evaluated the glycaemic, insulin and glucagon-like peptide-1 (GLP-1) responses to intraduodenal (ID) glucose in patients with T2D, and compared these to our previous data from 10 healthy controls (HC).
Materials and methods: 8 males with well-controlled T2D (age 57 ± 4 yrs; BMI 26.5 ± 1.4 kg/m^2; glycated haemoglobin 6.2 ± 0.3 %), managed by diet alone, were studied on four separate occasions in single-blind, randomized order. Data were compared to HC (age 32 ± 4 yrs; BMI 25.1 ± 0.4 kg/m^2). Blood glucose and plasma hormones were measured during 120 min ID glucose infusions at (i) 1 ("G1"), (ii) 2 ("G2"), and (iii) 4 ("G4") kcal/min, or (iv) saline control ("S"). Data are presented as mean ± SEM.
Results: Results are summarized in the figure. T2D patients had higher basal (P < 0.0001) and incremental (P < 0.0001) blood glucose responses to each glucose load, compared to HC. In HC, there was no significant difference between the glycaemic response to G2 and G4, whereas in T2D, blood glucose was greater after G4, compared to G2 (P < 0.005). In both groups, the

increases in insulin and GLP-1 to increasing glucose loads were not linear; responses to G1 and G2 were minimal, whereas the responses to G4 were much greater than to G1 and G2 (P = 0.0001 for each). In T2D, the insulin responses to both G2 and G4 were greater than HC (P < 0.05 for each).
Conclusion: In patients with well-controlled T2D, blood glucose, insulin, and GLP-1 responses are critically dependent on the duodenal glucose load, and GLP-1 responses are not deficient, when compared to HC.



Supported by: NHMRC

243

The role of GLP-1 in the enteroinsulinar axis in type 2 diabetes mellitus
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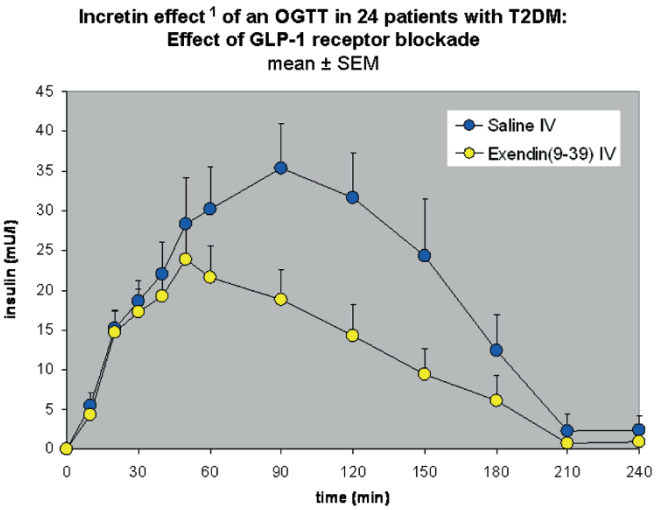
Background and aims: The incretin effect as part of the enteroinsulinar axis constitutes the difference between the total postprandial (PP) and the isoglycemic fasting insulin response. The insulinotropic gut born hormones GLP-1 and GIP account for the incretin effect in healthy humans. The incretin effect is reduced in type 2 diabetes mellitus (T2DM). Because several studies have indicated a failure of synthetic GIP to stimulate insulin secretion in T2DM in contrast to GLP-1, a defect of the GIP action has been suggested.
To quantify the contribution of endogenous GLP-1, we examined the insulinotropic action and the incretin effect of an oral glucose tolerance test (OGTT) in patients with T2DM during GLP-1 receptor blockade using exendin(9-39) (Ex-9).
Materials and methods: Double-blind, placebo-controlled, randomized, cross-over study in 24 patients with T2DM (61±7 y, HbA1c 6.2±0.2, BMI 27.7±0.9). On two separate days blood glucose and plasma insulin concentrations were measured during the four hours after ingestion of an OGTT (75g) with and without an intravenous background infusion of Ex-9 (900 pmol/kg/min) or saline. This dose of Ex-9 blocks the insulinotropic effect of GLP-1 in human by at least 95%. On two further days the blood glucose excursions obtained during the OGTTs were mimicked by intravenous glucose (isoglycemic clamp). The incretin effect was calculated as the difference between the postprandial and the isoglycemic fasting plasma insulin concentrations.
Results: During fasting, Ex-9 increased blood glucose concentrations (120±4.3 vs 132±5.4 mg/dl, saline vs Ex-9, P<0.001) without change in plasma insulin. In addition, after ingestion of the OGTT Ex-9 increased the mean PP blood glucose excursion (77±5 vs 84±5 mg/dl, P=0.038) and the PP incremental glucose peak (156±5 vs 167±6 mg/dl, P=0.008). In spite of increasing PP glucose, Ex-9 decreased the mean PP plasma insulin excursion significantly (MPI, 32±6 vs 26±4 µU/ml, P=0.007). The isoglycemic clamps mimicked postprandial glucose excursions of the OGTTs with R^2=0.932±0.009 under saline and R^2=0.943±0.008 under Ex-9, respectively. The calculated PP incretin effect amounted to 18.6±3.4 µU/ml under saline control (59±5% of MPI) which was significantly reduced but not eliminated under Ex-9 (10.5±1.7 µU/ml, 44±4% of MPI, P=0.005 vs saline).
Conclusion: In patients with T2DM, endogenous GLP-1 significantly reduces fasting and postprandial glycemia. Because of its insulinotropic properties GLP-1 contributes to the postprandial incretin effect in T2DM patients. However, similar to healthy subjects, GLP-1 accounts for only about 50% of the PP incretin effect in T2DM. We suggest, that GIP accounts for the remaining incretin effect in T2DM. Since the relative contribution of GLP-1 and non-GLP-1 incretins is preserved in T2DM we suggest that the reduced incretin effect in T2DM may represent a general failure of the B-cell rather than a specific defect of the enteroinsulinar axis.



[1] the difference between the plasma insulin concentrations after an OGTT and the respective isoglycermic clamp during intravenous glucose, i.e. the glucose-independent intestinal stimulation of insulin

Supported by MSD

244

Glucagon responses to increasing oral loads of glucose and corresponding isoglycaemic i.v. glucose infusions in patients with type 2 diabetes and healthy subjects
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Background and aims: Type 2 diabetes mellitus (T2DM) is associated with inappropriate glucagon response (hypersecretion and delayed and reduced suppression) to 50-g OGTT whereas isoglycaemic i.v. glucose infusion (IIGI) results in a completely normal glucagon response in these patients. This suggests that glucagonotropic signaling originates from glucose stimulation of the gastrointestinal tract. Therefore, we aimed to evaluate glucagon responses to increasing amounts of glucose given orally and IIGIs in patients with T2DM and in control subjects.
Materials and methods: Glucagon responses were measured on six separate days during three 4-hour OGTTs with increasing loads of glucose (25 g, 75 g

and 125 g) and three corresponding IIGIs in 8 patients with T2DM (age: 57±4 years; BMI: 29.5±1.0 kg/m^2; HbA_1c: 7.0±0.3% (mean±SEM)) and 8 healthy subjects (age: 57±4 years; BMI: 28.9±0.7 kg/m^2; HbA_1c: 5.4±0.1%).
Results: Isoglycaemia corresponding to the OGTTs was obtained during i.v. challenges. In the control subjects no difference in glucagon suppression during the first 60 minutes of the 25-g OGTT and the corresponding IIGI (-249±49 *vs.* -208±35 pM·1h; *p*=NS) was observed. Contrary, patients with T2DM exhibited significant glucagon suppression only following the IIGI (-2±36 *vs.* -228±29 pM·1h; *p*=0.006). At higher oral glucose loads this difference increased and became evident in healthy control subjects also (Table 1)
Conclusion: In patients with T2DM increasing amounts of orally ingested glucose (25, 75 and 125 g) elicit progressively inappropriate glucagon responses as compared to IIGIs, which result in normal glucagon suppression. This is also observed in control subjects when ≥75 g glucose is ingested orally, suggesting it to be a physiological phenomenon which has deteriorated pathologically in T2DM.

Table 1. Values are mean iAUC (0-60 min) for plasma glucagon responses (pM•1h) ± SEM

	T2DM	CTRL	*T-test (P)*
25-g OGTT	-2±36	-249±49	*0.001*
IIGI	-228±29	-208±35	*0.562*
Difference (T-test (P))	*226±58 (0.006)*	*-42±75 (0.598)*	
75-g OGTT	1±21	-122±42	*0.048*
IIGI	-223±32	-250±19	*0.517*
Difference (T-test (P))	*224±35 (0.000)*	*128±37 (0.010)*	
125-g OGTT	24±35	-88±46	*0.068*
IIGI	-277±53	-321±56	*0.533*
Difference (T-test (P))	*300±84 (0.009)*	*233±59 (0.005)*	

Supported by: the Investigator-Initiated Studies Program of Merck & Co., Inc.

245

Development of an *in silico* model to extrapolate whole body glucose - insulin homeostasis by altering tissue specific effects, simulation of SGLT-2 inhibition in type 2 diabetes
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Background and aims: The inhibition of gut and renal sodium-glucose co-transporters (SGLTs) has been proposed as a novel therapeutic approach for the treatment of diabetes. SGLT-2 inhibitors acutely induce renal glucose excretion by a change in the renal threshold. HOmeostatic Model Assessment (HOMA) is a model based on the interaction of organs or tissues (liver, muscle, pancreatic beta-cells, brain, gut) represented by equations derived from empirical data. It has become an international standard for measuring beta-cell function and insulin resistance in large studies where detailed physiology is not possible. We have developed an interactive Homeostatic Model of Assessment (iHOMA) in which the parameters of the individual models are modifiable through an onscreen interface using visual analogue controls. The iHOMA model can be used for *in silico* calculations of the specific effects drugs may have on glucose metabolism. The aim was to mathematically model the effect of an SGLT-2 inhibitor in patients with Type 2 diabetes (T2DM) using the iHOMA program.
Material and methods: A total of 428 patients (239 Male) with T2DM were retrospectively analysed from the Diabetes in Families (DIF) study. The DIF study was a population-based collection of patients with T2DM and their siblings sampled from GP practices in Oxfordshire and Northamptonshire. Although patients were on a variety of drugs none of these were likely to interfere with their renal glucose excretion. They had a mean BMI 30.4 (±5.6) kg/m2 , mean age 62(±11) years, mean HbA1c 7.9 (±1.6), mean Fasting Plasma Glucose(FPG) of 9.6 (±3.1) mmol/l and geometric mean Fasting Plasma Insulin (FPI) of 112.0 (range 65.8 - 190.5) pmol/l. Beta-cell function and insulin resistance were calculated for each subject using HOMA and used as inputs into the interactive model. The iHOMA program was developed by the authors at the University of Oxford and has been validated against the original HOMA algorithms. The iHOMA program was used to mathematically simulate the effect of an SGLT-2 inhibitor enhancing glucose excretion by the kidneys. FPG and FPI were then calculated for each subject using iHOMA adjusted for the effects of the SGLT-2 inhibitor.
Results: Using the iHOMA program, which can account for tissue-specific glucose metabolism effect, the simulated whole body effect by altering renal excretion of glucose was moderate. A paired-sample t-test revealed a significant difference in the mean FPG (9.6 vs. 8.9, p<0.01) mmol/l a decrease of 0.7 mmol/l. The FPG change in concentration was accompanied by a change in the geometric mean FPI (112.0 vs. 99.3, p<0.01) pmol/l, a decrease of 12.7 pmol/l.
Conclusion: The development of the iHOMA program enables the investigation of pharmacological effects of a novel therapy on glucose metabolism *in silico*. The model suggests that SGLT-2 inhibitors have the potential to effect a moderate change in glucose in people with T2DM. However, it is important that these data are replicated and validated in a prospective randomised control trial.
We would like to acknowledge the support of the NIHR Oxford Biomedical Research Centre

246

Reduced amylin (IAPP) secretion in response to hyperglycaemia and arginine are characteristic of impaired glucose tolerance and type 2 diabetes
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Objective: Islet amyloid deposition is a characteristic feature of T2DM. Islet amyloid polypeptide (IAPP) is co-secreted with insulin, but its secretion profile and relationship to other islet hormones (insulin, C-peptide) in response to glucose and non-glucose stimulus has not been clearly defined.
Methods: Forty subjects, 13 NGT (age=42, BMI=28±2, HbA1c=5.1±0.1%), 12 IGT (age=52, BMI=33±1, HbA1c=5.8±0.1) and 15 T2DM (age=53, BMI=34±1, HbA1c=8.1±0.2) received OGTT and 2 steps hyperglycemic (+125 and +400mg/dl) clamp followed by IV arginine (5g) bolus. The acute insulin (AIR), C-peptide (ACPR) and IAPP (AIAR) responses during each hyperglycemic step and following arginine (AIR_{Arg}) were assessed and Matsuda index of insulin sensitivity was calculated from OGTT.
Results: As expected, AIR and ACPR during both hyperglycemic steps (0-10 and 80-90 min) and after arginine (160-170 min) progressively decreased from NGT to IGT to T2DM. Fasting IAPP concentrations were 13±3, 9±1 and 16±3 pM in NGT, IGT and T2DM. Incremental IAPP AUC from 0-10 min of the clamp was 46±6, 37±17 and -5±8pM (p<0.05) in NGT, IGT and T2DM, and IAPP response to hyperglycemia + arginine was 271±72, 507±69 and 186.2±11 pM (p<0.05), respectively. The ratio of IAPP/C peptide during the first 10 min of hyperglycemia was higher in T2DM (3.9±0.5) and IGT (3.0±0.4) than NGT (2.0±0.2) (p<0.05) subjects, but after prolonged (80 min) and severe hyperglycemia (160 min) as well as after arginine stimulus (160-166 min) it was lower in T2DM versus NGT and IGT. The acute amylin response ($AIAR_{0-10}$) correlated with $ACPR_{0-10}$ (r=0.665, p<0.001), AIR_{0-10} (r=0.543, p<0.001), FPG (r= -0.508, p<0.005) and 2-h glucose (r= -0.571, p<0.005).
Conclusion: In conclusion, early IAPP secretion (0-10min) is higher in T2DM and IGT vs NGT but is reduced in response to prolonged hyperglycemia and arginine. IAPP/C-peptide ratio is higher during early hyperglycemia but with more prolonged and severe hyperglycemia it is decreased in T2DM. Defects in IAPP processing and secretion may lead to amyloid deposits and beta cell dysfunction in T2DM.

OP 42 Metabolic effects of incretins

247

Central GLP-1 receptor antagonism partially reduces the action of peripheral GLP-1 to improve insulin sensitivity in high-fat-fed mice

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Background and aims: Continuous infusion of glucagon-like peptide-1 (GLP-1) improves insulin sensitivity in both humans and rodents. The improvement of insulin sensitivity may result solely from GLP-1's actions in the periphery by a direct impact on tissues like muscle and fat. On the other hand, compelling evidence supports a role for the GLP-1 receptor (GLP-1R) in the brain in modulating glycemic control. Here, we evaluated the role of the central GLP-1R in improving insulin sensitivity in diet-induced insulin resistant mice.
Materials and methods: High-fat-fed insulin resistant C57Bl/6 mice were implanted a cannula in the lateral cerebral ventricle, which was connected to an osmotic minipump for the continuous delivery of the GLP-1R antagonist exendin-9 (EX-9) or cerebral spine fluid (CSF). After 5 days recovery a second minipump was implanted for the continuous s.c. delivery of GLP-1 or PBS. After 2 weeks of intervention a hyperinsulinemic euglycemic clamp was performed to monitor insulin sensitivity.
Results: The rate of glucose infusion necessary to maintain euglycemia was significantly increased by GLP-1 when compared to control animals (CSF/PBS: 17 ± 10; CSF/GLP-1: 43 ± 9 µmol/min/kg; P<0.01). Blocking the central GLP-1 receptor in combination with continuous GLP-1 infusion diminished the effect on GIR by 38% (EX-9/GLP-1: 27 ± 11 µmol/min/kg; P<0.01 vs. CSF/GLP-1). Central GLP-1R antagonism alone did not affect GIR compared to controls (EX-9/PBS: 20 ± 13 µmol/min/kg). GLP-1 treatment significantly increased insulin's action to stimulate glucose disposal compared to both CSF/PBS and EX-9/PBS mice (CSF/PBS: 30 ± 17; CSF/GLP-1: 54 ± 21; EX-9/PBS: 31 ± 16 % from basal; P<0.05). Central Ex-9 infusion tended to diminish the capacity of GLP-1 to stimulate insulin mediated glucose disposal (EX-9/GLP-1: 40 ± 18 % from basal, p=0.22 vs. CSF/GLP-1). GLP-1 reinforced the capacity of insulin to suppress endogenous glucose production (CSF/PBS: 24 ± 22; CSF/GLP-1: 65 ± 24; EX-9/PBS: 34 ± 25 % from basal), and central GLP-1 receptor antagonism tended to diminish this effect of GLP-1 (EX-9/GLP-1: 43 ± 26 % from basal; P=0.061 vs. CSF/GLP-1).
Conclusion: Activation of central GLP-1 receptors partially mediates the effect of peripheral GLP-1 administration to beneficially impact on insulin action in insulin resistant mice.
Supported by: an EFSD/Amylin grant

248

Effect of replacing pre-meal insulin for exenatide on body weight, insulin secretion and hepatic/muscle fat in well-controlled insulin-treated patients with type 2 diabetes

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Background and aims: Intensive insulin therapy with long-acting basal plus rapid-acting pre-meal insulin may lead to optimal glycemic control in patients with type 2 diabetes mellitus (T2DM) at the expense of weight gain and an increased risk of hypoglycemia. Exenatide, a GLP-1 receptor agonist known to lower postprandial and day-long plasma glucose in T2DM, may help avoid these side effects while maintaining good metabolic control. However, this has not been carefully studied and little is known about the mechanisms that are involved when combining insulin plus exenatide. Therefore, we investigated in well-controlled T2DM patients on basal long-acting insulin the impact of replacing rapid-acting pre-meal insulin with exenatide twice daily.
Materials and methods: We recruited 24 patients with T2DM (age=58±3, gender=21/3 (M/F), BMI=32.8±4.4 kg/m^2, FPG=121±35 mg/dl, A1c=7.1±0.9%) well-controlled after 6 months of insulin detemir plus pre-meal insulin aspart (INS). While all patients continued insulin detemir, the pre-meal insulin was discontinued and replaced with exenatide twice daily for 6 months (INS+EXE). Before and after treatment all patients were admitted to the research unit for the following metabolic measurements: 1) plasma glucose/insulin secretion during a 4-hour mixed meal and during a day-long metabolic profile (day 1); 2) insulin secretion during a hyperglycemic clamp (day 2; exenatide given only on day 1 and not the morning of the test); 3) plasma hsCRP levels; 4) liver (LFAT) and intramyocellular (IMCL) fat content by magnetic resonance spectroscopy (MRS).
Results: Adding exenatide to insulin detemir insulin (INS+EXE; results available in 17) allowed to maintain similar glycemic control after 6 months (A1c: 7.1±0.9%vs. 6.8±0.7%; NS), even as pre-meal rapid-acting insulin was discontinued (42±5 units). The mean insulin detemir dose at bedtime was no different at the end of the study. Use of INS+EXE was associated with a substantial reduction in body weight (-4.6 kg±1.1, p<0.001). During the 4-hour mixed meal, mean plasma glucose (190±9 vs. 138±7 mg/dl) and the glucose increase above baseline (83± 8 vs. 32±8 mg/dl, p<0.001) were both significantly lower after INS+EXE compared to INS (both p<0.001). During the hyperglycemic clamp, second phase insulin secretion increased significantly with INS+EXE (+19%; p<0.05) compared to insulin treatment. INS+EXE significantly reduced systemic inflammation as plasma hsCRP concentration decreased by 50% from 3.8±0.9 to 1.9±0.5 mg/L (p<0.04 vs. INS). Liver fat by MRS (data available in 12 patients) was reduced by 17% (from 12.1±1.3 to 10.0±1.1%; p=0.09) but intramyocellular lipids were unchanged (from 0.79±0.1 to 0.74±0.1%; NS). INS+EXE was well tolerated. Adverse events: INS+EXE was not associated with clinically significant hypoglycemia; 3 patients reported mild nausea that resolved within 4 weeks.
Conclusion: In well-controlled insulin-treated T2DM patients, replacement of pre-meal rapid acting insulin for exenatide twice daily is well accepted and allows equivalent glycemic control to intensified basal/pre-meal insulin therapy. Beneficial features of INS+EXE include significant weight loss, enhancement of insulin secretion, amelioration of systemic inflammation and reduction hepatic steatosis.
Supported by: Amylin Pharmaceuticals, Lilly, Novo-Nordisk and the Veterans Affairs Medical Research Fund

249

Glucose-dependent insulinotropic polypeptide leads to upregulation of inflammatory cytokines and calcitonin peptides in human adipocytes involving the PKA pathway

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Background and aims: Glucose-dependent insulinotropic polypeptide (GIP), a 42-amino acid peptide, is released from entero-endocrine K-cells upon nutrient ingestion. Plasma GIP levels are increased by high-fat diet and GIP has recently been shown to directly promote fat accumulation in adipocytes via a specific GIP receptor. Inhibition of GIP signalling in mice was able to prevent the development of obesity-induced diabetes. Adipose tissue produces proinflammatory cytokines such as interleukin (IL)-1β and IL-6 which are involved in obesity related insulin resistance. We have shown recently that upon inflammatory stimulation adipose tissue expresses CALC-I gene products including procalcitonin (Pro-CT) and calcitonin gene-related peptide (CGRP). Now we hypothesized that GIP is a mediator of low grade inflammation by increasing production of inflammatory cytokines and subsequently calcitonin peptides. In addition we assessed the question whether the GIP effects were mediated via the protein kinase A (PKA) pathway.
Materials and methods: Human preadipocytes obtained from surgical biopsies and preadipocyte-derived adipocytes were exposed to human GIP recombinant peptide GIP(1-42) (1h, 1nM), the GIP receptor antagonist GIP(6-30) (pre-incubation 15min, 10µM) as well as H-89, a PKA inhibitor (pre-incubation 1h, 20µM) and their combinations. mRNA-expression of IL-1beta, IL-6, Pro-CT and CGRP were analyzed by real-time PCR. CGRP protein concentrations were determined in supernatants by human-specific radioimmunoassay kits.
Results: In GIP-treated preadipocytes, mRNA-expression of IL-6, IL-1β, Pro-CT and CGRP was not altered. In differentiated adipocytes, 1h GIP-treatment increased mRNA-expression of IL-6 to 255%±41% of control (p<0.001). IL-6 expression was reduced by GIP(6-30) to 29%±3% (p<0.001) and by H-89 to 47.93%±8% (n.s.) of GIP alone. GIP (1nM) increased mRNA-expression of IL-1β to 145%±37% of control (n.s.) and was not significantly reduced by GIP(6-30). Induction of CT mRNA-expression to 1433%±17% of control (p<0.001) due to GIP (1nM) treatment was down-regulated to 10%±4% (p<0.001) by pre-incubation with GIP(6-30) and to 70.26%±11% (n.s.) by H-89, respectively. CGRP expression was increased to 187%±57% (P<0.01)

by GIP-stimulation and reduced to 53.77%±12% (p<0.01) by GIP(6-30) and to 3.44%±2% (p<0.001) by H-89. Measurement of CGRP-protein release revealed an induction to 170%±50% (p<0.01) due to GIP treatment.
Conclusion: These data demonstrate the potential of GIP to induce mRNA expression of pro-inflammatory cytokines and CALC-I gene products in human adipocytes involving the PKA-pathway. This indicates a potential role of GIP in the initiation of low grade inflammation in obesity and related insulin resistance and might represent an "entero-adipose-axis".

250

Effects and mechanism of glucagon-like peptide-1 on injury of mice cardiomyocytes induced by hypoxia-reoxygenation
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Background and aims: Although the insulinotropic role of glucagon-like peptide-1 (GLP-1) in type 2 diabetes mellitus has been substantiated, its role in cardioprotection remains largely unknown. This study aimed to determine effects of GLP-1 on injury of mice cardiac myocytes induced by hypoxia-reoxygenation (H/R) and possible mechanisms.
Methods: The cultured neonatal mice cardiac myocytes were randomly divided into seven groups: normal control group, H/R group, GLP-1+H/R group, GLP-1+H/R+UO126 group, GLP-1+H/R+LY294002 group, H/R+UO126 group, H/R+LY294002 group. Lactate dehydrogenase (LDH) activity, apoptosis rate of cardiac myocytes, caspase-3 activity were detected after the injury of H/R.
Results: Compared with normal control group, the activity of LDH , cardiac myocyte apoptosis rate ,caspase-3 activity all increased significantly in H/R group (p<0.01). Compared with H/R group, these three indexes all decreased in H/R+GLP-1 group (p<0.01). However those changes of LDH activity, apoptosis rate, caspase-3 activity were inhibited by LY294002 (phosphatidylinositol 3-kinase inhibitor, PI3K inhibitor) and UO126 (mitogen-activated protein kinase inhibitor, MAPK inhibitor) respectively.
Conclusion: GLP-1 can directly act on cardiac myocytes and protect them from H/R injury mainly by inhibiting their apoptosis,the mechanism of which may be concerned with GLP-1's effects on apoptosis or anti-apoptosis may be through phosphatidylinositol 3-kinase (PI3K)-Akt pathway and mitogen-activated protein kinase (MAPK) signaling pathway.

251

Exendin-4 stimulates proliferation of human coronary artery endothelial cells through eNOS- and PI3K/Akt-dependent pathways but independently of GLP-1 receptor
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Background and aims: We previously showed that human coronary artery endothelial cells (HCAECs) express the glucagon-like peptide 1 (GLP-1) receptor and that GLP-1 ameliorates endothelial dysfunction in type 2 diabetic patients with coronary heart disease. Exendin-4 is a stable GLP-1 receptor agonist and has been approved for clinical use against type 2 diabetes. In contrast to its effect on metabolism and endothelial function, the role of GLP-1 and its analogue on endothelial cell growth is not known. In the present study, we have investigated the effect of Exendin-4 on cell proliferation and its underlying mechanisms in HCAECs *in vitro*.
Materials and methods: HCAECs were incubated with Exendin-4 (10 nM), GLP-1 (7-36) (100 nM) or the major GLP-1 metabolite GLP-1 (9-36) (100 nM), respectively, in serum-deficient medium in the presence of 5 mM glucose for 48 h. Phosphorylation and expression of endothelial nitric oxide synthase (eNOS) and Akt were examined after 48 h incubation by Western blotting using anti-phospho-eNOS (Ser1177), anti-eNOS, phospho-Akt (Ser 473) and anti-Akt antibodies, respectively. ^{3}H-thymidine incorporation was assayed in 96-well plates as a measure of DNA synthesis after 48 h incubation. Cell viability was assessed using Trypan blue exclusion.
Results: Incubation of HCAECs with Exendin-4 for 48 h resulted in a dose-dependent increase in DNA synthesis; subsequent neogenesis was confirmed by an increased cell number. To investigate the roles of eNOS and PI3K in the Exendin-4 induced proliferation, the specific eNOS inhibitor L-NAME and the PI3K inhibitor LY294002 were used. The Exendin-4 induced proliferation was completely abolished by L-NAME and significantly suppressed by LY294002. Western blot showed that Exendin-4 dose-dependently stimulated phosphorylation of eNOS. The Exendin-4-stimulated activation of eNOS was associated with an increased NO production and was blocked by L-NAME or LY294002. Application of GLP-1 (7-36) or its major metabolite GLP-1 (9-36) also resulted in eNOS activation, to same extent as Exendin-4 alone. The effect of Exendin-4 on eNOS activation was further examined in the presence of GLP-1 (7-36). Results from Western blotting revealed that Exendin-4 induced eNOS phoshorylation was unaffected by co-incubation with GLP-1 (7-36). The GLP-1 receptor antagonist Exendin (9-39) alone suppressed the basal phosphorylation of eNOS. However, co-incubation of Exendin (9-39) with Exendin-4 did not interfere with the Exendin-4 induced eNOS phosphorylation. In addition, incubation of HCAECs with Exendin-4 caused an increased Akt phosphorylation, an effect which was inhibited by LY294002.
Conclusion: Our results suggest that Exendin-4 stimulates proliferation of human coronary endothelial cells. The effect of Exendin-4 seems to be GLP-1 receptor-independent and is mediated through the PI3K/Akt and eNOS activation pathways. The beneficial effects of Exendin-4 on human coronary artery endothelial cells add yet another salutary property to incretin-based antidiabetic therapy, increasing its clinical utility in type 2 diabetic patients in whom endothelial dysfunction is a salient feature that adversely affect their survival.
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252

Impairment of synaptic plasticity and learning in GLP-1 receptor knockout mice: interaction between type 2 diabetes and Alzheimer's disease
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Background and aims: Type 2 diabetes is a known risk factor for Alzheimer's disease (AD), with impairments in insulin signalling contributing to neurodegeneration. The incretin hormone Glucagon-like peptide-1 (GLP-1) has been shown to normalise insulin signalling in type 2 diabetes. Importantly, GLP-1 also plays a role in neuronal activity and brain functions. The aim of this study was to elucidate the specific role of GLP-1 receptors in synaptic plasticity and cognitive processes in the GLP-1 receptor knockout (GLP-1R KO) mouse model.
Materials and methods: Male GLP-1R KO mice and age-matched, control, wild-type (WT) animals with the same C57/Bl6 genetic background were used to assess exploratory behavior and learning and memory in open field, object recognition and spatial water maze tasks. Long term potentiation (LTP), was measured in GLP-1R KO and WT mice, in area CA1 of the hippocampus using a high frequency stimulation protocol.
Results: In open field assessment a small increase in speed of movement in GLP-1R KO mice (p<0.05) was observed. GLP-1R KO animals also exhibited slightly reduced anxiety levels (p<0.05), compared with WT controls. In an object recognition task GLP-1R KO animals displayed similar exploratory behavior to WT mice, but failed to differentiate between novel and familiar objects (p<0.05). No impairment was detected in an object relocation task. In a water maze task, GLP-1R KO mice were impaired in the acquisition phase (p<0.001) and in the probe trial (p<0.05). LTP in area CA1 of the hippocampus was severely impaired in GLP-1 KO mice (p<0.0001). Paired-pulse facilitation was also impaired at 25ms interstimulus interval (p<0.05), but not at intervals of increasing duration.
Conclusion: These results demonstrate that lack of GLP-1 receptor function impairs synaptic plasticity and some forms of learning and memory formation. They show that GLP-1 receptor signalling plays a specific role in neurotransmission and cognition. GLP-1 receptors appear to play a role in synaptic activity and neurotransmitter release, similar to the effects on insulin release in beta-cells. The results also help to explain previous results that show neuroprotective effects of GLP-1 analogues in models of Alzheimer's disease.
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PS 1 Genetics of type 1 diabetes

253

Bayesian network to investigate the dependency and interaction between HLA, INS, PTPN22 and CTLA4 genes in type 1 diabetes

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Background and aims: Type 1 diabetes (T1D) is a multifactorial and polygenic disease with four major susceptibility genes (HLA (DR/DQ), CTLA4, INS, PTPN22) that by interacting with each other and with environmental factors, determine disease onset. These genes are known to be involved in immune regulation whereas INS modulates insulin expression in human thymus. The incidence rate of T1D is increasing uniformly in Italy with an annual average increase of 3.6%. The genetic profile of individuals who develop diabetes appear to change in countries with a high T1D incidence shifting from predominantly high-risk HLA genotypes towards higher percentage of median and low-risk HLA genotypes. The aim of this study was to investigate in T1D.
Materials and methods: Genetic data (HLA, PTPN22, INS, CTLA4) were analysed from a group of 391 T1D patients and 100 controls (M/F 1.06, age 1-41) diagnosed by participating centres of the IMDIAB group in continental Italy. Diagnosis of T1D was based on the ADA classification criteria. The control group was recruited from the Blood Transfusion Service in Rome. We divided HLA alleles in high, moderate and low risk for T1D, CTLA4 and PTPN22 alleles in susceptibility/non susceptibility alleles, and INS gene alleles in susceptibility/protection. We estimated a Bayesian network to study dependencies and interactions between the different alleles in relationship to group status. Bayesian networks, also called belief networks, are probabilistic graphical models that represent a set of variables and their probabilistic dependencies. In the present study the model was trained on genetic variables and group status (T1D / control). A hill climbing algorithm was used for fitting the model.
Results: The frequency of the HLA genotypes with high, moderate and low risk was 18.9%, 55.2% and 25.8% in T1D patients (2%, 5%, 93% in controls). The model shows that group status is directly influenced by HLA ($p = 1.4*10^{-30}$) and INS ($p=3.0*10^{-8}$), and that there is a dependency of INS on HLA ($p = 3.0*10^{-5}$). For high, moderate and low risk HLA corresponding conditional frequencies of 0.74, 0.76 and 0.55 for INS susceptibility alleles were found. Separating the data group wise, leads to corresponding conditional probabilities of 1, 0.4, 0.34 for the control group and 0.73, 0.76, 0.75 for the patient group for high, moderate and low risk respectively. No significant connection between HLA and PTPN22 ($p = 0.07$) and CTLA4 and INS ($p = 0.1$) could be found.
Conclusion: This study confirms that HLA (DR/DQ) and INS susceptibility alleles are the major determinants of the disease. By analysing all 491 subjects a higher probability for INS susceptibility alleles was found in the presence of high and moderate risk HLA alleles. However, if we consider INS susceptibility alleles only in the T1D group, these are less frequent in high risk HLA alleles. Further substantiation is needed for the identification of the statistically significant conditional dependency of INS on HLA.
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254

Both isolated type 1 diabetes and polyautoimmune beta cell failure of APS2 share the same susceptibility profile at gene loci: HLA DR, CTLA4, and INS in the T1DGC dataset

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Background and aims: Type 1 diabetes (T1D) may occur as isolated immune mediated ß-cell disease or as part of polyglandular syndrome, mostly type 2 (APS2) where other endocrine and non-endocrine autoimmune disorders develop in the later course or are already present at T1D manifestation. These APS2 associated T1D patients often suffer from complicated metabolic control due to the comorbidities or medication. We reasoned whether the established genetic susceptibility markers differ in isolated T1D from APS2 associated T1D.
Materials and methods: Data from the RR 2008.07 dataset of the T1DGC were used to study transmission rates of the susceptible HLA DR, CTLA4 and INS alleles either to T1D only or T1D with APS2 manifestations (autoimmune thyroid disease, Addison´s disease, multiple sclerosis, vitiligo, alopecia, rheumatoid arthritis).
Results: Altoghether 5125 individuals had sufficient phenotypic and genotypic information. We identified 2820 T1D and 341 T1D/APS2 patients. In both groups a strong susceptibility was conferred by HLA DR3, DR4 (transmission rate 75.2 % and 75.7% , p=2,e-110 and 1,e-15, but no difference between the grouops) whereas DR1 and DRX were associated with significant protection. Whereas a non-significant trend was observed for the CTLA4+49 SNP in isolated T1D (p=0.0624), no difference in transmission was found in T1D/APS2 patients. The association at the INS locus however is significant only in T1D (p=1,e-15) but not in T1D/APS2 (p=0.0516) transmissions.
Conclusion: In conclusion the isolated form of T1D and the one with APS2 comorbidities do not appear to differ at major susceptibility loci. Interaction of genetic and environmental factors is therefore assumed to determine multiorgan autoimmunity in T1D patients.
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255

HLA Class I allele B39 defines the rate of progression of autoimmunity in children with HLA-DRB1*0404-DQB1*0302 haplotype

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Background and aims: HLA associated susceptibility to type 1 diabetes is mainly defined by alleles of class II DR and DQ genes but contribution of some class I alleles in A and B loci has been also demonstrated. It has been proposed, that class II genes determine the initiation of autoimmunity whereas class I genes might determine the progression of beta-cell damage. This would fit to the antigen recognition in context of class II alleles by CD4 cells initiating the immune response whereas cytotoxic CD8 cells respond to antigenic peptide presented by class I molecule. We have previously found that the risk for type 1 diabetes conferred by HLA-DRB1*0404-DQB1*0302 haplotype is dependent on the presence of HLA-B39 allele in the genotype.
Materials and methods: Children participating the Finnish Diabetes Prediction and Prevention (DIPP) study who developed at least 2 different diabetes associated autoantibodies (ICA, IAA, GADA or IA-2A) and were positive for DRB1*0404-DQB1*0302 haplotype were selected for the analysis. Children were followed at regular visits including blood samples at 3-12 month intervals since birth and at as a rule 3 month intervals since converting positive for an autoantibody. HLA-DRB1 and DQB1 alleles were defined using lanthanide labelled sequence specific oligonucleotides in microtitre plate based assay and B39 was typed using sequence specific primers for specific amplification and visualisation of the product on agarose gel. Autoantibodies were analysed using standard methods.
Results: The progression of autoimmunity after the time-point of appearance of the first autoantibody positive sample (IAA, GADA or IA-2A) was compared between children positive and negative for B39 allele. Kaplan-Meier analysis demonstrated more rapid development of clinical type 1 diabetes among children positive for B39 compared to those negative for this allele (P=0.00042). 9 of 40 (22.5%) children with B39 developed the disease compared to 5 of 114 (4.4%) children without B39. Mean survival time for B39 positive children with type 1 diabetes was 1.7 years and for B39 negative 2.4 years.
Conclusion: The result confirms the significance of this particular class I association in the progression of established autoimmunity into clinical diabetes.

256

Capture of type 1 diabetes-susceptible HLA-DR-DQ haplotypes in Japanese by tag single nucleotide polymorphisms

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Background and aims: HLA-DR and -DQ loci in MHC class II region most strongly confer the susceptibility to type 1 diabetes. However, DNA typing of HLA-DR and -DQ alleles are complicated. We tried to develop the methods to identify type1 diabetes-susceptible HLA-DR-DQ haplotypes rapidly using tag single nucleotide polymorphisms (SNPs) for HLA class II alleles, and estimated the disease risk using these SNPs.
Materials and methods: A total of 211 subjects including 201 patients with type 1 diabetes (men/women: 115/86, age at onset: 34 ± 14 years, mean ± SD) and 10 patients with other diseases were typed for tag SNPs and HLA-DRB1, -DQA1, and -DQB1 alleles. In addition, 300 control subjects were typed for tag SNPs. All subjects were Japanese. Based on the report of de Bakker et al. (Nat Genet 38:1166, 2006), we chose rs2395185 (T) and rs411326 (C) as tag SNPs for DRB1*0405, rs3129888 (G) as tag SNP for DRB1*0802, rs6457617 (T) as tag SNP for DQA1*03, and rs3998159 (A) as tag SNP for DQB1*0303. These SNPs were typed by TaqMan assay. HLA-DRB1, -DQA1 and -DQB1 alleles were typed by PCR-RFLP methods.
Results: One hundred and twenty-six of 128 DRB1*0405 haplotypes had T allele in rs2395185 (sensitivity: 98.4 %), whereas only 150 of 294 non-DRB1*0405 haplotypes had G allele (specificity 51.0 %). One hundred and sixteen of 128 DRB1*0405 haplotypes had C allele in rs411326 (sensitivity: 90.6 %), whereas only 87 of 135 non-DRB1*0405 haplotypes had T allele (specificity 64.4 %). However, T allele of rs2395185 captured all DR4 haplotypes involving not only DRB1*0405 but also other DR4 specificities including DRB1*0405-DQA1*03-DQB1*0401, 0303, 0302, or 0402, DRB1*0406 -DQA1*03-DQB1*0302, and DRB1*0403-DQA1*03-DQB1*0302 etc. and all DR9 haplotypes including DRB1*0901-DQA1*03-DQB1*0303 or 0302 at sensitivity of 98.5 % (262 /266) and specificity of 94.9 % (148/156). In addition, G allele of rs3129888 captured DRB1*0802 haplotypes including DRB1*0802-DQA1*03-DQB1*0302 and DRB1*0802-DQA1*0401-DQB1*0302 or 0402 at sensitivity of 92.3 % (24/26) and specificity of 99.0 % (390/394). As for haplotypes involving DQA1*03, sensitivity of T allele of rs6457617 was 98.9% (282/285), but specificity was only 64.4 % (87/135). Sensitivity of A allele of rs3998159 for capturing DRB1*0303 haplotypes was 99.1 % (108/109), but specificity was only 17.4 % (51/311). Frequency of T allele of rs2395185 was increased in patients with type 1 diabetes (64.4 %, 259/402) compared with normal controls (38.7 %, 232/600, p<0.0001) [Odds ratio (95% confidence interval) (OR: 95% CI): 2.87 (2.21-3.74)]. When GG genotype of rs2395185 was regarded as reference group, OR (95% CI) for developing type 1 diabetes in TT or GT genotype of rs2395185 was 9.49 (5.37-17.3) (p<0.0001) or 2.72 (1.65-4.62) (p<0.0001), respectively. On the other hand, frequency of G allele of rs3129888 did not differ between patients with type 1 diabetes and normal controls [7.0 % (28/402) vs. 6.5 % (39/597), p=0.80].
Conclusion: Tag SNPs of rs2395185 and rs3129888 could capture type 1 diabetes-susceptible HLA-DR-DQ haplotypes involving all DR4 and DR9 specificities and DRB1*0802, respectively. Especially, rs2395185 showed high odds ratio enough to estimate the risk for type 1 diabetes. This is the valuable resource for screening subjects at risk in prevention trial for type 1 diabetes.

257

KIR genes confer susceptibility to type 1 diabetes in Swedish patients positive for Coxsackie virus B antibodies

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Background: Natural Killer (NK) cells are potent modulators of innate (and adaptive) immune responses. Killer Ig-like receptors (KIR) are a group of activatory and inhibitory receptors expressed on NK cells, which bind to specific motifs of Human Leukocyte Antigen (HLA) Class I molecules. The activation or inhibition of NK cell activity depends on the overall signal generated as a result of the dynamic balance between activatory and inhibitory KIRs. The varying KIR/HLA genotypes in each individual in a population and among different populations are a mechanism by which immune system fights pathogens. However, this might also modify susceptibility related to autoimmune diseases. KIR genes have been associated with Type 1 Diabetes (T1D) in certain populations. Several enteroviruses such as Coxsackie virus B (CVB), Ljungan virus, rota virus, mumps virus, cytomegalovirus, and rubella virus have been associated with T1D.
Aims: In the present study we had tested the hypothesis that: i) presence (or absence) of certain KIR genes along with their HLA-C ligands confers susceptibility to T1DM, and ii) KIR genes confer higher risk to T1DM in subjects positive CVB antibodies.
Subjects and methods: The study included a total 315 consecutively diagnosed new onset T1DM patients and 249 age, gender and region matched healthy controls belonged to the age group 0-14 years, collected as a part of two major studies previously done in Sweden. DNA extracted previously was whole genome amplified using REPLI-g mini kit (Qiagen, Hilden, Germany) as per the manufacturer's instructions. A quality control was performed to access the quality of the amplified DNA. The amplified DNA was tested for the presence of framework genes, KIR2DL4, KIR3DL2 and KIR3DL3. Only those samples which were positive for all the three framework genes were included in the study. KIR and HLA-C ligand genotyping was performed using PCR-sequence specific primer (SSP) methodology. The subject material has been previously tested for high risk HLA, autoantibodies to GAD65, IA-2 and IAA, CVB antibodies and alleles of MICA and SUMO4.
Results: i) KIR genes frequencies were investigated in patient and healthy control populations belonging to the age groups 0-14 years. No significant difference was observed. ii) KIR and CVB: Of the 315 T1DM patients in 0-14 year age group, 223 were previously tested for CVB antibody and 55 out of 223 were positive for CVB. KIR and HLA-C ligand frequencies were compared among CVB antibody + and CVB antibody - patients. KIR2DL5 (OR (95%CI) =1.95 (1.06-3.61) P=0.03) and KIR2DS5 (OR (95%CI) =1.94(1.03-3.66) P=0.04) were positively associated with T1DM in patients positive for CVB antibody. The combined presence of KIR2DL5 and KIR2DS5 was significantly increased in CVB antibody positive patients when compared to CVB antibody negative patients, in the 0-14 year age group (OR (95%CI) =2.11 (1.11-4.03), P= 0.02). Combined absence of KIR2DL5 and KIR2DS5 was however significantly high in CVB antibody negative patients compared to CVB antibody positive patients.
Conclusion: KIR2DL5 and KIR2DS5 are important predisposing genes in CVB antibody positive Swedish patients.
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258

Novel polymorphism in the PTPN2 gene is associated with type 1 diabetes mellitus in Russian patients

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Background and aims: Last generation genomic scans for type 1 diabetes mellitus (T1DM) susceptibility revealed a group of genes coding protein tyrosine phosphatases of non-receptor type (PTPNs). At least 3 members of this family (*PTPN2*, *11* and *22*) were found to be located in the regions of maximum linkage to T1D on chromosomes 18, 1 and 12, respectively. In the current study we aimed to perform a mapping of one of these genes, *PTPN2*, using tag-SNP approach, in a group of T1DM patients from Russia. The use of taq-SNPs helps to evaluate association not only for single SNPs but for linkage blocks inside the gene region.
Materials and methods: A group of 176 T1DM patients and 206 unrelated controls of Russian origin were recruited in Endocrinology Research centre, Moscow. Genotyping was performed using Taqman assays on ABI 7500 thermocycler. Allele and genotype distributions in T1DM and control subjects, significance of association and the odds ratios were calculated using SPSS 11.0 software.
Results: A set of 4 tag SNPs (rs2542151, rs2847281, rs3737361, rs547268) was designed within *PTPN2* gene and 5' and 3' flanked regions using Hapmap web site. Minimum allele frequencies for affected SNPs were assumed to be 0.2. One of these polymorphisms, rs2542151, was previously found to be highly associated with T1DM. T1DM patients and controls were genotyped for all 4 polymorphisms and for HLA DR3/DR4 susceptible haplotypes.

Analysis of genotyping data demonstrated that none of these *PTPN2* polymorphisms were in significant linkage disequilibrium and all of them were in Hardy-Weinberg equilibrium in a control group. Analysis of association revealed significant difference in allele and genotype frequencies for one of *PTPN2* polymorphisms, rs2847281, which represents *C*/*T*. Particularly, we found that allele *T* and genotype *TT* appeared more frequently in T1DM patients (73% vs. 63%, OR=1.58, Pc=0.004; 59% vs. 42%, OR=1.93, Pc=0.006, respectively). In contrast, "traditional" rs2542151 polymorphism was not associated with T1DM in Russian patients living in Moscow. In order to reduce an effect of HLA genes we calculated the difference between T1DM and control group after removal of all carriers of DR3/DR4 heterozygous genotype. We observed stronger association in this case (76% vs. 63%, OR=1.81, Pc=0.0004 for allele *T*; 63% vs. 42%, OR=2.28, Pc=0.0007 for genotype *TT*). Polymorphism rs2847281 is located in intron 5 of *PTPN2* gene and unlikely represents a functional variant. In order to assess functional significance of association we searched for known polymorphisms being in strong linkage with rs2847281, and we have not found any coding or regulatory SNP. Thus, further sequencing of linkage blocks is required to find new SNPs involved in disease progression pathway.
Conclusion: We found a novel polymorphism in *PTPN2* gene which is associated with type 1 diabetes mellitus. Our data suggest that rs2847281 SNP is one of the strongest genetic markers of T1D in Russian patients living in Moscow.

259

CNDP1 gene polymorphism predicts progression from nephropathy to end-stage renal disease in type 1 diabetes mellitus
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Background and aims: Homozygousity of a 5-leucine repeat (5L-5L) in the carnosinase gene CNDP1 has been found to be associated with diabetic nephropathy in cross-sectional studies, mainly in type 2 diabetes. However, longitudinal data on renal function are limited. We prospectively investigated whether allelic variation in CNDP1 gene is associated with risk for progression from nephropathy to end-stage renal disease (ESRD) in patients with type1 diabetes.
Materials and methods: Prospective observational study in patients with type1 diabetes and nephropathy defined by persistent albuminuria 300 mg/24h. GFR was measured after a single injection of ^{51}Cr-EDTA. Leucine repeat in the CNDP1 gene was assessed by fluorescent DNA analysis system. ESRD was defined as need for start of chronic dialysis or kidney transplantation.
Results: 410 patients (59.3% male, age 41.9 10.3 years) were included. At baseline, duration of diabetes was 28 8.7 years, HbA1c 9.4 1.5%, serum creatinine 101 (interquartile range (IQ) 81-134) mol/L, GFR 77 33 ml/min and urinary albumin excretion 593 (IQ 267-1518) mg/24h. 5L-5L was found in 156 (38%) patients. After 9.5 (IQ 7.1-12.6) years of follow-up, 28 patients (17.9%) with 5L-5L developed ESRD vs. 42 (16.5%) with any other allelic variant [Cox-regression hazard ratio (HR)=1.1 (95% CI 0.7-1.8), P=0.69]. After adjustment for potential confounders, including baseline GFR, albuminuria, HbA1c, and blood pressure in a multivariate Cox-regression model, 5L-5L was associated with increased risk for progression to ESRD [HR= 1.8 (1.0-3.0), P=0.02].
Conclusion: In this prospective study on allelic variation in CNDP1 gene, homozygousity of a five-leucine repeat was associated with an increased risk for progression from nephropathy to ESRD in patients with type 1 diabetes. Interference in carnosine metabolism could be renoprotective in diabetic nephropathy.In this prospective study on allelic variation in CNDP1 gene, homozygousity of a five-leucine repeat was associated with an increased risk for progression from nephropathy to ESRD in patients with type 1 diabetes. Interference in carnosine metabolism could be renoprotective in diabetic nephropathy.
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260

Genetic effects of MCF2L2 Leu359Ile polymorphism on diabetic nephropathy
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Background and aims: MCF2L2 (MCF.2 cell-line derived transforming sequence-like 2) is a Rho family guanine-nucleotide exchange factor (GEF), and Rho protein plays an important role in the pathogenesis of cardiovascular disorders. The aim of this study was to investigate the genetic effect of Leu359Ile variant of MCF2L2 on diabetic nephropathy (DN).
Materials and methods: Americans subjects of European descent, including 574 (female 264/male 310) type 1 diabetes (T1D) patients with DN and 594 (353/241) T1D patients without DN were selected from Genetics of Kidney Diseases in Diabetes (GoKinD) study. Leu359Ile (T1165G) is a detectable non-synonymous single nucleotide polymorphism (nsSNP) in the MCF2L2 gene. Genotyping experiments were performed with TaqMan allelic discrimination.
Results: The G allele frequency in female T1D patients with DN was lower compared to female T1D without DN (0.165 vs. 0.220, P=0.017, OR=0.701). The association between Leu359Ile polymorphism and DN among female T1D patients was significant (P=0.005, OR=0.615 by the dominant model and P=0.018, OR=0.757 with Armitage's trend tests). Further analysis indicated that female T1D-DN patients carrying with GG genotype had lower creatinine (1.44±0.24 mg/mL vs. 1.95±0.13 and 1.96±0.19) and cystatin (1.73±0.33 mg/L vs. 2.21±0.12 and 2.20±0.21) levels compared to the carriers with TT and TG genotypes. The association of this polymorphism with DN among male T1D patients was not statistically significant.
Conclusion: The present study provides the evidence that MCF2L2 Leu359Ile variant is associated with decreased risk of DN in female T1D patients. Questions of gender difference and GEF biology have been taken into our consideration with replication and functional studies.
Supported by: all subjects included in the GoKinD study, Novo Nordic Consortium and Family Erling-Persson Foundation

261

Polymorphism of glutamate-cysteine ligase catalitic subunit (*GCLC*) and glutathione peroxidase 3 (*GPX3*) genes as risk factors for overt nephropathy in type 1 diabetes mellitus
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Background and aims: Diabetic nephropathy (DN) represents one of the leading causes of end-stage renal disease and affects approximately one third of type 1 diabetic patients during their lifetime, at times irrespective of the glycemic control. These findings, taken together with results of family and epidemiological studies point to the existence of genetic susceptibility to the renal lesions induced by the metabolic disturbances triggered by chronic hyperglycemia. The aim of this study was to test whether genes involved in glutathione (GSH) metabolism are candidates for nephropathy susceptibility in patients with type 1 diabetes mellitus. To this end, two functional polymorphisms were genotyped in Brazilian type 1 patients: -129C→T in *GCLC*, the gene coding for the catalytic subunit of glutamate-cysteine ligase, the rate-limiting enzyme in GSH synthesis, and -65T→C in *GPX3*, the gene coding for plasma glutathione peroxidase, an important antioxidant enzyme
Materials and methods: 263 patients were divided into two groups according to the presence (n = 87) or absence (n = 176) of overt diabetic nephropathy (DN), defined by persistent macroalbuminuria or proteinuria, or renal replacement therapy
Results: The allelic distribution of both polymorphisms was consistent with the Hardy-Weinberg equilibrium. The presence of at least one T allele of *GCLC* –129C→T (OR = 2.84; CI 95% = 1.33 -6.06; p = 0.007) and of at least one C allele of *GPX3* -65T→C (OR = 2.61; CI 95% = 1.31 - 5.20; p = 0.006) were independent risk factors for overt DN after adjustment for other risk factors. The combination of the wild type genotypes of *GCLC* and *GPX3* poly-

morphisms (CC/TT) was protective for overt DN (OR = 0.37, CI 95% = 0.20 - 0.68; p = 0.001)
Conclusion: this study provides evidence that the evaluated polymorphisms, associated to a lower transcriptional activity of *GCLC* and *GPX3* genes, and probably to a smaller GSH antioxidant pool, constitute risk factors for overt DN in type 1 diabetic patients.

Frequencies of GCLC and GPX3 genotypes and genotype combinations according to nephropathy status

	Without overt diabetic nephropathyn=176	With overt diabetic nephropathyn=87	*P* value
Genotypes			
GCLC (CT + TT)	22 (12.5)	20 (23.0)	0.029
GPX3 (TC + CC)	33 (18.8)	32 (36.8)	0.001
Genotype combinations (*GCLC*/*GPX3*)			
CC/TT	122 (69.3)	40 (46.0)	<0.001

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PS 2 Prediction and prevention of type 1 diabetes

262

Prognostic relevance of autoantibodies against IA-2beta in IA-2A-positive school children from the general population
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Background and aims: Autoantibodies (AAb) against islet autoantigens like Glutamate decarboxylase (GADA), Protein tyrosine phosphatase (IA-2A) and Insulin (IAA) are established serological markers of beta-cell specific autoimmunity in the prediabetic phase and are therefore used for prediction of Type I diabetes mellitus (T1DM). In the mid-1990s, AAbs against another Protein tyrosine phosphatase (IA-2beta) which is structurally similar to IA-2 (74% amino acid sequence homology for the intracellular domains) were also found to be associated with T1DM. The aim of the present study was to assess the prevalence of AAbs against IA-2beta (IA-2betaA) in IA-2A-positive children from the general population with no first-degree T1DM heredity, and to examine whether the detection of IA-2betaA could be used to further stratify the risk for progression to T1DM in IA-2A-positive subjects.
Materials and methods: Serum samples from 71 IA-2A-positive schoolchildren from the Karlsburg Type-1-Diabetes Risk Study, a general population-based combined AAb screening for GADA, IAA and IA-2A, were examined for the presence of IA-2betaA by radio-ligand binding assay using [^{35}S] methionine-labelled human recombinant intracellular portion of IA-2beta. Additionally, PCR and DNA hybridization with specific non-radioactive probes were used for determination of the HLA-DQB1 genotype.
Results: IA-2betaA were detected in 32.4% (23/71) of the IA-2A-positive children. 95.7% of them (22/23) had multiple diabetes-associated AAbs (GADA, IA-2A and/or IAA), only 1/23 (4.3%) has singular IA-2A ($p<0.0001$). 21/23 (91.3%) of the IA-2betaA-positive children also had a T1DM-associated HLA-DQB1 haplotype (HLA-DQB1 *0302 and/or *02) while only 2/23 (8,7%) had neutral HLA-DQB1 alleles ($p<0.0001$). The HLA-DQB1*0302 haplotype was more common (12/21 subjects, 57.1%) than the HLA-DQB1*02 haplotype (9/21 subjects, 42.9%). No IA-2betaA-positive child had the dominant-protective HLA-DQB1 allele *0602. 18 children progressed to T1DM, of those 11 (61.1%) were IA-2betaA-positive and had also multiple AAbs. The risk for IA-2A-positive children to develop T1DM was therefore significantly higher with detection of IA-2betaA than without IA-2betaA reactivity ($p<0.01$). All IA-2betaA-positive subjects with at least one diabetes-associated HLA-DQB1 allele developed T1DM. Testing for IA-2betaA in IA-2A-positive subjects had a specificity of 61%, a sensitivity of 77%, a positive predictive value of 48% and a negative predictive value of 85%.
Conclusion: The occurrence of IA-2betaA is associated with known T1DM risk factors like positivity for multiple AAbs and presence of T1DM-associated HLA-DQB1 alleles as well as with progression to T1DM. In IA-2A-positive subjects from the general population, the detection of IA-2betaA can help to further stratify the risk for progression to T1DM by identifying probands with a higher risk. Therefore, based on specificity and sensitivity as well as positive and negative predictive value, testing for IA-2betaA in IA-2A-positive subjects should be included in AAb-based risk assessment for prediction of T1DM.
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263

Decrease in GAD-65, IA-2 and insulin autoantibodies during non-diabetic pregnancy
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Background and aims: To investigate seroconversion occurrence of type 1 diabetes associated autoantibodies in non-diabetic pregnancies concurrently explore if pregnancy alters the circulating levels of these autoantibodies.

Materials and methods: Obtained blood samples from all mothers in the Skåne region during the first trimester of pregnancy and at the time of delivery between September 2000 and August 2004 have been analyzed for type 1 diabetes associated autoantibodies GADA, IA-2A and IAA. Altogether we compared 244 non-diabetic mothers positive for at least one of the three autoantibodies with 1419 control mothers matched by age and first trimester sampling date ± seven days.
Results: The level differences in GADA was significantly lower (p<0.0001) from the first trimester to delivery. Although the number of mothers was fewer the IA-2A levels showed a similar development in level differences as GADA. The level difference in IA-2A was significantly lower (p<0.0001) from the first trimester to delivery. The level difference IAA was significantly lower (p<0.005) from the first trimester to delivery. The decreasing levels of IAA were not as pronounced as the decrease in GADA and IA-2A levels. Furthermore the increasing levels off IAA were more numerous and greater in difference between the first trimester and delivery compared to the other two autoantibodies.
Conclusion: This present study supports the notion of decreasing levels of all three detected autoantibodies in samples taken at the time of the delivery compared to the samples taken at the end of the first trimester.
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264

Insulin antibodies and selected markers of insulin resistance during the initial 2 years of type 1 diabetes in children
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Background and aims: The aim of the study was to estimate the relation between insulin antibodies (IA) and selected markers of insulin resistance in children with type 1 diabetes mellitus (T1DM).
Materials and methods: IA were measured as total anti-insulin antibodies (expressed in %) by a radioimmunometric assay in 71 patients (age at T1DM onset 6.8-17.6 years; 44 males) at 3 time-points: T1DM onset (baseline), after 6 months (6.mth) and after 24 months (24.mth). Following parameters were included into statistical analysis as markers of insulin resistance: M-index (i.e. glucose disposal rate determined by euglycemic hyperinsulinaemic clamp technique by de Fronzo; mg/kg/min; the lower M-index - the higher insulin resistance), daily insulin dose (U/kg/day), Z-score of BMI (body mass index). Other parameters included into multivariate analysis were: age at T1DM onset, glucagon-stimulated C peptide after 6 minutes and HbA1c. Associations with a final value of p<0.05 were considered statistically significant.
Results: Baseline: Initial values of IA correlated only with age at T1DM onset (R=-0.23; p=0.049). 6.mth: The following parameters correlated with IA level: M index (R=0.29; p=0.02), insulin dose (R=0.24; p=0.02), Z-score of BMI (R=-0.23; p=0.06) and age at T1DM onset (R=-0.26; p=0.03). In multivariate analysis only M-index and insulin dose remained significant. R^2 for whole model equaled 0.19 (p <0.001). 24.mths: Age at T1DM onset (R=-0.29; p=0.01) was correlated with IA titer. Z-score of BMI (R=-0.19; p=0.14) showed borderline significance. In multivariate analysis age at T1DM onset was confirmed to influence IA titer, Z-score of BMI showed borderline significance (p=0.09). R^2 for whole model equaled 0.07 (p<0.001).
Conclusion: 1) IA do not increase insulin resistance assessed as M-index, 2) Positive correlation between IA and M-index after the 6.mth may indicate that IA can promote glucose disposal rate at same time-points of T1DM course 3) Negative correlations between IA and Z-score of BMI after the 6.mth and after the 24.mth of T1DM require further clarification.
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265

Autoantibodies to REG family proteins in Japanese diabetes patients
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Background and aims: Reg, an autocrine/paracrine growth factor for β-cell regeneration was first isolated from regenerating islet cDNA. Recombinant REG ameliorated diabetes in 90% depancreatised rats and non-obese diabetes mice. The human *REG* family consists of five genes (viz; *REG Iα*, *REG Iβ*, *REG III*, *HIP/PAP* and *REG IV*), which share a common gene structure with 6 exons and 5 introns encoding homologous 158-175 amino acids secretory proteins. REG proteins are expressed at the event of tissue injury and regeneration. We have previously identified autoimmunity against REG Iα in Japanese Type 1 and Type 2 diabetes patients. Autoantibodies to REG Iα showed a correlation with the duration of diabetes and ages of patients at the onset of the disease. Owing to sequence homology and similarity in protein expression between various REG family members, it is of great interest to study the occurance of autoimmunity against other REG family members and understand its association with autoimmune diseases, if any. In the present study we have analysed the presence of autoimmunity to REG Iβ, REG III, HIP/PAP and REG IV in diabetes patients.
Materials and methods: Over 300 subjects with diabetes and 75 healthy controls were selected from Tohoku University Hospital (Sendai, Japan). All controls and patients were informed of the purpose of the study and their consent was obtained. The cDNA of each *REG* gene was cloned into pTriEX-4 multiple expression system vector (Novagen®) and proteins were expressed in *E. coli*, with conjugated S-tag sequence at N-Terminal to fascilitate one-step affinity purification on S-Protein agarose (Novagen®). Autoantibodies against REG proteins were detected by Western Analysis. Each purified REG protein was electrophoresed on SDS-polyacrylamide gel, and electrotransfered on PVDF membrane. After blocking with milk solution the membranes were incubated with patient sera as primary antibody, at appropriate fold dilutions. HRP conjugated goat Anti-human monoclonal IgG (American Qualex) were used as secondary antibody, and signal was detected by ECL Plus Western Blotting System (GE Healthcare). Signal intensity from each patient serum was measured by densitometric analyses, using ImageJ software. Statistical significance of the occurance of anti-REG autoimmunity in patients' sera and its correlation with the general characteristics of diabetes patients was analysed by Chi-squared test, Student's t-test, simple regression analyses and ANOVA analyses.
Results: Significant autoimmunity was found against REG Iβ in 10% of Type1, 32% of Type 2, and 2.7 % non-diabetes controls (P = 0.001), and against REG III in 15% of Type1, 20% of Type 2, and 1.4 % non-diabetes controls (P = 0.0001). There was significant difference between anti-REG Iβ positive (39.8 ± 1.6) and negative (33.5 ± 1.0) groups in age at onset of diabetes (P = 0.0024). Autoantibodies against HIP/PAP and REG IV antigens were not detected in diabetes patient sera.
Conclusion: These results suggest that diabetes patients may carry autoantibodies against REG Iβ and REG III. Thus, autoimmunity against REG family proteins may be associated with diabetes, and different REG family members may serve as separate biomarkers.
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266

Composite multi-antigen probes enhance detection of type 1 diabetes autoantibodies
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Background and aims: A major goal in Type 1 Diabetes (T1D) is to conduct population based screening of genetically-at-risk individuals prior to clinical disease onset. Autoantibody assays provide highly sensitive and specific measures of auto-immunity and assays of insulin, GAD65, IA-2, and ZnT8 autoantibodies detect greater than 90% of newly diabetic subjects. However, the current methodology requires multiple single target assays. We reasoned that combining multiple epitopes into a single "poly-antigen" probe would improve efficiency and may simultaneously enhance specificity and/or sensitivity, especially for low affinity antibodies.
Materials and methods: We constructed probes of T1D autoantigen homo- and hetero- multimers. Compound ZnT8 and IA-2/phogrinÂ¸ fusion proteins were combined into a single chain analyte using polypeptide linkers of different lengths and amino acid composition. We further optimized probe parameters by "trimming" epitope components, re-ordering individual domains, and comparing synthetic dimers with monomers joined by coiled/coils. Constructs were tested in radioimmunoprecipitation assays against a panel of sera derived from new onset T1D patients and HLA and age matched controls.
Results: Analysis with monomer compared to homo-dimer probes showed enhanced reactivity to the dimeric fusion proteins, most significantly in sera

having marginal reactivity. Hetero-dimeric probes containing both polymorphic variants (325R and W) of ZnT8 C-terminal domain showed enhanced sensitivity as compared to monomers (78% vs 53% (325R) / 48% (325W)) to a panel of new onset sera without compromising specificity (>99%). Probes containing homo-dimers of the N-terminal domain also displayed increased sensitivity relative to monomer N-terminal constructs. Binding to N-terminal dimers increased autoimmunity detection from 5.0% with a monomer probe to 16.8% with dimeric constructs, however specificity declined to 95%. Expanding the linker between monomers improved assay sensitivity, but their order appeared benign. Homo-dimeric constructs of IA-2 and phogrin also had improved sensitivity. Heterodimeric IA-2/phogrin fusion proteins detected both antigenic specificities from a single probe without loss of specificity, and provided higher sensitivity. In contrast, heterodimers comprising IA-2 and ZnT8 were able to detect mono-specific sera to each antigen, but did not show enhanced sensitivity over the individual monomers. Comparison of inter- and intra-assay variation indicated that both the homo- and heterodimeric probes showed greater consistency (CV <20%) providing a "tighter" cut-off than their monomer counterparts. Consequently, sera on the cusp of scoring positive readily exceeded the cut-off with the dimeric probes.
Conclusion: Our data show a significant advantage of employing epitope multimers. Combining two ZnT8 C-term variants, two N-term domains, or the two IA-2 family orthologues improves assay sensitivity. The lower CV achieved with dimeric probes could not entirely account for the improved sensitivity of these assays suggesting that additional factors such as increased avidity due to duplication of monomer epitopes, or detection of dimer-specific epitopes play a role. We conclude that our strategies improve the sensitivity of current RIAs without conceding specificity.
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267

Involvement of the Ig heavy chain locus in autoantibody reactivity in Portuguese type 1 diabetes patients
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Background and aims: Autoantibodies are linked to Type 1 Diabetes (T1D), but their role in disease pathogenesis has long been disputed. Autoantibodies are mostly seen as a by-product of the autoimmune attack that targets the pancreatic Beta cell. Nevertheless, autoantibodies are frequently detected before disease onset and non-pancreatic Beta cells are frequently observed in the in Type 1 Diabetes patients. These observations suggest that autoantibodies may be related take part in early phases of the disease process. The aim of this study was to analyze the contribution of specific single nucleotide polymorphisms (SNPs) in Immunoglobulin Heavy Chain loci (IgHG, IgHD, IgHM and IgHV) to autoantibody reactivity and to disease susceptibility.
Materials and methods: A group of 102 diabetics and their relatives, collected at the Associação Protectora dos Diabéticos de Portugal, were genotyped for 15 genetic markers in IgH loci, specifically 4 SNPs in IgHG locus, 4 SNPs in IgHD locus, 5 in IgHM locus and 2 in IgHV locus. Genetic association with T1D and with the pattern of autoantibodies was evaluated through Transmission Disequilibrium Tests (TDT) and case-control analysis.
Results: TDT analysis suggested that SPNs in the heavy chain locus, mapping on the IgHG and IgHM regions were associated to T1D in a cohort of portuguese patients (P-value=0,0455 and P-value=0,006, respectively). The T1D patients were studied for the presence of autoantibodies (including anti-ICA; IA2; GADA and gliadin) and by comparing patients showing one autoantibody versus patients showing multiple-autoantibodies we also found out that SNPs on the IgH locus, over the region of IgD, IgM and IgHV were controlling antibody multireactivity in T1D patients (P-value=0,0021, P-value=0,0020, P-value=0,0032, respectively). Furthermore, TDT analysis revealed that the IgH locus was associated to disease in T1D patients showing autoantibody mono-reactivity but not multi-reactivity (P-value(IgHM)=0,0047 and P-value(IgHV)=0,014).
Conclusion: These results suggest that the IgH locus may influence disease genetic susceptibility through influencing autoantibody reactivity, raising the possibility that inherited ability to generate auto-antibodies is a component of T1D pathogenesis.
Supported by: FCT

268

Phenotype versus immunotype and endogenous insulin production in diabetes in adults - does the clinical assessment still have a meaning in classifying patients?
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Background and aims: The category of diabetes in adults, clinically diagnosed as type 2, includes a number of subtypes and groups of patients. The various presence, configuration and concentration of autoimmune diabetogenic process markers, insulin secretion, insulin resistance and Body Mass Index makes this group of patients very heterogeneous. Despite our present knowledge about the etiology of diabetes, clinical phenotypic assessment is frequently the first and sometimes the only one criterion of classification and treatment choice. The aim of this study was to analyze whether there is a correlation between clinical phenotype, endogenous insulin production and immunotype in the polish population of adults with newly diagnosed diabetes and to establish simple algorithm of additional studies supplementing clinical evaluation when needed.
Materials and methods: The study included 142 patients, aged 20-79 yrs, with diabetes diagnosed on the basis of current WHO criteria as diabetes type 2. Patients underwent detailed physical examination, and biochemical studies: fasting glucose, insulin and glucagon test with peptide C assessment. The concentration of autoantibodies against pancreatic beta-cells (glutamic acid decarboxylase antibodies - GADA and protein tyrosine phosphatase-like protein antibodies -IA-2) was measured. Insulin resistance was established on the basis of HOMA.
Results: The studied group of patients classified as diabetes type 2 (according to WHO criteria) was very heterogeneous in age, BMI (normal in 52,8%; overweight - 26,5%; obesity 20,7%), insulin resistance (confirmed in 42,9%) and endogenous insulin production (fasting insulin 1,1 to 21 mU/l), established on the basis of glucagon test (fasting peptide C:0,1-4,39 ng/ml, after glucagon stimulation:0,1-10,3 ng/ml). GADA were found in 38,7% and IA-2 in 24,7% of patients. GADA were twice as common as IA-2 in patients <40yo and at the age of 47 yrs the frequency of IA-2 was higher than GADA (p=0,003). IA-2 were more common in overweight patients (67vs33%, p=0,004). There was no correlation between the presence of antibodies and insulin resistance, in 33% of patients both abnormalities were present.
Conclusion: Diabetes in adults is very heterogeneous disease, in which the clinical assessment is not enough for appropriate classification. Presence of phenotypic symptoms of diabetes type 2 and insulin resistance does not exclude autoimmunity. Also reversely lean body mass does not exclude the presence of insulin resistance. Therefore we suggest the algorithm for appropriate classification and choice of treatment besides clinical examination should include assessment of: 1) beta cell function (glucagon test);2) insulin resistance (HOMA);3)autoimmunity a)GADA <40 yrs ;b)IA-2 >50 yrs; c) IA-2 in BMI>25 kg/m^2. This would permit the optimal choice of proper treatment plans.
Supported by: Warsaw Medical University Grant

269

Analysis of CD25+ and CD45RA+ regulatory subsets of CD4+ T lymphocytes: comparison between nondiabetic first degree relatives and recent onset type 1 diabetes
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Background and aims: Previous studies have reported an important role of the changes in CD4+ T cell subsets in the initial phase of Type 1 diabetes (T1D). However, the changes in CD4+CD25+ and CD4+CD45RA+ T suppressor subsets of the regulatory T cells in nondiabetic first degree relatives (FDR) of patients with T1D, have not yet been clarified. Therefore, the aim of this study was to analyze the percentage of the regulatory CD4+CD25+ and CD4+CD45RA+ T cell subsets in 50 nondiabetic FDR (group A), 30 recent-onset T1D patients in insulin-requiring state (IRS) at the onset (group B) and 19 T1D patients in the state of clinical remission (CR) (group C), as well as in 25 healthy, age-matched control subjects (group D).
Materials and methods: T1D was diagnosed in accordance to WHO criteria. The CR was defined as optimal metabolic control without insulin lasting >30

days. The percentage of CD4+CD25+ and CD4+ CD45RA+ T cell subsets was analysed by using two-color immunofluorescence staining and flowcytometry.
Results: When the percentage of CD4+CD25+ T lymphocytes was analyzed, we found that in group A it was higher vs group B (A: 5.30±0.10 vs B: 2.24±0.25, p<0.05). Moreover, the percentage of CD4+CD25+ T cells in group A reached the values similar to those in group C, being slightly lower than in group D (A: 5.30±0.10 vs C: 5.17±0.14; D:6.20±0.84%, p=NS). Simultaneously, the percentage of CD4+CD45RA+T cells was found to be higher in group A vs group B (A: 25.56±2.08 vs 20.3±1.6, p<0.05). In addition, the percentage of CD4+CD45RA+T cells in group A became similar to those in C and not significantly lower compared to group D (A: 25.56±2.08 vs C:24.8±1.2; D:27.0±2.0, p=NS).
Conclusion: Our results have demonstrated that nondiabetic FDR of patients with T1D, showed higher levels of the suppressor CD4+CD25+ and CD4+ CD45RA+ subsets of T regulatory lymphocytes compared to the patients at onset of T1D, but similar to those in CR. The results imply that the onset and clinical course of T1D might be modulated on the level of those T regulatory cell subsets.

270

High risk vs low risk nondiabetic first degree relatives of type 1 diabetics: differences in CD4+CD25+ and CD4+CD161+ T regulatory subsets but not in insulin sensitivity levels

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Background and aims: It has been previously suggested that changes in $CD4^+$ T lymphocyte subsets might be associated with the onset of Type 1 diabetes (T1D). However, the relevance of immunological changes in CD25+ and CD161+ suppressor subsets of the CD4+ T regulatory cells, and metabolic changes in insulin sensitivity levels, for the development of T1D have not yet been elucidated in nondiabetic first degree relatives (FDRs) of patients with T1D. Therefore, the aim of this study was to compare the changes in (a) CD25+ and CD161+ subsets of $CD4^+$ T lymphocytes in peripheral blood and (b) insulin sensitivity levels among two groups of nondiabetic FDRs exhibiting difference in risk for developing T1D in accordance to presence or absence of glutamic acid decarboxylase antibody (GADA) positivity and the preservation of the capacity of first-phase insulin response (FPIR, insulin levels 1+3 min after IVGTT) and the group of healthy controls. Thus, in the study we included 15 high-risk nondiabetic FDRs (GADA+, FPIR < 45mU/l) (group A) and 35 low-risk nondiabetic FDRs (GADA-, FPIR > 45mU/l) (group B) and 17 healthy unrelated control subjects (GADA-, FPIR > 45mU/l) (group C).
Materials and methods: T1D and glucose intolerance were excluded in the study by using WHO criteria. GADA levels were determined by ELISA. The percentages of CD4+CD25+ and CD4+CD161+ T cell subsets were analyzed in peripheral blood by using two-color immunofluorescence staining and flowcytometry. Insulin sensitivity was tested by using euglycemic hyperinsulinemic clamp method. This test was implemented by infusing insulin at the rate of 1 mU/kgbw/min during 120 min and glucose infusion was adjusted manually, at 5 minute intervals, to maintain target glycemia at 90 mg/dl. Total glucose uptake was calculated on the basis of the amount of glucose infused during steady state period (80-120 minutes).
Results: When the percentage of CD4+CD25+ T lymphocytes was analyzed it was found to be lower in group A vs groups B and C (A: 3.58 ±0.82 vs B: 5.80±0.55; C:6.10±0.75%, p<0.05). Simultaneously, the percentage of CD4+CD161+ T cells was found to be lower in group A vs groups B and C (A: 4.68±0.43 vs B 6.18±1.01; C: 6.30±0.91%, p<0.05). However, when insulin sensitivity was tested, total glucose uptake did not differ significantly in group A compared to groups B and and C (A: 5.87±0.11 vs B: 6.58±0.27; C: 6.78±0.28 mg/min/kg, p=NS).
Conclusion: Our results have demonstrated that high risk vs low risk FDRs showed lower leves of both CD25+ and CD161+ subsets of CD4+ T regulatory lymphocytes, and did not differ in insulin sensitivity levels. The results also imply that the risk for developing T1D might be strongly influenced on the level of the activity of these subsets of T regulatory cells but not by the changes in insulin sensitivity.

271

Vitamin D supplementation increases the frequency of regulatory T cells in apparently healthy humans

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Background and aims: Several epidemiological studies support potential beneficial effects of vitamin D supplementation during infancy and childhood on the incidence of type 1 diabetes later in life. Vitamin D is thought to have an important regulatory role in modulating the immune response by increasing the frequency or function of regulatory T cells (Tregs). Tregs are critical for maintaining immunologic homeostasis and downregulation of undesired immune responses to self-antigens. Thus, Tregs might have the potential to prevent autoimmune-mediated destruction of insulin-producing beta-cells in the pancreas. In the present study, we aimed to elucidate, whether Treg frequency could be enhanced by vitamin D supplementation in apparently healthy individuals.
Materials and methods: We determined the percentage of Tregs (%Tregs) within 20000 $CD4^+$ T cells isolated from peripheral blood of 20 healthy donors before and 4 weeks after vitamin D supplementation (140000 IU as a single dosis). Fluorescence-activated cell sorting (FACS) was used to measure cell surface expression of CD4, CD25 and CD127 as well as intracellular expression of the transcription factor FoxP3. Serum 25-hydroxyvitamin D levels were measured by means of radioimmunoassay.
Results: Our study cohort consisted of 20 study participants [13 females, 7 males; median age (with interquartile range): 31.4 (28.1-34.5) years]. 25(OH)D levels increased from a median level of 23.7 (17.9-25.1) ng/ml serum at baseline to 46.5 (39.9-51.2) ng/ml serum four weeks after vitamin D supplementation. Accordingly, the median percentage of Tregs increased significantly from a median baseline level of 5.1 (3.6-6.1) % to a level of 6.4 (5.0-7.6) % ($p \leq 0.001$ for Wilcoxon rank sum test). Levels of C-reactive protein were within the normal range before and after vitamin D supplementation and did not significantly vary between the two study visits.
Conclusion: We showed that vitamin D supplementation significantly increased the frequency of Tregs in peripheral blood of apparently healthy humans over a short time period. This immune-modulatory effect of vitamin D might support the previously observed associations of vitamin D deficiency and autoimmune diseases such as type 1 diabetes mellitus. Hence, our finding provides a rationale for further studies to investigate the effects of vitamin D on beta cell survival in type 1 diabetes.

272

Smoking predicts short partial remission of type 1 diabetes

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Background and aims: Factors predicting the duration of partial remission of type 1 diabetes are known to be associated with the degree of initial metabolic disturbances and also with features of insulin resistance. Cigarette smoking is associated with decreased insulin sensitivity but its influence on the length of remission is unknown. Therefore, the aim of this study was to assess the relationship between cigarette smoking and duration of partial remission of type 1 diabetes.
Materials and methods: We followed prospectively 149 patients (48 women and 101 men, aged 16-35, mean age 25 ± 5), admitted to the Department with newly diagnosed type 1 diabetes and then attending the outpatient clinic. Thirty patients presented with diabetic ketoacidosis (DKA), mean glycaemia at diagnosis was 23.2 ± 8.4 mmol/l, HbA_{1c} 10.9 ± 2.5 % and BMI 21.9 ± 3.0 kg/m^2. In all patients intensive functional insulin therapy in multiple injections was introduced. Partial remission was defined as an insulin dose ≥0.3 U/kg/24h and an HbA_{1c} value <7% with baseline serum C-peptide concentration over 0.5 µg/l. Duration of remission was calculated from the onset of the disease. Statistical analysis was performed using Statistica 7.0 software. Mann-Whitney U test, Fisher's exact test and Cox proportional-hazards method were used.
Results: Sixty-eight patients remained in partial remission after one year of observation, in 39 patients partial remission ended before 12 months of dis-

ease and 42 patients did not enter remission. Group with remission exceeding one year did not differ from the remaining patients (with no or short remission) in age, initial: BMI, glycaemia, serum TC, LDL-C and HDL-C, serum C-reactive protein (hsCRP) concentration and presence of diabetes-related autoantibodies (ICA, GADA, anti-IA2). In the group with remission exceeding one year significantly lower prevalence of cigarette smoking (28% vs. 51%, p=0.007), higher male predominance (79% vs. 58%, p=0.008), lower incidence of DKA at onset (11% vs. 29%, p=0.005), lower initial HbA_{1c} (10.3 ± 2.1% vs. 11.3 ± 2.4%, p=0.022) and lower serum triglyceride concentration (1.68 ± 2.20 vs. 1.70 ± 1.30 mmol/l, p=0.028) were noted. In the Cox proportional-hazards model, cigarette smoking was associated with shorter duration of remission, independently from gender and presence of DKA at diagnosis.
Conclusion: Cigarette smoking may be associated with shorter duration of partial remission of type 1 diabetes.
Supported by: Polish Ministry of Science and Higher Education

PS 3 Type 1 diabetes - epidemiology

273

Age-period-cohort analysis of temporal trend of diabetes incidence in age group 0-14 years in Italy: the RIDI study group

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Background and aims: Epidemiological studies on type 1 diabetes have shown geographical and temporal variations in incidence of the disease, suggesting the effect of environmental determinants on the pathogenesis of the disease, which are still unknown. Few data are available in tha Mediterranean area. RIDI was set up in 1997 aiming to co-ordinate pre-existing registries for the incidence of T1DM in Italy and to promote the establishment of new registries in uncovered areas.
Materials and methods: Our data are regularly updated from 12 local registries: 7 regional registries and 5 province registries, covering a population at risk of 15 million of inhabitants (35% of the whole Italian population). All registries report newly diagnosed insulin-treated children, defined as the date when the first insulin injection was give and each registry employed at least 2 independent data sources for case ascertainment. This report is based on 5289 incident cases registered in period 1990-2003. Poisson regression models have been used to estimate the effects of sex, age (five three-years age groups: 0-2, 3-5, 6-8, 9-11, 12-14), calendar time (four three-year periods: 1990-92, 1993-95, 1996-98, 1999-2001, and one two-year period: 2002-03) and birth cohorts (nine six-year birth cohorts: 1978, 1981,…1999, 2001 mid-years).
Results: In the period 1990-2003, 5289 incident cases of type 1 diabetes have been identified among children aged 0-14 years. The incidence rate was 12.52 per 100,000 person-year, significantly higher in boys (13.34; 95% CI: 12.86-13.83) than in girls (11.66; 95% CI: 11.20-12.14). Large geographical variations in risk within Italy were evident, with the highest risk in Sardinia, an intermediate risk in Central-Southern Italy, and the highest risk in Northern Italy, particularly in the Trento Province, where an incidence rate of 30.48/100,000 was registered. A steep increasing risk from age 0-2 years was evident, with a doubling incidence rates in the age group 3-5 years, a quite similar risk in age 6-8, a slight further increase in age group 9-11 years. An increasing temporal trend was evident both examining all Italian data and separately the three Italian macroareas. Controlling for age and sex, the annual increase was 3.50% (2.80-4.19). With respect to the calendar period 1990-1992, the incidence rates were higher in the following four time periods. With respect to the birth cohort 1987-1993, the incidence rates increased approximately linearly from the cohort 1975-81 to for the cohort 1999-2003, RR=1.52 (1.18-1.96). The best model was the one with sex, age and a linear time trend (drift).
Conclusion: Increasing temporal trend is evident in Italy. Age period-cohort analysis, however, shows that the variation over time has a linear component that cannot be ascribed to either the calendar period or the cohort.
Supported by: Piedmont Region

274

The dynamics of incidence of type 1 diabetes in Polish children, 1983-2020: where are we?

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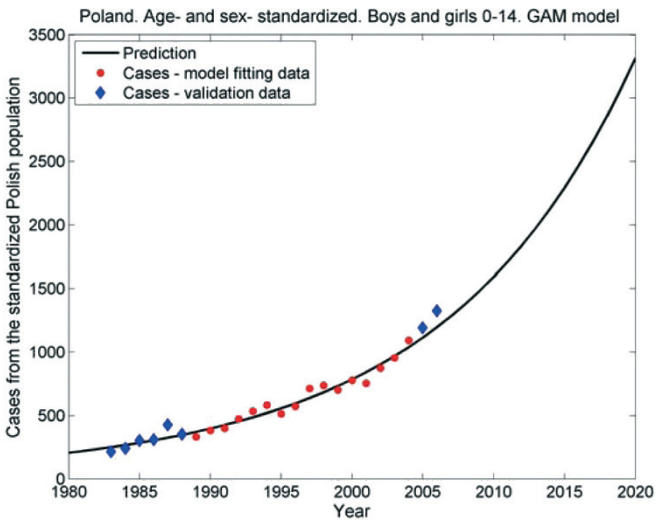
Background and aims: The incidence of childhood type 1diabetes (T1DM) has been increasing world-wide. The aim of this study was to evaluate the time trends in the incidence of childhood T1DM in Poland and to develop a predictive model for the near future.
Materials and methods: Regional registers that covered 35% of the Polish population ascertained prospectively using the EURODIAB criteria incident cases of T1DM under the age 15 years, between 1989-2004. Generalized additive modeling (GAM) was used to predict the annual number of new cases in the overall Polish population between 1989 and 2020.
Results: The primary study population included 4268 incident T1DM cases (2230 boys and 2038 girls). In 1989-2004, the average age-sex-standardized incidence was 9.9 per 100 000 per year (95% CI 8.5-11.6). The incidence has increased more than 3-fold, from 5.2 to 17.2 per 100 000 per year, between 1989 and 2004. The incidence has increased the most among children aged 5-9 years, on average by nearly 1.0/100 000 per year (95% CI 0.8-1.1). There was a significant non-linear component to the time trend ($p<0.000001$). Figure shows the standardized number of T1DM cases in the overall Polish population 0-14 years of age, during the observation period, as well as the best-fitting time trend. Based on this model, assuming constant population size, 32375 Polish children are expected to develop T1DM between 2005 and 2020. Datasets comparable to the primary register, preceding or following the study period, were analyzed to estimate the validity of this model. The observed number of new cases in the Silesia and Lodz register during 2005-2006 was similar to that predicted from the generalized time trend model: 371 vs. 390, error 5.1%. The prediction error of 1.2% for the Lodz register incidence in 1983-1988 further validated the model.
Conclusion: During the recent 15-years, the incidence of childhood T1DM in Poland has increased 3-fold, with predicted additional at least 3-fold increase between 2005-2020. While 12465 cases were diagnosed in 1989-2004, more than 32000 cases are expected in the following 15 years.



The work was partially financed by KBN grant no 402279134 and EUBIROD grant no 2007115

275

Age-specific trends of type 1 diabetes incidence in childhood differ between sexes in Germany

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Background and aims: A large risk population and a sufficiently long observation period are important preconditions for valid estimation of type 1 diabetes incidence in childhood and its temporal trend. Aim of the present study was to estimate incidence and time trend of type 1 diabetes in children 0-14 years of age in the large risk population of the German federal state North Rhine-Westphalia (NRW) during 1996-2007.
Materials and methods: During the study period the average risk population was 2.84 million children. The North Rhine-Westphalian diabetes incidence register ascertains newly diagnosed cases of type 1 diabetes by means of three data sources: the prospective hospital-based active surveillance system ESPED, annual inquiries among paediatric, internal, and general medical practices, and the computer-based documentation system DPV founded for quality control and scientific research in paediatric diabetes care. Completeness of ascertainment was estimated by the capture-recapture-method using log-linear modelling. Point and interval estimates (95%-CI) of incidence rates (per 100,000 person-years) were based on Poisson distribution. Age-and sex-standardized rates were estimated by the direct method using equal weights. Poisson regression analysis was applied to assess time trends.
Results: During 1996-2007 a total of 7,049 newly diagnosed diabetic children aged 0-14 years (3,678 boys, 3,371 girls) were registered. Ascertainment was estimated to be 98.2% (95%-CI: 98.0%-98.4%) complete. The overall age- and sex-standardized incidence rate was 20.4 (20.0-20.9). The age-standardized incidence among boys (20.8, 20.1-21.5) was slightly higher than among girls (20.1, 19.4-20.8, $p=0.124$), among 10-14 years old children the difference was significant (25.2, 23.9-26.5 vs. 22.1, 20.9-23.4, $p<0.001$). Incidence depended significantly on age ($p<0.001$). Age-specific estimates for age groups 0-4, 5-9, and 10-14 years were 14.8 (14.1-15.6), 22.9 (22.0-23.8), and 23.6 (22.8-24.5), respectively. Annual incidence rates ranged between 16.0 in 1997 and 24.4 in 2007. The average annual incidence increase was estimated as 4.1% (3.4%-4.8%). Overall, there were no significant differences in incidence trends among boys and girls (annual increase: 4.2% vs. 3.9%, $p=0.715$) or among age groups (0-4, 5-9 and 10-14 years: 4.7%, 4.3% and 3.5%, $p=0.402$). However, the annual incidence increase in 10-14 years old girls (1.9%) was significantly lower than among younger girls and boys of the same age ($p<=0.01$).
Conclusion: This study based on a large risk population and high case ascertainment confirms that incidence of type 1 diabetes in childhood is steadily increasing in Germany. Interestingly, differential trends between sexes were observed among children 10-14 years old. Based on the actual incidence rate, there are annually about 2,800 children newly diagnosed with type 1 diabetes in Germany underlining the public health importance of childhood diabetes care. Further research is needed to identify causes of the continuous rise of diabetes incidence and differential trends between sexes.
Supported by: German Ministry of Health, Ministry of Innovation, Science, Research and Technology of North Rhine-Westphalia

276

No association between vitamin D levels and the risk for islet autoimmunity and type 1 diabetes: the Diabetes Autoimmunity Study in the Young (DAISY)

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Background and aims: Vitamin D has been implicated as having a protective effect on the risk for islet autoimmunity (IA) and type 1 diabetes (T1D) because of its functions as a modulator of the immune system. The aims of this study were to investigate the associations between plasma 25-hydoxyvitamin D (25[OH]D) levels and potential gene by 25[OH]D interactions respectively, and the risk for developing IA and subsequent T1D in children at increased risk for T1D.

Materials and methods: Since 1993 the Diabetes Autoimmunity Study in Young (DAISY) in Denver, Colorado has been following children at increased risk of T1D for the development of IA and T1D. Plasma 25[OH]D was measured in DAISY children using a case-cohort study design. In addition, 25[OH]D was measured every 3-6 months once children became IA positive. The *BsmI* variant of the vitamin D receptor (*VDR*), the *-23 HphIA* variant in the insulin gene (*INS*), *R620W* variant in the *PTPN22* gene, and the *JO27_1* variant in *CTLA4* were genotyped in all children. Cox proportional hazards analyses with 25[OH]D as a time-varying covariate were conducted to examine predictors of the following outcomes of interest: 1) IA, as defined by positivity for insulin, glutamic acid decarboxylase, or insulinoma-associated antigen-2 autoantibodies on 2 consecutive visits (n= 70 IA cases) and 2) T1D (n= 42 T1D cases in children with IA after a mean age of follow-up of 6.8 years). Mixed modeling was performed to examine the predictors of 25[OH]D over time.
Results: An avergage of 5 visits were analyzed per child. Plasma 25[OH]D levels showed an expected seasonal pattern with a peak in summer. In addition, dietary intake of vitamin D, age of study participant, and body mass index were associated with 25[OH]D levels. (figure 1). Adjusting for HLA genotype, family history of T1D, and ethnicity, plasma vitamin D was not associated with the risk for IA (HR: 1.02, 95 CI: 0.98-1.05, p-value: 0.34), nor was it associated with progression to T1D in IA positive children (HR: 0.90, 95 CI: 0.64-1.26, p-value: 0.54). There were no significant interactions between 25[OH]D and the polymorphisms listed above on the risk for IA or T1D.
Conclusion: 25[OH]D levels are not associated with IA or T1D in our population of children at increased risk for T1D.

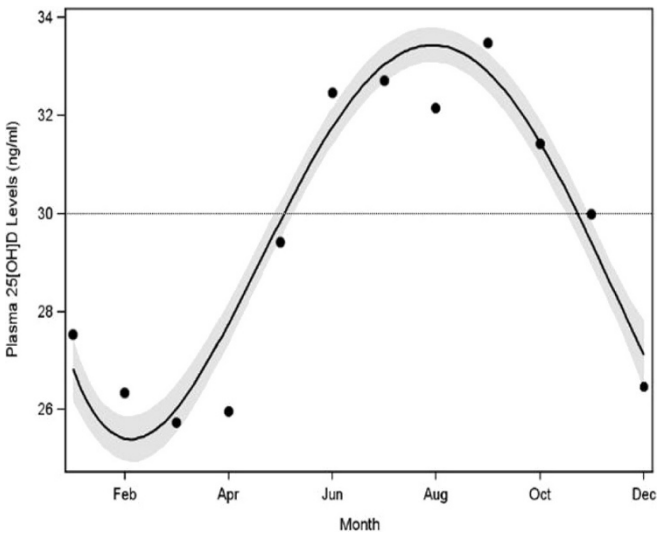

Figure 1: Plasma vitamin D levels vs onth of the year • represents mean 25[OH]D levels for each month. The curve is the yearly predicted curve for vitamin D, adjusting for age at the time of the blood draw (p<0.001), standardized body mass index (p=0.04), dietary intake of vitamin D (p=0.06), and ethnicity (p=0.011). The horizontal line represents the cutoff for insufficiency (30 mg/ml).

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277

Seroconversion in the DPT-1 Study: evidence of triggering of autoimmune process at different ages

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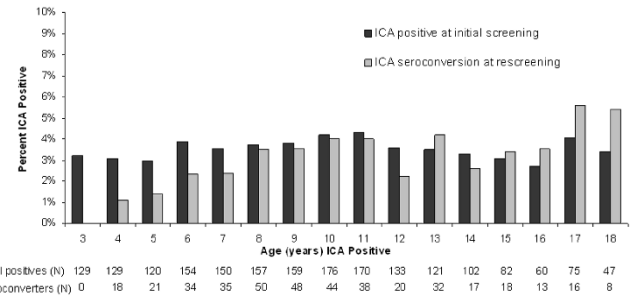
Background and aims: The purpose of this study was to determine the timing of islet cell antibodies (ICA) autoimmune seroconversion in first- and second- degree relatives of patients with type 1 diabetes (T1D).
Materials and methods: Study subjects were identified through the Diabetes Prevention Trial - Type 1 (DPT-1) which screened 55,476 subjects 3-18 years of age for ICA for potential accrual to a study to delay or prevent T1D. 1964 were ICA positive (≥ 10 JDF units) at the time of initial screening. 16,218 subjects who were initially ICA negative were recalled for annual rescreening; and 412 seroconverted to ICA positive subsequently.
Results: The median ICA seroconversion age was 9.5 years. The age distribution of seroconversion closely mirrored that of those ICA positive at initial screening. There was no significant difference in risk of ICA seroconversion by age (p=0.75), although there appears to be two peaks at 10-11 years and 17-18 years. Human leukocyte antigen (HLA) haplotypes were not significantly different between those children ICA positive at time of initial screening and those that seroconverted at a rescreening (p=0.15). Siblings of patients with T1D had a 24% greater risk of ICA seroconversion compared to other relatives (HR: 1.24, 95%CI 1.0-1.5).
Conclusion: While the risk of ICA seroconversion varies little across the age spectrum studied, it does appear that young (< age 8) children have a lower risk of seroconversion as compared to those initially screened, but as age increases the risk increases and eventually surpasses that of those initially screened. Public health implications of this finding suggest more than one autoantibody screening should be conducted to capture those that seroconvert and that by age 8 years the yield reaches or exceeds the magnitude of the prevalence.



The DPT-1 was supported by the NIH, NCRR, ADA and JDRF

278

Association of type 1 diabetes at onset with enterovirus infections in probands, siblings and parents

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Background and aims: Type 1 diabetes (T1D) is primarily a cell-mediated autoimmune disease with autoantibodies to several islet antigens. What triggers the destruction of islet beta-cells is uncertain, but viruses are prominent among candidates. A number of studies have shown epidemiological links between the onset of childhood diabetes and enterovirus (EV) infections. At the time of T1D diagnosis, the occurrence of EV infections has been investigated in six families having one diabetic child. The aim was to evaluate whether, at presentation, EVs were present in diabetic probands and whether these agents could spread among family members.
Materials and methods: Twenty-three subjects (12 children and 11 parents) belonging to six families have been investigated. The age of diabetic children was 3 to 14 years (n=8). EV genomes and infectivity were searched for in serum samples and peripheral blood leukocytes (PBL). To this end, RT-PCR with a panel of primers directed to the conserved 5`UTR and 3D regions of different EVs was used together with cell culture methods aiming at detect-

ing virus infectivity and the expression of viral proteins. Partial molecular identification of EV types was obtained by direct sequencing. Using the above methods, EV genomes and infectivity were not detected in the blood of 20 healthy controls.
Results: HLA typing showed that all members of the six investigated families carried the high-risk alleles DR3 and/or DR4. At diagnosis, EV genomes and infectivity were detected in 16/23 investigated subjects (12/12 children and 4/11 parents). Two siblings who were infected but not diabetic at the time of diagnosis, developed diabetes during follow-up (latency of 3 and 9 months from the index case). Six out of 8 diabetic children carried the DR3/DR4 phenotype (75%). Different EVs belonging to the A and B species were detected. However, each family was infected by only one EV type. According to the traditional nomenclature, only members of the coxsackie A group and echoviruses were represented. At diagnosis, the median HbA1c level was 10.9%. Glucagon-stimulated C-peptide was undetectable in 4/8 children. One year after diagnosis, all patients were under insulin treatment. The insulin requirement was <0.4 IU/kg/day in 2/8 patients. In the remaining 6 children, the median insulin requirement was 1.0 IU/kg/day.
Conclusion: At the time of diagnosis, a high prevalence of EV sequences and viral infectivity has been detected not only in diabetic children, but also in family members. Different EV types appeared to be involved. Development of multiple cases within the same family is an uncommon event. However, among the six families that have been investigated, two additional diabetes cases developed in previously normoglycemic EV-infected siblings. Thus, in genetically susceptible individuals, EV infections may represent a triggering factor of T1D.
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279

Similar frequencies of HLA risk genotypes in type 1 diabetes over the past six decades in a southern European Caucasian population
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Background and aims: The major genes responsible for at least 50% of the type 1 diabetes (T1D) genetic component are human leukocyte antigens (HLA) linked. The incidence of childhood onset T1D increased progressively over the past half century. Recent data showed that high risk HLA genotypes are becoming less frequent over time in youths with T1D of non Hispanic white and Hispanic origin. In the present study we evaluated the frequency of high, moderate and low risk HLA genotypes in a southern European Caucasian population of T1D that showed an increase in incidence in the past decades.
Materials and methods: We analysed 723 randomly T1D patients born from 1950 to 2008 and diagnosed at the mean age of 14.9 ±7.8 years (age range 5-36). Typing for HLA-DRB1 and DQB1 loci was performed by a reverse line blot assay. HLA genotypes were classified in three risk categories, based on the absolute risk values previously estimated in our population: high risk for DRB1*03-DQB1*0201/DRB1*04-DQB1*0302 genotype (DRB1*04 not 0403,06,11);moderate risk for DRB1*04-DQB1*0302/DRB1*04-DQB1*0302,DRB1*03-DQB1*0201/DRB1*03-DQB1*0201, DRB1*04-DQB1*0302/X (X not DRB1*02, 03, DRB1*04-DQB1*0302 (DRB1*04 not 0403, 06, 11) or DQB1*0602) genotypes and DRB1*03/X (X not DRB1*02, 03, DRB1*04-DQB1*0302 (DRB1*04 not 0403, 06, 11) or DQB1*0602); low risk for the remaining genotypes. Subjects were subdivided by years of birth in two cohorts: 1950-1989 and 1990-2008. The HLA genotypes frequencies between cohorts were analyzed using the chi2 test.
Results: We observed that the proportion of the three HLA risk categories (22.3% high, 61% moderate and 18.4% low, vs. 20.4% high, 59.3% moderate and 18.6% low, respectively) was similar between the two cohorts.
Conclusion: We demonstrated that the distribution of T1D HLA risk categories did not change over the past six decades in this Caucasian population in contrast with what reported elsewhere in Caucasians from Australia and in non Hispanic white and Hispanic patients. This indicates that in our population, despite the increase in incidence of the disease, the distribution of HLA genotype categories among T1D patients remains the same.

280

The Novosibirsk city type 1 diabetes registry among children: trends for 1980-2008 study periods
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Background and aims: Novosibirsk is a big industrial City in Western Siberia, Russia. The population of City takes third place among Russian Cities. The aim of the study was to describe trends of Type 1 Diabetes Mellitus (T1DM) incidence among children from 0 to 14 years old in Novosibirsk City during 1980-2008 study periods.
Materials and methods: Data for 1980-1989 and 1990-1999 study periods was collected according the standards of WHO program "DiaMond" and data for 2000-2008 years according the National Russian Registry of DM. Two sources of information have been used during the both studies: data from all Novosibirsk hospitals and data from all Novosibirsk children' endocrinologists. The criteria of diagnosis were based on the Diagnosis of Diabetes, age of onset from 0 to 14 years, citizenship of Novosibirsk City for at least 1 year before manifestation.
Results: During 1980-2008 study period 651 children of 0-14 years old were diagnosed and registered (324 boys, 327 girls) with total incidence of 9,7 cases per 100000 children (95% CI:7,9-11,6). The mean T1DM incidence among boys in 1980-1989, 1990-1999 and 2000-2008 study periods was 5,7 per 100000 boys (4,2-7,1), 8,5 (6,1-10,9) and 15,0 (11,8-18,1) accordingly. The incidence among girls for the same study periods was 5,1 (3,7-6,5), 8,5 (7,4-9,6) and 17,2 (14,1-20,2). For both sexes the incidence was 5,4 (4,1-6,6), 8,5 (7,1-9,9) and 16,0 (14,0-18,0) accordingly. We have revealed the peculiarities of T1DM incidence growth among 0-4, 5-9 and 10-14 age groups of boys and girls during study periods. We have found significant growth of incidence among girls 0-4 years old during 1980-1989, 1990-1999 and 2000-2008 study periods: 2,3 (0,6-4,0), 5,9 (3,2-8,6) and 10,8 (6,6-15,0). We have found significant growth of incidence among boys 0-4 years old only for 1990-1999 study period: 8,3 (5,5-11,2), compare with 2,7 (1,2-4,2) for 1980-1989 and 8,4 (3,7-13,1) for 2000-2008 study periods. For the 5-9 years old children the incidence growth was significant as for boys: 4,9 (2,2-7,5), 8,9 (5,1-12,7) and 19,5 (15,4-23,6) for 1980-1989, 1990-1999 and 2000-2008 study periods, so as for girls: 6,0 (3,6-8,4), 10,3 (6,9-13,8) and 22,5 (16,8-28,7). We have found significant growth of incidence among 10-14 years old children only for 2000-2008 study period: 18,1 (6,8-29,3) for boys, so as for girls: 18,3 (14,7-22,0), compare with 10,0 (6,3-13,7), 8,2 (4,7-11,6) for boys and 7,3 (4,7-9,9), 8,7 (6,9-10,5) for girls in 1980-1989 and 1990-1999 study periods. There were no significant differences in incidence among boys and girls (9,5(7,6-11,4) and 10,0(7,9-12,2) during 1980-2008 study periods. We also have found picks of manifestation of T1DM in March, June and September during 1980-2008 study periods with lowest incidence in July.
Conclusion: T1DM incidence among 0-14 years old children of Novosibirsk City is high and constantly growing as for boys, so as for girls. The highest T1DM incidence rates were revealed in 5-9 and 10-14 years old groups. But the highest incidence growth was revealed for 0-4 and 5-9 years old groups for both sexes. We think that environmental factors responsible for seasonal manifestations played a major role in such unfavourable trends of T1DM incidence in Novosibirsk City.

281

Low-grade inflammation and endothelial dysfunction precede the increase in pulse pressure in type 1 diabetes: a 20-yr longitudinal study
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Background and aims: Arterial ageing is accelerated in diabetes. This is illustrated by steeper increases in pulse pressure (PP), a marker of arterial stiffening, with ageing, in diabetic than in non-diabetic individuals, which may partially explain their increased cardiovascular risk. The mechanisms involved in accelerated arterial ageing remain unknown, however, but low-grade inflammation and/or endothelial dysfunction have been suggested to

play an important role. We have therefore investigated whether the longitudinal increases in markers of inflammation (CRP and sICAM-1) and endothelial dysfunction (sICAM-1 and sVCAM-1) were associated with and *preceded* the increases in PP in an inception cohort of individuals with type 1 diabetes (T1D).
Materials and methods: The study population consisted of 277 T1D patients (114 women) from an outpatient diabetic clinic (Gentofte, Denmark), who were consecutively admitted, upon diagnosis, between Sep'79-Aug'84 (mean age at diagnosis 27.5 ± 13.8 yrs; 216 adults). These patients attended the outpatient clinic every 3-4 months throughout a total follow-up period of >20 yrs, as part of their routine evaluation, which included measurement of blood pressure (BP) and other risk factors. PP (the pulsatile component of BP) was calculated as systolic - diastolic pressure. CRP, sICAM-1 and sVCAM-1 were measured in serum samples collected throughout the whole longitudinal period. We used generalized estimating equations (GEE) to investigate, longitudinally, the associations hypothesized; in order to ascertain 'possible causality', we also investigated the associations between levels of CRP, sICAM-1 and sVCAM-1 at any time point and changes in PP 2 years thereafter (autoregressive GEE model). All analyses were adjusted for sex, age at diagnosis, smoking, anti-hypertensive treatment and mean arterial pressure (the steady component of BP). Results hereby obtained are expressed as longitudinal regression coefficients (per SD increase in serum marker) and respective 95%CI.
Results: PP increased by 0.53 mmHg/yr (95%CI: 0.47; 0.60), CRP by 0.033 mg/l/yr (0.028; 0.039), sICAM-1 by 1.8 ng/ml/yr (1.0; 2.6) and sVCAM-1 by 1.8 ng/ml/yr (0.3; 3.2) over the 20-yr longitudinal period. Higher levels of lnCRP, sICAM-1 and sVCAM-1 were all positively associated with higher levels of PP: 0.53 mmHg (0.05; 1.00), 1.09 mmHg (0.53; 1.66) and 0.94 mmHg (0.40; 1.48) per SD, respectively. In auto-regressive GEE models, sICAM-1 and sVCAM-1, but not lnCRP, were also associated with PP: 0.56 mmHg (0.18; 0.94), 0.53 mmHg (0.17; 0.90) and -0.01 mmHg (-0.38; 0.35) per SD, respectively. Adjustments for other risk factors, i.e. HbA1c, cholesterol, BMI, creatinine and urinary albumin excretion, did not change these associations.
Conclusion: Life-course increases in low-grade inflammation and endothelial dysfunction are associated with and *precede* increases in pulse pressure. Our findings thus support, for the first time with a truly longitudinal design, the view of early involvement of these mechanisms in the premature development of arterial stiffness in diabetes.
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PS 4 Prediabetes and screening for type 2 diabetes

282

New approach to diabetes screening
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Background and aims: Leading international organizations (WHO, ADA, IDF) offer different models of screening of glucose metabolism abnormalities (GMA). Aims: 1) the comparison of different GMA screening models via determination of their sensitivity and specificity for diagnosing T2DM, IGT and IFG; 2) to develop new screening model, which simplify screening procedure and to reduce expenses on its conduction while keeping high sensitivity and specificity.
Materials and methods: Population-based screening for GMA among 2508 individuals of Moscow County. Standard 2-h 75 g OGTT was performed using HemoCue analyzers in subjects previously undiagnosed with diabetes. 626 subjects (24,9%) with GMA were found followed the 1999 WHO criteria. Depending on diagnostic criteria (ADA-2003 or WHO-1999) and research method (only FBG, OGTT) 5 screening models were considered: Model 1. Screening according to WHO, 1999 criteria (with using OGTT in all persons);Model 2. Screening according to ADA, 2003 criteria (OGTT not used); Model 3. Use of OGTT only for subjects with IFG to be measured by ADA criteria; Model 4. Use of OGTT only for subjects with IFG according to WHO criteria; Model 5. Use OGTT only for subjects with FPG > 5.0 mmol/l; Sensitivity (Se) and specificity (Sp) of screening models is compared based on definition of T2DM, IGT, IFG on formula: Se=TP/(TP+FN), Sp=TN/(TN+FP),TP-True Positive, TN-True Negative, FN-False Negative, FP-False Positive.
Results: Screening conducted according to model 1 makes it possible to discover the highest percentage of patients with T2DM and IGT. Removal of OGTT or limited use of OGTT as suggested in other screening models leads to a decrease in the diagnosing of T2DM and IGT in the population. Therefore, using screening model 2 we cannot find persons with IGT and 4% of T2DM patients (among some patients T2DM is diagnosed at the 2nd point of OGTT). A decrease in the diagnosing of IGT could worsen the prognosis of T2DM development among this category of people. When there is no information about the presence of IGT, corresponding preventative measures to decrease the risk of T2DM will not be taken. We suggest that the risk of glycemia increasing following a 75 g load of glucose is minimal given a certain level of FPG. Therefore, no people with hyperglycemia were found after loading given FPG of up to 4.0 mmol/l it was minimal given glycemia from 4.1 to 5.0 mmol/l. Given glycemia from 5.1 to 5.5 mmol/l, the risk of IGT appearing grew by 2.26 times. Given glycemia from 5.6 to 6.0 mmol/l, the risk grew by nearly 4 times. Correspondingly, the risk of discovering T2DM and IGT at the second point after loading could be considered minimal among people with glycemia of up to 5.0 mmol/l and OGTT could be excluded from the screening model. According to our data, 23.3% of those examined had FPG of less than 5.0 mmol/l. Excluding OGTT among this category could make it possible to save funds spent on the conduction of screening. We suggest a new model: use OGTT only for subjects with FPG > 5.0 mmol/l.
Conclusion: Screening model 5 (use OGTT only for subjects with FPG >5.0 mmol/l) displays high sensitivity for diagnosing T2DM (0.99) and for IGT (0.93). New screening model allows to reduce expenses by excluding OGTT among subjects with FPG < 5.1 mmol/l, share of which makes 23.3% among participants.

283

A comparison of screening strategies for impaired glucose tolerance and type 2 diabetes mellitus in a UK community setting: a cost per case analysis
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Background and aims: Universal screening for vascular risk is to be introduced in the UK starting in April 2009, for those aged over 40 years. This will have a substantial impact on health care resources, therefore it is essential to

investigate different methods for pre-screening people at high risk. Currently, there is no systematic or structured screening policy for Type 2 Diabetes (T2DM) in most countries. The aim of this study was to assess effectiveness and cost efficiency of a combination of different screening methods for undiagnosed T2DM and impaired glucose regulation (IGR included IGT and/or IFG) in a multi-ethnic community setting.

Materials and methods: A random sample of people aged 40-75 years from 24 general practices in Leicestershire were invited for a 75g Oral Glucose Tolerance Test (OGTT), as the ADDITION (Leicester) screening study. Participants provided a detailed medical history, anthropometric measurements and completed the FINDRISC questionnaire. Clinical effectiveness was compared using sensitivity, specificity, and the area under the ROC curve. We compared 17 approaches to screening involving FINDRISC and our own Leicester self-assessment (LSA) and Leicester primary-care-data (LPD) risk scores, in addition to fasting glucose and HbA1c. The cost-effectiveness of tests was assessed by 'cost per case', i.e. the total estimated cost of screening a specified population, modeling cost components such as nursing cost rates, nursing time, administration costs, lab test costs. This is then divided by the number of cases identified, as estimated using test sensitivity values from the study sample data. As an improvement on previous screening cost-effectiveness models, these models were run within a Bayesian framework, using a simulation-based approach that incorporates uncertainty around both the estimated costs and sensitivity values.

Results: 6749 individuals participated: the age range was 25-75 years (SA) and 40-75 (WE); mean age was 56.1 (SD 10.8), 47.7% were male and 27.5% were of south Asian ethnicity. 6372 individuals (94.4%) had all relevant data recorded and were included in the analysis; of these 202 (3.2%) were diagnosed at OGTT with screen-detected T2DM, and an additional 902 (14.2%) were diagnosed with IGR. Estimated cost implications varied substantially for the 212 screens we considered; results for three screens are shown in the Table for illustration.

Conclusion: The suggested strategies provide a number of tools for screening people for T2DM and IGR. A stepwise screening strategy using self-assessment or practice routine data to calculate a risk score, followed by a FPG or HBA1c is an efficient screening strategy for detecting T2DM and T2DM/IGR in a community setting.

Table. Sample, 3 of the 212 screening methods modelled.

Stage 1	Stage2	Stage 3	Proportion to OGTT (%) (SE)		Est. cost per T2DM case detected (€) (SE)	
HbA1c ≥6.0	OGTT		22.3	(0.5)	1713	(55)
LSA≥14	HbA1c≥5.8	OGTT	29.8	(0.6)	1245	(51)
LPD≥5.00	HbA1c≥6.0	OGTT	18.8	(0.5)	931	(42)

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284

Diagnostic value of glycated haemoglobin as a screening test for diabetes mellitus

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Background and aims: To assess the validity of fasting plasma glucose (FPG) and/or glycated haemoglobin (HbA1c) as screening tests for diabetes mellitus.

Materials and methods: A total of 2298 subjects (956 male and 1342 female) in Shanghai area were included. All subjects underwent a 75g oral glucose tolerance test (OGTT) and HbA1c measurement. Receiver operating characteristic curve (ROC curve) analysis was used to examine the sensitivity and specificity of FPG and HbA1c for detecting diabetes, which was defined as a FPG≥7.0 mmol/L or a post-challenge 2-h plasma glucose≥11.1 mmol/L.

Results: 1. Based on 1999 WHO criteria,830 had normal glucose tolerance(NGT),110 had impaired fasting glucose(IFG),380 had impaired glucose tolerance(IGT),183 had IGT and IFG,795 had diabetes. The prevalence of newly diagnosed diabetes was 34.6% (*n*=795). 2. Based on the ROC curve,the optimal cut-point of FPG related to diabetes diagnosed by OGTT was 6.1mmol/L that was associated with a sensitivity and specificity of 81.5% and 81.0% respectively; The optimal cut-point of HbA1c related to diabetes diagnosed by OGTT was 6.1%,which was associated with a sensitivity and specificity of 81.0% and 81.0% respectively; The screening model using FPG≥6.1mmol/L or HbA1c≥6.1% had sensitivities of 96.5% for detecting undiagnosed diabetes;The screening model using FPG≥6.1mmol/L and HbA1c≥6.1% had specificity of 96.3% for detecting undiagnosed diabetes.

Conclusion: The simultaneous measurement of FPG and HbA1c might be a more sensitive and specific screening tool for identifying high-risk individuals with diabetes at an early stage.

sensitivitis and specificities of different screening models for detecting diabetes

screening model	sensitivity (%)	specificity (%)	positive likelihood ratio	negativelikelihood ratio
FPG≥5.6mmol/L	92.5	54.3	2.02	0.46
FPG≥7.0mmol/L	54.5	100	∞	0.46
FPG≥6.1mmol/L	81.5	80.5	4.18	0.23
HbA1c≥6.1%	81.0	81.0	4.26	0.23
FPG≥6.1mmol/L and HbA1c≥6.1%	66.0	96.3	17.84	0.35
FPG≥6.1mmol/L or HbA1c≥6.1%	96.5	65.2	2.77	0.05

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285

Differences between the 10-year incidence of retinopathy and chronic kidney disease in screening-detected and in usual care-detected type 2 diabetes patients. The Hoorn Study

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Background and aims: The onset of type 2 diabetes (T2DM) complications is known to occur in many patients several years before T2DM is diagnosed. Early detection of T2DM patients by screening would be meaningful if a reduction of complications like retinopathy and chronic kidney disease could be achieved. We investigated whether screening-detected T2DM patients carry a lower risk of retinopathy and chronic kidney disease than T2DM patients diagnosed in usual care.

Materials and methods: In the Hoorn Study, a population-based cohort study (n=2484, age 50-75) of diabetes and diabetes complications, 115 patients were classified as having newly diagnosed T2DM after screening at baseline, 44 of whom participated in the 10-year follow-up examination. The 10-year incidence of complications of these screening-detected patients was compared to the 10-year incidence of complications as estimated from cross-sectional annual medical examinations in usual care-detected T2DM patients (n=1336). The regression coefficient of the relation between the proportion of complications and the T2DM duration was used to compute an estimated 10-year incidence of T2DM complications in the usual care-detected T2DM patients.

Results: The 10-year incidence of retinopathy and chronic kidney disease in the screening detected patients were 12.2% (5/41) (95% confidence interval (CI): 4.1% to 26.2%) and 37.8% (14/37) (95% CI: 22.5% to 55.2%), respectively. In the usual care-detected patients the estimated 10-year incidence of retinopathy was 8% (95% CI: 3% to 13 %) and for chronic kidney disease 20% (95% CI: 13% to 26 %). The difference in 10-year incidence between screening and usual care-detected patients shows a 4.2% (95% CI: -5.9% to 14.3 %) higher incidence for retinopathy and 17.8% (95% CI: 2.1 to 33.6%) higher incidence for chronic kidney disease in screening-detected compared to usual care-detected patients. The screened subjects were characterized by a markedly higher blood pressure as compared to the usual care detected patients, at time of diagnosis and 10 years later.

Conclusion: Surprisingly, this study showed that the incidence rate of retinopathy and chronic kidney disease is higher in screening-detected patients compared to patients detected in usual care. This suggest that non-symptomatic hyperglycemia confers a high-risk for diabetic complications. Thus, screening for T2DM might help detect patients in an earlier stage of the disease with need to initiate strict treatment on multiple risk factors for retin-

opathy and chronic kidney disease in order to prevent the development of these complications.

286

Pre-diabetes is associated with abnormal ventricular diastolic function in Korean population

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Background and aims: Patients with diabetes have an increased incidence of congestive heart failure. The recent use of sensitive and less load-dependent techniques such as myocardial velocity by tissue Doppler imaging has demonstrated subclinical left ventricular (LV) dysfunction is common in diabetic patients without known cardiac disease. However, the impact of prediabetes on LV dysfunction has not been studied in a population-based study. In the present study, we aimed to evaluate the extent to which prediabetes is associated with LV structure and function.
Materials and methods: The subjects consisted of 1221 Koreans aged 40-69 years from the Korean Genome Epidemiology Study, which is an ongoing prospective population-based study. Data were collected from a comprehensive health examination and subjects with LV systolic dysfunction or known CVD were excluded.
Results: Glucose tolerance status was assessed by fasting (FPG) and 2 hour glucose after 75g glucose loading (2hPG). Categories of glucose regulation was defined by the 2003 ADA criteria; normal glucose tolerance (NGT, FPG <5.6 and 2hPG <7.8 mmol/l, n=581), prediabetes (FPG=5.6-6.9 and/or 2hPG=7.8-11.0 mmol/l, n=434) and diabetes (FPG≥7.0 or 2hPG ≥11.1 mmol/l or previous clinical diagnosis, n=200). Echocardiography was used to assess LV structure (LV mass) and systolic (LV ejection fraction) and diastolic function, by pulse-wave Doppler (E/A ratio; ratio of early to late diastolic mitral flow velocity) and tissue Doppler imaging (Ea; early diastolic velocity). From NGT to prediabetes to diabetes, LV mass increased, and E/A ratio and Ea decreased progressively [LV mass index (mean±SD), 86.5±17.5, 88.7±16.8, 91.2±19.4 g/m^2, p=0.001; E/A ratio, 1.23±0.40, 1.10±0.32. 1.02±0.32, p<.0001; Ea, 7.57±1.91, 7.02±1.63, 6.47±1.65 cm/s in NGT, prediabetes, and diabetes, respectively, p<.0001]. However, ejection fraction and other parameters of conventional Doppler indexes were comparable in the three groups. After adjusting for age, sex, body mass index, and blood pressure, Ea was lowest in subjects with diabetes, and significantly lower in those with prediabetes than with NGT (mean±SE, 7.26±0.07, 7.00±0.08, 6.66±0.11 cm/s in NGT, prediabetes, and diabetes, p<.0001). E/A ratio was also lower in those with prediabetes than NGT, but comparable in subjects with prediabetes and diabetes in the multivariate model (mean±SE, 1.17±0.02, 1.10±0.02, 1.05±0.02 cm/s in NGT, prediabetes, and diabetes, p<.0001).
Conclusion: Diastolic dysfunction was evident in subjects with prediabetes independently of body mass index and hypertension. Screening those with prediabetes for diastolic dysfunction may identify subjects with risk for cardiovascular morbidity and mortality.
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287

One third of the Portuguese population has diabetes or "pre-diabetes" - Diabetes Prevalence Study in Portugal

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Background and aims: The number of people with diabetes has been increasing all over the world to an extent that can be called as an epidemic. Until this moment the Portuguese data came from either Catalonian data (IDF), either from the Portuguese Statistics Institute (2006) by patients' self-reference.
The objectives of this study were to determine in the Portuguese population aged between 20 and 79 years the prevalence of type 2 diabetes and pre-diabetes defined as Impaired fasting glucose (IFG) levels and Impaired glucose tolerance (IGT)
Materials and methods: Taking into account the number of inhabitants (7.657.529 people between 20 and 79 years old), 122 sub-statistical units were selected with regional and national representativeness. Within each sub-statistical unit, the resident population was randomly chosen by age and gender. The total sample was constituted by 5.167 subjects, which corresponds to an acceptance rate of 63,2% of the invitations sent to participate in the Study. The national prevalence and comparative prevalence have been calculated. Fasting glycaemia and 2 hour OGTT were made to all non-diabetic subjects. The diagnostic criteria used were the referenced by WHO. The Study took place between January 2008 and January 2009.
Results: The national diabetes prevalence is 11,7%, (95% CI: 10,8% - 12,6%) with a significant difference between men: 14,2% (95% CI: 12,5% - 15,5%); and women: 9,5% (95% CI: 8,5% - 10,6%). 6,6% of the subjects had a previous diabetes diagnosis and 5,1% were undiagnosed. The comparative prevalence found was 9.8% (5.3% with previous diagnosis of diabetes and 4.4% undiagnosed). 905.035 people aged between 20 and 79 years have diabetes, from which 395.134 subjects (43,6% of the total) didn't know they had diabetes. By age groups, the results show that: 2,4% of the population aged between 20 and 39 years; 12,6% of the people from 40 to 59 years old and 26,3% of the people aged between 60 and 79 years have diabetes. 23,2% (1.782.663 people) have IGF and/or IGT. The population from Azores (an autonomous region) has the highest regional results, with a diabetes prevalence of 14,3% (9,2% with diagnosed diabetes and 5,1% with undiagnosed diabetes).
Conclusion: Diabetes is a chronic disease with a high prevalence in Portugal, which along with "pre-diabetes" reaches one third of the population over 20 years old, being among the highest numbers in Europe. 34.9% of the population aged between 20 and 79 years have diabetes or "pre-diabetes", corresponding to 2.687.698 Portuguese resident people. Concerning people with diabetes we observe an high number of males and as described in another countries a high number- 43,6% - of undiagnosed people with diabetes.
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288

Adiponectin trajectories in the 13 years prior to the onset of type 2 diabetes mellitus - The Whitehall II study

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Background and aims: Low levels of serum adiponectin have repeatedly been associated with the development of type 2 diabetes (T2D). To clarify the issues of timing and causality, we examined trajectories of adiponectin prior to T2D diagnosis compared to trajectories in non-cases, who did not develop diabetes during follow-up.
Materials and methods: Case-cohort study within the Whitehall II cohort with a total of 2809 participants (27.2% women, age at baseline [mean±SD] 49.3±5.9 years, BMI 25.3±3.5 kg/m^2). Serum levels of adiponectin were measured at up to three time points for the 335 cases with incident T2D during the follow-up period and 2474 controls remaining T2D-free (phase 3, the baseline: 1991-94, phase 5: 1997-99, and phase 7: 2003-04). In the analysis, year 0 was set at diabetes diagnosis for cases and at a randomly selected time point during follow-up for non-cases. Adiponectin levels were traced backwards to participants' first clinical screening to assess trajectories during 13 years prior to T2D diagnosis. Multilevel models adjusted for age, sex and ethnicity were based on 755 measurement points in cases and 5095 measurement points in the remaining cohort.
Results: Adiponectin levels were lower among diabetes cases 13 years before year 0 (mean difference 0.28 [95% CI 0.21-0.34] $\log_2$ mg/L). Adiponectin levels decreased log-linearly throughout the observation period in non-cases by 0.011 [95% CI 0.006-0.0.017] $\log_2$ mg/L/year from 9.3 [95% CI 9.1-9.5] to 8.8 [95% CI 8.6-9.0] mg/L. The decline in adiponectin from 7.9 [95% CI 7.5-8.3] to 7.1 [95% CI 6.8-7.4] mg/L among cases was significantly steeper than that among controls (mean difference in slopes 0.005 [95% CI 0.001-0.0.010] $\log_2$ mg/L/year). After adjustment for contemporaneous body mass index, the difference in adiponectin levels between cases and non-cases was halved but remained significant (mean difference 0.15 [95% CI 0.09-0.22] $\log_2$ mg/L) while the slopes were no longer different from each other (p>0.05).
Conclusion: Adiponectin levels differ markedly between those who develop T2D and those who do not, more than a decade before diagnosis, but do

not appear to be involved in the immediate changes that precede the disease onset. The more rapid decline in adiponectin levels among cases compared to non cases is explained largely by differences in BMI and BMI changes of the two groups.
Adiponectin measurements were funded by a Medical Research Council (UK) New Investigator Award

289

Work stress increases risk of type 2 diabetes in middle-aged Swedish women
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Background and aims: In cross-sectional analyses in the Stockholm Diabetes Prevention Program, a population-based cohort, stress factors at work and low sense of coherence (SOC), a factor for successful coping with stressors, were associated with type 2 diabetes in middle-aged Swedish women. We investigated these associations prospectively in both men and women in the Stockholm Diabetes Prevention Program.
Materials and methods: The study included 3205 Swedish women and 2227 Swedish men, aged 35-56 years, with normal glucose tolerance at baseline. At follow-up, type 2 diabetes was detected in 60 women after 8 years and in 111 men after 10 years. We analysed the associations of work stress by high demands, low decision latitude, job strain (a combination of high demands and low decision latitude), shift work, and overtime work with type 2 diabetes. Furthermore, we studied low SOC in association with type 2 diabetes. Baseline work factors and SOC were measured by questionnaire. We calculated odds ratios (OR) and 95% confidence intervals using multiple logistic regression analysis. ORs were adjusted for age and education, while analysis revealed that data were not confounded by factors like waist, BMI, physical inactivity, smoking and family history of diabetes.
Results: In women, we found no association between high work demands (age-adjusted OR 1.0 (0.5-2.0); fully adjusted OR 1.2 (0.6-2.4)), overtime work (age-adjusted OR 0.9 (0.5-1.6); fully adjusted OR 1.1 (0.6-1.9)), or low SOC (age-adjusted OR 0.9 (0.4-2.1); fully adjusted OR 0.8 (0.3-1.8)) and type 2 diabetes. Low decision latitude was associated with type 2 diabetes on its own (age-adjusted OR 2.7 (1.4-5.5); fully adjusted OR 2.3 (1.1-4.9)), and combined with high demands (job strain; age-adjusted OR 3.9 (2.1-7.4), fully adjusted OR 3.4 (1.7-6.5)). Shift work seemed associated with type 2 diabetes (age-adjusted OR 2.6 (1.2-5.3); age and education adjusted OR 2.4 (1.2-5.1)), but this increased risk was partly explained by BMI (age, education and BMI adjusted OR 2.2 (1.0-4.9). In men, high work demands seemed to protect against type 2 diabetes (age-adjusted OR 0.5 (0.3-0.9); fully adjusted OR 0.6 (0.3-1.0)), whereas low SOC seemed to increase diabetes risk (age-adjusted OR 1.9 (1.2-3.1); fully adjusted OR 1.7 (1.1-2.9)). There were no associations between decision latitude (age-adjusted OR 0.9 (0.7-1.3); fully adjusted OR 0.9 (0.6-1.2)), shift work (age-adjusted OR 1.2 (0.7-2.1)); fully adjusted OR 1.1 (0.6-2.0)), or overtime work (age-adjusted OR 0.8 (0.5-1.3)); fully adjusted OR 0.9 (0.6-1.4)) and type 2 diabetes.
Conclusion: This prospective study indicates that risk of type 2 diabetes is influenced by stress factors at work in middle-aged women and men. In women, type 2 diabetes risk is increased by low decision latitude, especially in combination with high work demands, and shift work, but not by overtime work or low SOC. In men, type 2 diabetes risk is decreased by high work demands, and increased by low SOC.
Supported by: Stockholm County Council, Swedish Research Council on Working Life and Social Sciences, Novo Nordisk Scandinavia

290

Trends in the epidemiological situation on diabetes among adult population in Russia
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Background and aims: All over the world is marked growth of number patients with diabetes (DM), finding epidemic scales. Monitoring of situation on DM in Russia carries out the State Register of DM (SRDM) which represents information-analytical system of the regional centers. The aim of this study was to estimate trends in the prevalence, incidence and mortality of adult diabetic patients in Russia.
Materials and methods: Using the regional centers SRDM database, we estimated trends in the prevalence, incidence and mortality of adult diabetic patients from 2002 to 2007. All patients with type 1 (T1DM) or type 2 (T2DM) DM were aged > 18 yrs.
Results: Number of diabetic patients (millions) in Russia increased considerably from 1.078 in 2002 to 2.807 in 2007. Prevalence of T1DM increased by 2.1% (from 217.9 (2002) to 222.5 (2007) per 100.000 adult person-years). Prevalence of T2DM increased by 30.5% (from 1696.1 (2002) to 2214.0 (2007) per 100.000 adult person-years). Incidence of DM increased 1.4-fold among adult population, basically at the expense of patients with T2DM. So incidence of T2DM increased by 45% (from 166.2 (2002) to 241.0 (2007) per 100.000 adult person-years), whereas incidence of T1DM decreased by 1.4% (with 14.3 (2002) to 14.1 (2007) per 100.000 adult person-years). Incidence of T1DM was higher (~1.5-fold) in males, than in females. Incidence of T2DM was higher (~2.3-fold) in females, than in males. Mortality of diabetic patients decreased from 31.3 (2002) to 28.9 (2007) per 100.000 adult person-years (for T1DM) and from 341.9 (2002) to 339.5 (2007) per 100.000 adult person-years (for T2DM). Average lifetime of diabetic patients increased from 54.9±0.75 yrs to 57.2±0.28 yrs (for T1DM) and from 69.2±0.11 yrs to 71.6±0.10 yrs (for T2DM).
Conclusion: Thus, prevalence of DM in Russia increased by 16.3%. Incidence of T2DM increased by 45%, whereas incidence of T1DM decreased by 1.4% and mortality decreased by 1.3%, lifetime of diabetic patients increased by 1%.
Supported by: The State Register of Diabetes

PS 5 Prevalence of type 2 diabetes

291

Prevalence of diabetes among adults aged from 18 to 74 years living in mainland France - the French Nutrition and Health Survey (ENNS 2006-2007)

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Background and aims: The purpose of this study was to investigate the prevalences of diagnosed and undiagnosed diabetes, and impaired fasting glucose in adults from 18 to 74 years living in mainland France.
Materials and methods: Data come from the French Nutrition and Health Survey conducted in 2006-2007 which describes the nutritional situation of children aged from 3 to 17 years and adults aged from 18 to 74 years living in mainland France. A three stages stratified probability sampling design was used to assure a nationwide basis analysis (*n*=2,413 adults included in our analysis). Diagnosed diabetes was identified by self-questionnaire (self-reported diabetes or oral hypoglycemic medication or insulin). Fasting plasma glucose was used to categorize people with undiagnosed diabetes (FPG≥7.0 mmol/l) and impaired fasting glucose (World Health Organisation criteria : 6.1≤FPG <7.0 mmol/l; American Diabetes Association criteria : 5.6≤FPG <7.0 mmol/l).
Results: The prevalence of total diabetes was 4.9% (3.9-6.2) for the total 18-74 years population. The prevalence of diagnosed diabetes (with medication or not) was estimated to 3.9% (3.0-5.1), so the prevalence of undiagnosed diabetes reached 1.0% (0.6-1.7). The prevalence of the treated diabetes by oral or insulin medication was 3.4% (2.5-4.4). The proportion of 18-74 years old adults with impaired fasting glucose was 5.6% (4.3-7.4) according to WHO criteria and 15.5% (13.2-18.1) according to ADA criteria. Prevalences of diagnosed diabetes and impaired fasting glucose were about twofold for men than women (5.4% versus 2.5% and 7.9% versus 3.4% (WHO) or 19.9% versus 11.2% (ADA) respectively), and no difference by sex was observed for the prevalence of undiagnosed diabetes.
Conclusion: On a nationwide basis sample, this study brings a first estimation of the prevalence of the total diabetes (diagnosed, with medication or not, and undiagnosed) and impaired fasting glucose in adults aged from 18 to 74 years living in mainland France. We found only one diabetic person on five was not diagnosed.

292

WITHDRAWN

293

Metabolic syndrome in patients with bipolar disorders and the general population of Spain: findings from a population-based case-control study: the BIMET-VIVA study

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Background and aims: To compare the frequency of Metabolic Syndrome (MS) and its components between patients with Bipolar Disorder (BD) under psychiatric pharmacological treatment with the general population in Spain.
Materials and methods: The BIMET study enrolled during a 12-month period 532 (321 females-60.3%), consecutive unselected patients (mean age 46.3±13 years), according to DSM-IV TR criteria, from several mental health centres across Spain. The VIVA Study is a multicentre (9 sites), population-based, epidemiological study investigating the Variability of Insulin with Visceral Adiposity (VIVA). Both studies were concurrent in time and performed between 2007-2008. A random sample of 1560 individuals (three controls per case), matched by age (mean age 47.6±9.4 years) and sex, were selected from the VIVA data set (n=2.828). Fasting blood glucose (FBG), HDL-cholesterol, triglycerides, BMI, waist circumference and blood pressure were measured in both study groups. The National Cholesterol Educational Program NCEP/ATP III and the International Diabetes Federation (IDF) definitions were used for MS. Bivariate and multivariate comparisons (conditional logistic regression for matched case-control studies) were performed.
Results: Overall, the prevalence of MS-NCEP was 25.1% (95%CI 21.1-29.5) in BD patients and 19.3% (95%CI 17.4-21.4) in controls (p<0.05). Corresponding figures for IDF were 34.8% (95%CI 30.1-39.6) vs. 29.8% (95%CI 27.5-32.1) (p<0.05). Gender-related differences between BD patients and controls were only observed in males (28.5% vs 16.8% for NCEP and 40.2% vs. 26.4% for IDF) (all p values <0.001). Among BD patients, males showed a higher frequency of MS than females (28.5% vs. 23.0% by the NCEP criterium and 40.2% vs. 30.9% by the IDF). There was a higher prevalence of IDF-abdominal obesity in the Spanish female population 78.7% in BD and 66.2% in controls) compared with male population (64.7% in BD and 49.8% in controls). BD patients, males or females, had also higher values than controls in BMI 28,2±5,4 vs. 27,6±4,5 (p=0.02); waist circumference: 96±17,1cm vs.88,4±11,5cm (p<0.001); triglycerides: 145,4±102,2 mg/dl vs.116,8±81,4 mg/dl (p<0.001); and systolic blood pressure in males:129±14,6 mmHg vs.125,9±19,2 mmHg (p=0.02). Logistic regression identified the waist circumference and triglycerides as the two main independent variables explaining the higher frequency of MS observed in BD patients than in controls.
Conclusion: One out of three BD patients under psychiatric pharmacologic treatment in Spain, have MS according to the IDF criteria. This is significantly higher than in the general population, where the prevalence of abdominal obesity is already very high, particularly in women. The components of the MS which better explain the observed higher prevalence of MS in BD patients than in the general population, are waist circumference and triglycerides.
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294

Changes in life expectancy of people with type 2 diabetes relative to non-diabetics over the last 40 years, from Reykjavik-AGES Study

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Background and aims: People with type 2 diabetes (T2D) have shorter life expectancy than non-diabetics. Recent studies have suggested that life expectancy of people with T2D has improved relative to non-diabetics. We examine changes in relative mortality of people with T2D in two age categories over a time span of 40 years.
Materials and methods: Participants from the Reykjavík Study in 1967-1996 and the AGES-Reykjavik Study in 2002-2005 were divided into four cohorts. Two middle aged cohorts, one from 1972 (n=9972), mean age 53 years, and the other from 1983 (n=4760), mean age 56 years, were followed for 20 years. Similarly two cohorts of elderly people were followed, one for 5.5 years from 1993 (n=1599), mean age 78 years, and the other for 3.5 years from 2003 (n=4952), mean age 76 years. Age and gender adjusted hazard ratio (HR) of dying from all causes and from cardiovascular disease (CVD) was estimated for people with T2D and compared to non-diabetics using Cox survival analysis
Results: For middle aged diabetics the HR of dying from any cause compared to non-diabetics was 1.64 (95%CI= 1.45-1.86) in 1972, and 1.58 (95%CI= 1.31-1.89) in 1983. For the elderly diabetics the calculated HR of dying from any cause compared to non-diabetics was 1.71 (95%CI= 1.31-2.22) in 1993, and 1.60 (95%CI= 1.30-1.98) in 2003. From 1972 to 1983 the decline in HR of middle aged diabetics of dying from all causes was 3.7%. From 1993 to 2003 decline in HR in elderly diabetics of dying from all causes was 3.5%. The data show a gradual decrease in relative mortality for diabetics compared to non-diabetics over a long time period, although not statistically significant. Similar trends are seen in HR of diabetics dying from CVD.
Conclusion: Since 1967 the general increase in life expectancy of the Icelandic population has also benefitted people with type 2 diabetes. However, the observed small decline in relative mortality and CVD rate for diabetics over the study period still shows a persistent difference in life expectancy between people with T2D and non-diabetics.
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295

Socioeconomic position and incidence of LADA and type 2 diabetes - 11-year follow-up of the Nord-Trøndelag Health Study

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Background and aims: It has been shown that type 2 diabetes is more prevalent in lower socioeconomic groups. Conversely, an increased risk of type 1 diabetes has been seen in children from families with a high socioeconomic position. The aim of this study was to investigate whether the risk of latent autoimmune diabetes in adults (LADA) also is associated with socioeconomic position, and if so, whether the association can be explained by traditional risk factors, such as overweight, smoking and physical inactivity.
Materials and methods: We used information from an 11 year follow-up of the population-based Nord-Trøndelag Health Study. In this cohort (n=38,725), 86 incident cases of LADA (anti-GAD positive and insulin independent at diagnosis), and 879 incident cases of type 2 diabetes were identified. Socioeconomic position was measured by self-reported information on education and occupation.
Results: High education (university level) was associated with an approximately two times increased risk of LADA (RR 1.9, 95% CI 1.1-3.6), in comparison with low education (primary school). Adjustment for lifestyle and psychosocial factors strengthened this association (RR 2.7, 95% CI 1.4-5.2). Conversely, the risk of type 2 diabetes was decreased in subjects with a high educational level (RR 0.6, 95% CI 0.5-0.9). This risk reduction was explained by differences in lifestyle and psychosocial situation, and primarily overweight (adjusted RR 1.0, 95% CI 0.7-1.3). Similar results were seen when socioeconomic position was measured by occupation, i.e. a high occupational position was associated with an increased risk of LADA and a reduced risk of type 2 diabetes.
Conclusion: This is the first study to indicate that the risk of LADA is increased in subjects with a high socioeconomic position. On the other hand, these subjects had a reduced risk of type 2 diabetes, which was explained by a healthier lifestyle in general, with lower prevalence of overweight, smoking and physical inactivity. The association between LADA and a high socioeconomic position was not explained by traditional risk factors, indicating that some other environmental or lifestyle factors must be involved. The similarity of the results to previous findings for type 1 diabetes suggests that the explanation includes factors related to the autoimmune process.
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296

The metabolic changes and diabetic complications in the Chinese patients with newly diagnosed type 2 diabetes

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Background and aims: To evaluate biochemical characteristics and the change trend of diabetic complications in the Chinese patients with newly diagnosed type 2 diabetes from 1994 to 2008
Materials and methods: We utilized the database of the diabetes complications assessment and analysed the metabolic disorder and the diabetic complications change in the patients with newly diagnosed diabetes.
Results: 2085 cases were collected, including male 1189 and female 896. The average age was significantly younger, 51.6 $\pm$ 13.1 vs. 54.6 + 7.9 yrs (2008 vs. 1994). No case with the age 20-29 yrs and 5% of the patients with age 30-39 in 1994, but 2% with the age 20-29 and 16% cases with the age 30-39 yrs in 2008. BMI was increased from the 24.48 $\pm$ 4.15 in 1994 to 26.03 $\pm$ 3.63 in 2008. Abnormal percentage of the patients with BMI ≥25kg/m^2, WHR ≥ 0.90 (male) or ≥ 0.85 (female) increased significant from 63.6 %, 75.0% and 71.4% in 1994 to 79.6 %, 95.2 % and 93.8 % in 2008. Both SBP and DBP were not significantly changed. The fasting blood and postprandial blood glucose,HbA1c decreased from 10.3 mmol/L, 15.2 mmol/L, 11.1% in 1994 to 9.0 mmol/L and 14.3 mmol/L, 8.6% in 2008, respectively. The average TG level increased from 1.7 mmol/L in 1994 to 2.1 mmol/L in 2008, however, TC and HDL level were no significant changed. The prevalence of patients with diabetic retinopathy decreased from 28.2%, 25.0%, 39.2%, 28.9%, 14.9%, 8.4%, 11.1%, 6.1%, 11.2%, 8.4%, 4.5%, 4.0%, 4.1%, 3.5%, 3.9% from 1994 to 2008. The prevalence of diabetic nephropathy increased from 17.7% in 1994 to 24.6% in 2008. The prevalence of diabetic cardiovascular disease increased from 14.3% in 1994 to 24.1% in 2008. Compared with the patients without microvascular complications, the patients with microvascular complications had higher SBP, DBP and HbA1c (136/78 vs. 130/77 mmHg, 9.41% vs. 9.11%). The patients with macrovascular complications had higher age, SBP, TC and TG than those without macrovascular complications (53.4 vs. 50.0 yrs; 132 vs. 129 mmHg; 5.3 vs. 5.1 mmol/L and 2.6 vs. 2.1 mmol/L).
Conclusion: The younger percentage of the newly diagnosed diabetic patients in china is bigger. The macrovascular factors impacts these patients, such as obese and hyper TC and TC levels, higher systolic blood pressure, even if the prevalence of diabetic retinopathy decreased significantly.
We thank the Beijing capital medical development grant for supporting this study

297

Sex and all-cause mortality in hospitalized patients with type 2 diabetes mellitus

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Background and aims: Many clinicians believe that diabetes mellitus exerts an excess risk of death among those patients in need of hospitalization for any reason. Women with diabetes in particular seem to suffer an excess risk of death due to cardiovascular risk factors, a risk that has not been adequately studied for patients hospitalized for other complications such as an infection.
Materials and methods: A retrospective cohort study was conducted, comparing all patients with any common infection hospitalized in our clinic during a decade, (1990-2000), to matched (for age and sex) patients hospitalized for any reason other than infection. The risk ratios of death according to sex, as well as that attributable to diabetes mellitus and to infectious disease, between those with and without diabetes were calculated, using multiple logistic regression analysis models.
Results: The cohort consisted of 4959 patients, 2583 hospitalized for any common infection (491(19.01%) with diabetes and 2092 without) and 2376 patients, matched for age and sex, hospitalized for any reason other than infection (337(14.18%) with diabetes and 2039 without). There were 96(11.59%) deaths among the 828 diabetic patients and 406(9.83%) deaths among the 4131 non-diabetic patients. The overall risk of death, for patients hospitalized for any reason, did not differ between patients with and without diabetes (OR=0.91 95% CI 0.53-1.56). There were 36(11.80%) deaths among 305 diabetic men and 60(11.47%) deaths among 523 diabetic women. The risk of death did not differ between diabetic and non-diabetic patients according to sex (interaction OR=1.58 95% CI 0.94-2.66). There were 174(14.77%) deaths among 1178 men and 149(10.60%) deaths among 1405 women hospitalized with any common infection. The risk of death did not differ between diabetic and non-diabetic patients with concomitant infection (interaction OR=0.95 95% CI 0.61-1.48), after sex was taken into account.
Conclusion: Diabetes mellitus did not seem to exert an excess risk of dying for those patients in need of hospitalization for any reason, a result that did not differ even for diabetic patients with concomitant complications, such as any common infection. Women with diabetes do not seem to suffer an excess risk of dying when in need of hospitalization for any reason compared to their men counterparts.

298

Is diabetes mellitus linked to an increased risk of cancer?

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Background and aims: Some studies have shown an association between obesity, hiperinsulinemia, insulin resistance and type 2 diabetes and an increased risk of developing certain kinds of cancer. It is our aim to evaluate the prevalence of cancer in diabetic and nondiabetic patients admitted to a central hospital in the north of Portugal between 1999 and 2008.
Materials and methods: We retrospectively analysed data from all inpatients that satisfied the International Classification of Diseases (9th version) criteria for cancer disease and diabetes between 1999 and 2008. Statistical analysis was performed with Student's t-test. A two-tailed p value < 0.05 was considered significant.
Results: During the period of the study there were a total of 980128 admissions, 4743 of which with the diagnosis of diabetes mellitus and cancer, and

23032 with cancer without diabetes. They were 20092 men and 14867 women with a mean age of 61,9 years old in the group of diabetic patients with cancer, and 20092 men and 14867 women with a mean age of 54,1 years old in the nondiabetic group with cancer. We found no positive correlations between diabetes and cancer. Instead, there were positive correlations between nondiabetic status and cancer. Prevalence of ear, nose, mouth and throat cancers (2.43% vs 2.47%; r=0.848, p=0.002), respiratory system cancers (2.34% vs 2.56%; r=0.708, p=0.033), digestive malignancy (1.78% vs 2.43%; r=0.763, p=0.017), bone and joint malignancies (2.39% vs 2,51%; r=0.805, p=0.009), breast cancer (2.44% vs 2.47%; r=0.942, p=0.000), kidney and urinary tract cancers (1.8% vs 2.5%; r=0.736, p=0.024), male reproductive system cancers (1.5% vs 2.4%; r=0.881, p=0.001) and female reproductive system cancers (2.59% vs 7.02%, r=0,888, p=0.001) was significantly higher in nondiabetic than in diabetic patients. There were no significant differences in the prevalence of nervous system, hepatobiliar and pancreatic malignancies in diabetic and nondiabetic patients.
Conclusion: Unlike other studies, we did not find any positive correlation between diabetes mellitus and cancer. Although many issues may be responsible for these results, we also think that it is possible that the more recent advised use of insulinsensitizers, such as metformin or glytazones, could be responsible for the mitigation of our results.

299

New onset diabetes mellitus after kidney transplantation in Denmark
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Background and aims: To investigate the development of new onset diabetes mellitus (NODM) in a prospective study of 89 non-diabetic patients on the Danish waiting list for kidney transplantation.
Materials and methods: Included were 48 kidney recipients (Tx-group, age 39±13 years, males/females 33/15). 41 uremic patients on the waiting list for kidney transplantation served as controls (controls, age 47±11 years, males/females 29/12). All were examined at baseline before transplantation and after 12 months. The prevalence of diabetes, pre-diabetes, insulin sensitivity index (ISI) and insulin secretion index (ISecr) were estimated, using an oral glucose tolerance test with measurements of plasma-glucose and -insulin.
Results: NODM was present in 13% (6/48, p=0.03) at one year after transplantation when 44 % received tacrolismus, 56 % ciclosporin, 94 % prednisolon (average 6 mg) and 96 % mycophenolate mofetil. Only one patient in the control group developed NODM. At baseline 27% had prediabetes in the Tx-group. ISI in the Tx-group deteriorated from 7.4±4 to 5.3±3 at 0 and 12 months after transplantation (p=0.005). In the controls, ISI was 8.2±5 and 8.5±5 (NS). The ISecr improved in the Tx-group compared with controls, (p=0.01 between groups). Within groups, ISecr in the Tx-group changed from 35 ±19 to 42±25 between 0 to 12 months (p=0.06) compared with 30 ±17 to 27±15 in controls (NS).
Conclusion: One year after kidney transplantation NODM was present in 13%. This was mainly due to a further increase in insulin resistance and was observed despite of an improved insulin secretion.
Supported by: The Danish Kidney Foundation, Ejner and Helen Bjørnow Foundation and Rigshospitalet fellowship

PS 6 Epidemiology of cardiovascular disease

300

Cardiovascular risk factors in the Netherlands over 17 years: results from the Hoorn Study and the New Hoorn Study
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Background and aims: Since repeated data on the level of cardiovascular risk factors from epidemiological studies are lacking, little is known on the changes in cardiovascular risk factors in the general population of the Netherlands. Therefore, our aim was to describe the changes in blood glucose, lipid profile, blood pressure and adiposity over 17 years in the Netherlands.
Materials and methods: Data from two random samples of the Dutch population (50-65 years old) were used: 1592 participants from the Hoorn Study (1989) and 1718 participants from the New Hoorn Study (2006). Fasting plasma glucose (FPG), plasma glucose 2 hours after an oral glucose tolerance test (OGTT) with a 75 g glucose load (2hrPG), HbA1c, cholesterol (total, HDL and LDL) and triglycerides were determined. In addition, weight, height, systolic blood pressure, diastolic blood pressure and waist circumference were measured.
Results: Between 1989 and 2006, mean HbA1c increased significantly in both sexes, while mean 2hrPG increased in men only and no change was observed in the prevalence of diabetes (Table 1). Significantly better mean levels of cholesterol (lower total and LDL cholesterol, higher HDL cholesterol) and triglycerides were present in 2006. Mean systolic blood pressure increased significantly, especially in men. Mean body mass index (BMI) and waist circumference showed a significant increase in men only.
Conclusion: A significant increase in mean HbA1c, 2hrPG, systolic blood pressure, BMI and waist circumference was observed between 1989 and 2006 in the general 50-65-year old population of the Netherlands, especially in males. In contrast, lipid profile became more favourable in both sexes and no change was observed in the prevalence of diabetes.

Table 1.

	TOTAL		MALES		FEMALES	
	HS 1989	**NHS 2006**	**HS 1989**	**NHS 2006**	**HS 1989**	**NHS 2006**
N (%)	1592	1718	755 (30.4)	821 (32.9)	837 (33.7)	897 (36.0)
Age (years)	57.2 (4.2)	57.0 (4.3)	57.0 (4.2)	57.3 (4.3)	57.3 (4.3)	56.8 (4.3)*
FPG (mmol/l)	5.7 (1.4)	5.7 (1.0)	5.8 (1.2)	5.8 (1.1)	5.6 (1.6)	5.5 (0.9)
2hrPG (mmol/l)	5.7 (2.7)	6.0 (2.5)*	5.7 (2.6)	6.1 (2.8)*	5.8 (2.7)	5.9 (2.3)
HbA1c (%)	5.4 (0.8)	5.5 (0.5)*	5.4 (0.7)	5.5 (0.6)*	5.4 (0.8)	5.5 (0.5)*
Diabetes (%) (FPG ≥ 7.0 mmol/l and/or 2hrPG ≥ 11.1 mmol/l or known diabetes)	7.8	7.9	8.8	9.1	6.9	6.7
Total cholesterol (mmol/l)	6.6 (1.2)	5.7 (1.0)*	6.5 (1.1)	5.4 (1.0)*	6.8 (1.2)	5.7 (1.0)*
HDL cholesterol (mmol/l)	1.3 (0.4)	1.5 (0.4)*	1.2 (0.3)	1.4 (0.3)*	1.4 (0.4)	1.7 (0.4)*
LDL cholesterol (mmol/l)	4.6 (1.1)	3.4 (0.9)*	4.5 (1.0)	3.3 (0.9)*	4.7 (1.2)	3.5 (0.9)*
Triglycerides (mmol/l)#	1.6 (1.1)	1.5 (1.0)*	1.7 (1.1)	1.6 (1.2)*	1.5 (1.0)	1.4 (0.7)*
Systolic blood pressure (mmHg)	130.9 (18.4)	135.5 (18.2)*	131.8 (17.0)	137.4 (16.5)*	130.2 (19.6)	133.7 (19.4)*
Diastolic blood pressure (mmHg)	82.1 (10.2)	77.6 (10.3)*	83.8 (9.3)	80.5 (9.8)*	80.7 (10.7)	75.4 (10.3)*
BMI (kg/m^2)	26.4 (3.5)	26.4 (3.9)	26.2 (3.0)	26.7 (3.5)*	26.5 (3.9)	26.2 (4.2)
Waist (cm)	90.1 (10.8)	90.9 (11.4)*	94.7 (10.0)	95.7 (10.0)*	86.0 (10.7)	86.5 (10.9)

Data are presented as mean (standard deviation) or percentage
* $p \leq 0.05$
# log transformed before testing

301

Central obesity and cardiovascular risk factors in south Asians and white Europeans with type 2 diabetes. A report from the United Kingdom Asian Diabetes Study (UKADS)

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Background: The relation between waist circumference (WC) and cardiovascular risk factors (CVD-RF) has not been well-studied in South Asians (SA) with type 2 diabetes (T2D).
Aims: To assess and compare CVD-RF between SA and White Europeans (WE) with T2D at different degrees of central obesity.
Methods: A cross-sectional study. SA and WE, above 35 years of age, with T2D from the UKADS were included. CVD-RF defined as hypertension (HTN) (BP>140/80mmHg or the use of anti-hypertensives), hypercholesterolemia (total cholesterol>4mmol/l or on lipid lowering treatment) and/or nephropathy (ACR>2.5 and 3.5 for men and women respectively). WC was divided into 5 groups in women (≤80cm, 80.01-90, 90.01-100, 100.01-110, >110, categories 1-5 respectively) and men (≤90cm, 90.01-100, 100.01-110, 110.01-120, >120, categories 1-5 respectively). Data presented as medians or frequencies
Results: 1486 SA and 492 WE (52% and 58% males respectively). Participants' characteristics: SA vs. WE: age (56 vs 66 years. P<0.001), BP-systolic (139vs 142mmHg, p<0.001), BP-diastolic (83 vs 83 mmHg, p=0.2), LDL (2.3 vs 1.9mmol/l, p<0.001), HDL (1.2 vs 1.4 mmol/l, p<0.001), Trigs (2.49 vs 2.03 mmol/l, p<0.001), HbA1c (7.9 vs 6.9%, p<0.001), diabetes duration 6 vs 4 years, p<0.001), HTN (83 vs 93%, p<0.001), hypercholesterolemia (90 vs 91%, p=0.4), nephropathy 27 vs. 26%, p=0.4). CVD-RF in SA and WE in relation to WC categories is summarised in Table 1 (data presented as medians). The prevalence of HTN, hypercholesterolemia or nephropathy was not different between SA and WE across WC categories. The differences in HDL, trigs and HbA1c could not be accounted by differences in medications between groups. WE were on more statins than SA. However, linear regression showed that ethnicity is independently related to LDL levels (B: 0.13, p=0.003) in a model that includes all anti-lipid and anti-diabetic therapy.
Conclusions: In T2D, SA had higher LDL, HbA1c, Trigs and lower HDL levels compared to WE with a similar WC despite being younger. Aggressive CVD-RF management in this group is needed. Despite having higher LDL levels, fewer SA were prescribed statins.

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CV risk factors in South Asians (SA) and White Europeans (WE) in regard to waist circumference (WC).

WC category	CVD-RF	Women			Men		
		SA	WE	P	SA	WE	P
1	BP-systolic	136	136	0.6	138	134	0.7
	BP-diastolic	76	77	0.7	82	78	0.003
	LDL	3.9	2.6	0.4	2.4	2	0.2
	Trigs	1.9	1.1	0.1	2.1	0.9	<0.001
	HDL	1.1	2.4	0.003	1.2	1.6	<0.001
	HbA1c	8.4	7.1	0.08	8.1	6.4	<0.001
2	BP-systolic	134	146	0.09	140	143	0.01
	BP-diastolic	81	80	0.4	83	82	0.9
	LDL	2.5	2.0	0.03	2.3	1.9	0.03
	Trigs	1.8	1.6	0.3	2.1	1.4	<0.001
	HDL	1.3	1.7	<0.001	1.1	1.4	<0.001
	HbA1c	7.8	6.4	<0.01	8	6.9	<0.001
3	BP-systolic	134	140	0.1	141	146	0.008
	BP-diastolic	82	85	0.4	85	85	0.7
	LDL	2.9	1.7	<0.001	2.3	1.7	<0.001
	Trigs	2	1.7	0.009	2.2	2	0.2
	HDL	1.3	1.6	<0.001	1.1	1.3	0.002
	HbA1c	8	6.7	<0.001	7.6	6.7	<0.001
4	BP-systolic	138	141	0.2	145	144	0.5
	BP-diastolic	83	80	0.06	86	82	0.2
	LDL	2.4	1.9	0.003	2.1	1.7	0.049
	Trigs	2.2	2	0.4	2	2.1	0.9
	HDL	1.3	1.4	0.001	1.1	1.2	0.4
	HbA1c	7.9	6.9	<0.001	7.9	7	0.004
5	BP-systolic	136	139	0.3	140	141	0.6
	BP-diastolic	84	86	0.7	86	84	0.6
	LDL	2.2	1.9	0.1	2.3	2.2	0.6
	Trigs	2.2	1.9	0.01	1.8	2	0.9
	HDL	1.2	1.4	<0.001	1.1	1.1	0.5
	HbA1c	8	7.2	<0.001	7.3	7	0.4

302

Changes in all-cause and CVD mortality in three Finnish middle-aged population cohorts with and without diabetes during a 10-year follow-up

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Background and aims: When diabetes is increasing in many populations, it may result in a leveling off of the decrease in all-cause and cardiovascular disease (CVD) mortality. This may be called 'diabetes-cardiovascular risk paradox'. The objective of this study was to assess changes in all-cause mortality rates among people with and without diabetes between three large study cohorts with baseline assessments 10 years apart and followed up for 10 years.
Material and methods: Six population surveys were carried out in 1972, 1977, 1982, 1987, 1992 and 1997 in randomly selected independent cohorts in North Karelia and Kuopio, Eastern Finland. For the analyses the 1972 and 1977 cohorts (cohort 1), the 1982 and 1987 cohorts (cohort 2) and the 1992 and 1997 cohorts (cohort 3) were combined and followed-up for 10 years regarding all-cause and CVD mortality. The patients developing incident dia-

Table 1. Multiple adjusted[1] Hazard Ratio (HR) of cardiovascular disease (CVD) mortality among men and women by diabetes status and by age group.

	Cohort 1			Cohort 2			Cohort 3		
	Deaths	(HR)	95% CI	Deaths	(HR)	**95% CI**	**Deaths**	**(HR)**	**95% CI**
Men									
No diabetes									
All	417	1.0	Ref	117	0.75	0.60-0.93	26	0.37	0.25-0.57
25-49 years-old	157	1.0	Ref	27	0.75	0.49-1.15	7	0.52	0.24-1.15
50-64 years old	260	1.0	Ref	90	0.76	0.59-0.98	19	0.32	0.20-0.53
Diabetes[2]									
All	31	1.0	Ref	12	0.61	0.30-1.23	3	0.25	0.07-0.87
25-49 years-old	9	1.0	Ref	0	-	-	1	0.33	0.04-3.12
50-64 years old	22	1.0	Ref	2	0.76	0.35-1.65	2	0.22	0.05-1.03
Women									
No diabetes									
All	117	1.0	Ref	27	0.60	0.38-0.93	7	0.31	0.14-0.70
25-49 years-old	33	1.0	Ref	4	0.45	0.15-1.32	2	0.49	0.11-2.28
50-64 years old	84	1.0	Ref	23	0.57	0.35-0.92	5	0.24	0.09-0.62
Diabetes[2]									
All	32	1.0	Ref	23	1.50	0.84-2.68	1	0.14	0.02-1.04
25-49 years-old	2	1.0	Ref	3	8.34	0.77-90.480		-	-
50-64 years old	30	1.0	Ref	20	1.33	0.72-2.45	1	0.13	0.02-1.04

[1]adjusted for age, education, smoking, systolic blood pressure, serum cholesterol and BMI
[2]baseline diabetes or diabetes during 10-year follow-up

betes during the follow-up or having diabetes at baseline were derived from the national drug reimbursement records of the Social Insurance Institution and the hospital admission records of the National Hospital Discharge Register by computer-based record linkage.
Results: A total of 17 361 men and 18 707 women were followed up for 10 years. The risk of all-cause mortality decreased in cohort 3 compared to the first two cohorts in both men and women who did not develop type 2 diabetes during the follow-up. CVD mortality decreased in cohort 2 and 3 compared to cohort 1 in both gender. In men with diabetes, all-cause mortality did not significantly change during the follow-up. The Hazard Ratio (HR) for all-cause mortality after adjustment for age, education, smoking, systolic blood pressure, BMI and serum cholesterol was 0.84 in cohort 2 (95% CI 0.51-1.38) and 0.57 in cohort 3 (95% CI 0.28-1.18). No statistically significant changes regarding all-cause mortality were observed in women in cohort 2 (HR 1.54; 95% CI 0.96-2.49) or cohort 3 (HR 0.59; 95% CI 0.23-1.49), either. The respective HRs for CVD mortality was 0.61 (cohort 2; 95% CI 0.3-1.23) and 0.25 (cohort 3; 95% CI 0.07-0.87) in men and 1.50 (cohort 2; 95% CI 0.84-2.68) and 0.14 (cohort 3; 95% CI 0.02-1.04) in women.
Conclusions: While all-cause and CVD mortality seems to decrease in the non-diabetic segment of the population, no changes have been observed in women with diabetes. Only men of the latest cohort had a reduced CVD mortality rate. Special attention should be given to prevent the onset of diabetes in the population and to intensify the management of patients with diabetes.
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303

Impact of quality of HbA_{1c} control and changes in HbA_{1c} level on cardiovascular events in a population based study with type 2 diabetes: The Diabetes In Germany (DIG) Study
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Background and aims: Significance of HbA1c control in patients with long-term diabetes is still a matter of debate. So far little is known on significance of HbA1c and its change in late phases of the disease as independent risk factor in populations with HbA1c in the range of 7%.
We therefore analyzed incidence of major cardiovascular events (MACE) (1) in relation to established targets of HbA1c and (2) in relation to change over follow-up.
Material and methods: 4020 unselected patients with type 2 diabetes aged 35-80 years from 238 sites, follow-up 3.7 years, 2959 completed the study or died (n=175, 5.98%), 2213 (89.8%) had no MACE at baseline. MACE was history of myocardial infarction and/or coronary revascularisation and/or stroke. Change of HbA1c was greater or equal +0.3% or lower or equal -0.3% from HbA1c at baseline versus stability: no change in the interval ±0.3%.
Results: Average HbA1c at baseline was 7±1.2%, after 3.7 years 7.03±1.08%, diabetes duration 8.4 years. 251 (10.2%) patients reported a first event during follow-up. 845 patients had HbA1c lower than 6.5% with an event rate of 6.9%. The corresponding figures for HbA1c between 6.5% and 7% were 463 (7.2%), for HbA1c between greater or equal than 7 and 8% 550 (7.3%) and for HbA1c greater or equal than 8% 354 (9%). Change was associated with a significantly higher event rate: greater than +0.3% 8.1% events, lower than -0.3% 7.9% vs. stability (4.9%, p=0.03). In stepwise regression analysis with major risk factors age, smoking, male sex and HbA1c change in both directions were independent predictors of newly reported cardiovascular events.
Conclusions: In a German population based cohort of patients with average HbA1c of 7% level of diabetes control had no effect on cardiovascular event rate. However, change of HbA1c in both directions was associated with a significantly increased cardiovascular risk. Event rate was in the same range whether IDF or ADA target level was applied.
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304

Prediciton of recurrent cardiovascular events in a general, pre-diabetic and diabetic population. The Hoorn Study
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Background: Several prediction formulas exist to estimate the risk of a first cardiovascular event in a general population as well as in a population with type 2 diabetes. Characteristics such as blood pressure, cholesterol, smoking status and diabetes are known predictors for a first cardiovascular event, with addition of glycemic level (HbA_{1c}) in the diabetes population. Because of the increase in obesity and diabetes prevalence worldwide, and improved care after a first cardiovascular event, the number of people at risk for a recurrent cardiovascular event is growing. Less is known about predictors of a recurrent cardiovascular event in the general population, in a population with intermediate hyperglycemia or persons with type 2 diabetes. The aim of this study is to investigate 1) differences in absolute risk of a recurrent event between persons with normal glucose tolerance, intermediate hyperglycemia, and type 2 diabetes, and 2) which characteristics are predictors of a recurrent cardiovascular event in the general population, and in persons with intermediate hyperglycemia or type 2 diabetes.
Methods: The Hoorn study is a population-based cohort study among 2484 men and women, aged 50-75 at the baseline examination in 1989. Of these, 515 participants had a history of cardiovascular disease at baseline or developed a first event during 10-years of follow-up (ICD-codes: 390-459), and were at risk for a recurrent event. We identified significant predictors of a recurrent cardiovascular event during follow-up (ICD-codes: 390-459, 789) by Cox proportional hazards models using stepwise backward strategy.
Results: During a mean of 5.3 years of follow-up, 176 (34%) second cardiovascular events had occurred. In the total population, male sex, increasing age, higher level of triglycerides and higher glycemic level predicted a recurrent cardiovascular event. When stratified by glucose status, a recurrent event occurred in 31% of persons with normal glucose tolerance and in 34% of persons with intermediate hyperglycemia. The highest percentage of recurrent

Table. Predictors of a recurrent cardiovascular event stratified by glucose status group

General population		HR	p-value
Male sex		1.59 (1.16 - 2.16)	0.004
Age (years)		1.06 (1.03 - 1.09)	<0.001
Triglycerides (mmol/L)		1.26 (1.09 - 1.45)	0.002
HbA_{1c} (%) (continuous)		1.16 (1.01 - 1.32)	0.030
Normal glucose tolerance			
Male sex		1.54 (1.02 - 2.30)	0.038
Age (years)		1.07 (1.04 - 1.10)	< 0.001
Triglycerides (mmol/L)		1.31 (1.02 - 1.67)	0.032
Intermediate hyperglycemia			
HbA_{1c} (%)	≤ 5.5	1 (referent)	0.030
	> 5.5	2.24 (1.08 - 4.65)	
Male sex		2.76 (1.34 - 5.69)	0.006
Triglycerides (mmol/L)		1.50 (1.17 - 1.93)	0.001
Type 2 diabetes			
HbA_{1c} (%)	≤ 6.0	1 (referent)	0.026
	6.0 - 7.0	3.01 (1.14 - 7.91)	0.008
	>7.0	3.11 (1.35 - 7.19)	
Total/HDL cholesterol ratio		1.17 (1.00 - 1.38)	0.054
Intermediate hyperglycemia or type 2 diabetes			
HbA_{1c} (%)	≤ 6.0	1 (referent)	0.031
	6.0 - 7.0	1.87 (1.06 - 3.31)	0.001
	>7.0	2.62 (1.48 - 4.63)	
Total/HDL cholesterol ratio		1.16 (1.04 - 1.28)	0.006

cardiovascular events was observed in the population with type 2 diabetes (46%). In participants with intermediate hyperglycemia or type 2 diabetes, glycated hemoglobin was a main predictor.
Conclusions: The results of this study suggest that risk factors for a recurrent cardiovascular event differ from the known risk factors for a first event. Furthermore, the predictors for the population with intermediate hyperglycemia or type 2 diabetes were different compared to subjects without glucose abnormalities. Glycemic level was an important predictor in the general population as well as in persons with intermediate hyperglycemia or type 2 diabetes.

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305

Association between the metabolic syndrome and coronary artery disease or ankle-arm index: mediation by low-grade inflammation and/or endothelial dysfunction (the CODAM study)
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Background: The Metabolic Syndrome (MetS) is associated with both coronary artery disease (CAD) and peripheral arterial disease and these associations may be related to low-grade inflammation and/or endothelial dysfunction.
Aim: To address the role of low-grade inflammation and endothelial dysfunction in the associations between the metabolic syndrome and CAD or ankle-arm index (AAIx; an available and reproducible method for the detection of peripheral arterial disease).
Methods: The study population consisted of 574 subjects (age: 59.6 ± 7.0 years, 61.3% men, 54.7% MetS; NCEP-ATP 2005 criteria) from the Cohort Study Diabetes and Atherosclerosis (CODAM) cohort. Presence of CAD was assessed by extensive questionnaires and ECG. The AAIx was calculated by dividing the highest of the ankle pressures (dorsalis pedis or tibial arteries) in either leg by the highest pressure at the brachial artery. The following biomarkers markers were measured in fasting plasma: (1) markers of inflammation (C-reactive protein, IL6, serum amyloid A, ceruloplasmin, haptoglobin, soluble intercellular adhesion molecule) and (2) markers of endothelial dysfunction (soluble vascular cell adhesion molecule, e-selectin, von Willebrand factor). Each biomarker was transformed into a Z-score and an inflammation score and an endothelial score were then calculated as the average of the Z-scores of the six inflammation markers and as the average of the three markers of endothelial dysfunction, respectively. Multiple logistic and linear regression analyses were used to determine the associations of the metabolic syndrome, low-grade inflammation and endothelial dysfunction with CAD or AAIx (outcomes), adjusted for age, sex and smoking.
Results: The MetS was associated with CAD (OR (95% CI) = 1.75 (1.14-2.69), p=0.011). Adjusting this association for the inflammation score, the strength of this association was reduced by 26%, but adjustment for endothelial dysfunction did not affect the strength of the association (attenuation <6%).
The MetS was also associated with AAIx (β (95% CI) = -0.036 (-0.056; -0.016), p<0.001). Adjusting for the inflammation score reduced the strength of this association by 28% and for endothelial dysfunction the strength of this association was reduced by 19%. When low-grade inflammation and the endothelial score were included simultaneously into the model, the strength of the association was reduced by 36% (β (95% CI) = -0.023 (-0.004; -0.002), p=0.035).
Conclusion: Low-grade inflammation, but not endothelial dysfunction can partly explain the association between the metabolic syndrome and CAD. On the other hand, both low-grade inflammation and endothelial dysfunction can each independently explain part of the association between the metabolic syndrome and AAIx.
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306

Metabolic syndrome vs Framingham risk score for association of coronary heart disease: the Korean Health and Nutrition Examination Survey, 2005
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Background and Aim: Although many studies have been shown that metabolic syndrome (MS) is associated with significantly increased risk of developing coronary heart disease (CHD), recent several studies in Western population, indicate that MS is inferior to the Framingham Risk Score (FRS), one of traditional risk scoring system, in predicting CHD. However there has been no study about predictability of MS vs. FRS for CHD in Far-East Asian population. We have assessed the relative associations of MS using National Cholesterol Education Program (NCEP) criteria vs. FRS with CHD in national representative cohort of South Korea.
Population and Methods: This study was based on the data obtained from the third Korea National Health and Nutrition examination Survey (KNHANES III) among non-institutionalized civilians in the Republic of Korea, which was conducted by the Korean Ministry of Health and Welfare in 2005. This survey is a nationwide representative study using a stratified, multistage probability sampling design for the selection of household units. A total of 34,145 individuals from these sampling frames were included in the health interview survey; among them, 5,497 persons aged 18 to 99 years were identified as participants in our study, with laboratory test and nutritional survey data. Trained interviewers visited participant's homes and administered a standardized questionnaire on daily life style. The participants were, also, asked to recall a physician's diagnosis of CHD (angina or myocardial infraction). MS was defined by NCEP criteria. The FRS for CHD was calculated for each participants.
Results: Median age in this study population were 46 (18-92) in men (n=2355) and 45 (18-99) years in women (n=3142). Body mass index were 24.0 3.1 in men and 23.5
3.4 kg/m^2 in women. Prevalence of self-reported CHD was 1.7% in men and 2.1% in women. Participants were categorized according to quintiles of FRS in each sex. Receiver-operating characteristics (ROC) curves and their respective areas under the curve (AUCs) were used to compare the ability of the FRS and the number of components of metabolic syndrome to predict CHD in each sex. In men, AUC of FRS was significantly larger than that of MS [0.763 (0.707-0.819) vs. 0.628 (0.542-0.714), p<0.01]. In women, AUC of FRS was comparable to that of MS [0.776(0.728-0.824) vs. 0.734 (0.674-0.795), not significant].
Conclusion: The study showed that FRS was more closely associated with CHD compared to MS in Korean men.

307

Prevalence of cardiovascular disease in people with diabetes with and without schizophrenia: a population-based cohort study
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Background and aims: Individuals with diabetes have a high prevalence of cardiovascular (CV) risk factors and cardiovascular disease (CVD), as do individuals with schizophrenia. It is unclear, however, whether the prevalence of CVD is further increased in people with diabetes and schizophrenia. The objective of this study was to evaluate prevalence of CV risk, CVD, and revascularization procedures in people with diabetes and schizophrenia compared to people with diabetes only.
Materials and methods: A population-based cohort study was used to evaluate the study objective. Information from the databases of Alberta Health and Wellness was used to create the cohort, and included all individuals aged 20 years and older with diabetes in the Canadian province of Alberta from 1995 to 2006. Individuals with diabetes were identified using criteria from the Canadian National Diabetes Surveillance System. Schizophrenia, CV risk (hypertension, dyslipidemia), CVD (congestive heart failure [CHF], stroke, acute coronary syndrome, ischemic heart disease, arrhythmia, old myocardial infarction), and revascularization procedures (coronary artery bypass grafting, percutaneous transluminal coronary angioplasty) were identified using physician claims data, ambulatory care data, and hospitalization data. Prevalence of CV risk, CVD, and revascularization was compared using mul-

tivariable logistic regression analysis while adjusting for age, sex, healthcare subsidy, and number of physician visits (to control for surveillance bias).
Results: We identified 129,438 people with diabetes, and 2,621 (2.0%) of these individuals had schizophrenia. Individuals with diabetes only were older (mean age 60.3 years vs. 57.6 years), more likely to be male (51% vs. 42.9%), and less likely to have subsidized health care (39.6% vs. 73.3%) compared to individuals with schizophrenia and diabetes. After multivariable adjustment, prevalence of dyslipidemia was not significantly different between groups (OR: 0.97; 95% CI: 0.89 - 1.05), and hypertension was less prevalent in people with diabetes and schizophrenia (OR: 0.60; 95% CI: 0.55 - 0.65). Individuals with schizophrenia and diabetes were more likely to have CHF (OR: 1.31; 95% CI: 1.18 - 1.46), stroke (OR: 1.27; 95% CI: 1.14 - 1.42), and CVD (OR: 1.19; 95% CI: 1.10 - 1.30), but were less likely to undergo revascularization (OR: 0.51; 95% CI: 0.41 - 0.63).
Conclusion: Despite having a higher prevalence of CVD, individuals with schizophrenia and diabetes were significantly less likely to undergo revascularization compared to people with diabetes only. Also, prevalence of CV risk factors was lower (hypertension) or no different (dyslipidemia) between groups. This may be due to a lack of screening, or other lifestyle risk factors increasing the risk of CVD in those with schizophrenia and diabetes.
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308

High prevalence of residual dyslipidemia in statin-treated patients with diabetes mellitus in Europe and Canada: results of the Dyslipidemia International Study

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Background and aims: Though statins are considered the essential and most widespread therapy for prevention of cardiovascular disease, statin-treated patients remain at increased cardiovascular risk. This study was designed to better explain the residual risk by assessing the prevalence of persistent lipid abnormalities.
Materials and methods: DYSIS was a cross-sectional study conducted by 2987 general practitioners, endocrinologists/diabetologists, cardiologists and internists in 12 countries. Patients were recruited consecutively who were ≥ 45 years of age, on statin therapy ≥ 3 months, agreed to a clinical exam and had a least one lipid value.
Results: 22, 063 were enrolled between April 2008 and February 2009. Of these, 8613 (39%) had diabetes, with an average age of 66.3 years (± 9.4), 41.2 % female. According to ATP III guidelines 76.5% had metabolic syndrome while the IDF criteria defined 86.8% as having metabolic syndrome. Of the total enrollment, 19,132 had sufficient lipid data to assess dyslipidemia.
Conclusion: Though the patients with DM were more likely to have LDL-C at target, they were less likely to have normal HDL-C and TGs

ESC Guidelines	Patients with DM N = 7558 (39.%%)	Patients without DM N = 11,574 (60.5%)
LDL-C at goal (1)	58.5%	47.5%*
LDL not at goal and:		
HDL-C normal & TG normal	16.2%	29.2%*
Low HDL-C & TG normal (2)	3.2%	3.7%
High TG & HDL-C normal (3)	13.9%	14.1%
Low HDL-C & high TG	8.3%	5.6%*
Low HDL-C &/or high TG	25.4%	23.4%*

LDL-C=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol; TG=triglycerides. (1) ≥ 2.5/2.0 mmol/L (high risk); ≥ 3.0 mmol/L (low risk); (2) < 1.0 mmol/L (male); <1.2 mmol/L (female); (3) > 1.7 mmol/L *statistically significant

Supported by: Merck & Co., Inc.

309

Vitamin D deficiency is associated with type 2 diabetes mellitus and insulin resistance in 3290 patients referred to coronary angiography

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Background and aims: There is accumulating molecular and epidemiological evidence that vitamin D is important for beta-cell function and glucose metabolism. We aimed to evaluate whether 25-hydroxyvitamin D (25[OH]D) is associated with type 2 diabetes mellitus and insulin resistance in patients with an indication for coronary angiography.
Materials and Methods: 25(OH)D was determined in 3290 patients from the LUdwigshafen RIsk and Cardiovascular Health (LURIC) study, a prospective cohort study among patients referred to coronary angiography. We measured fasting glucose and fasting insulin to calculate the homeostasis model assessment of insulin resistance (HOMA-IR). In addition, we performed a standardized 75-gram oral glucose tolerance test in the vast majority of study participants without known diabetes mellitus.
Results: Median 25(OH)D levels in ng/ml (with interquartile range) were 16.8 (11.0-24.1) in patients with normal glucose metabolism (n=1415), 16.4 (10.6-24.1) in patients with impaired glucose metabolism (impaired fasting glucose and/or impaired glucose tolerance) (n=827), 14.9 (10.0-21.2) in patients with newly diagnosed diabetes mellitus (n=476) and 12.7 (8.1-19.3) in patients with previously known type 2 diabetes mellitus (n=572) (P for trend <0.001 for ANCOVA adjusted for age, sex, BMI and season of blood draw). In a linear regression analysis including HOMA-IR as the dependent variable and age, sex, body mass index, season of blood draw and 25(OH)D as explanatory variables we observed a significant inverse association of 25(OH)D and HOMA-IR (β-coefficient = -0.070; p<0.001).
Conclusions: We have shown that a poor vitamin D status is associated with type 2 diabetes mellitus and insulin resistance. These results suggest that vitamin D supplementation might be useful for the prevention and/or treatment of type 2 diabetes mellitus.
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PS 7 Epidemiology of obesity and type 2 diabetes

310

How much do we know about obesity and diabetes? A survey of British male drivers

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Background: Obesity and type 2 diabetes (T2DM) are major public health challenges. Behaviour modification necessary to tackle these conditions requires individual awareness of the existing problem.
Objectives: We assessed adiposity perception, awareness of the relation between adiposity and T2DM, and the relation between adiposity and weight loss attempts.
Methods: A cross-sectional study. British male drivers were randomly recruited at Motorway service stations across the UK between May-July 2007. Participants completed a questionnaire and had body mass index (BMI), waist circumference (WC), body composition and random blood cholesterol and glucose measurements. BMI was classified into normal, overweight and obese based on the WHO criteria. WC >94cm was considered as a marker of central obesity as per the international diabetes federation criteria.
Results: 266 men, median age 52 years and BMI 28.25kg/m^2 participated. Obesity prevalence was 35% and 73% based on BMI and WC, respectively. 46% were overweight based on BMI. Based on the questionnaire 29% of the participants thought they were "thin" or "just right", 64% and 7% thought they were overweight and obese, respectively. There was no difference in age between the self-perception categories (53 vs. 50 vs. 51 years, p=0.35). Participants underestimated their WC (94.3±10.22 vs. 102.86±11.41cm, estimated vs. actual, p<0.001). Of participants with normal BMI, 18% thought they were overweight, while 26% of overweight thought they were "just right" and 19% of obese recognised their obesity. Based on WC, 30% of participants with normal WC thought they were obese and 9% of obese realised they were obese. Only 25% and 42% of participants recognised that T2DM is associated with obesity and large waist, respectively. 81% and 62% of overweight and obese participants (based on BMI) and 71% with central obesity believed that they were **not** at increased risk of T2DM. Adiposity self perception weakly predicted weight loss attempts (Lambda 0.28, p=0.008). Participants' BMI and WC did not predict attempts to lose weight (p=0.12, p=0.2 respectively). 43% and 18% who were either overweight or obese (based on BMI) and 33% with central obesity had not attempted to lose weight. On the other hand, 51% of normal subjects (based on WC) have attempted to lose weight. The majority of patients who thinks their weight is normal but who are overweight/obese based on BMI and/or WC did not attempt to lose weight. In contrast, individuals who perceived themselves to be overweight/obese, have attempted to lose weight even though they fall into the normal range based on BMI/WC. 92% of participants with central obesity and 97% and 87% of overweight and obese participants (based on BMI) had never sought medical advice regarding weight management.
Conclusion: Individuals significantly underestimate their degree of adiposity. A significant proportion of overweight/obese subjects had never attempted to lose weight. Participants underestimate their risk of T2DM. A significant proportion is not aware of the association between adiposity and T2DM. Participants self perception (rather actual) of adiposity predicted attempts of weight loss. The majority of participants never considered seeking medical advice regarding weight. Further public education regarding obesity, its associated health risks and what healthcare professionals can provide is needed.
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311

Differential effects of ethnicities on the relationship between body mass index and insulin resistance in Singapore

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Background and aims: Singapore has a high prevalence of diabetes mellitus, which is at least partially attributable to an increase in obesity, acting through its effects on insulin resistance (IR). The three ethnic groups in Singapore exhibit differences in the levels of obesity and IR. However, Malays, which have the highest body mass index (BMI), do not have the highest levels of IR, raising the possibility that the relationship between BMI and IR may differ between ethnic groups. Accordingly, the aim of this study was to examine the relationship between BMI and IR in a sample of Asian Indian, Malay and Chinese populations living in Singapore.
Materials and methods: 5472 individuals from 4 previous cross-sectional studies carried out in Singapore were followed up between 2004-2007. Anthropometric measures were taken and fasting blood samples were drawn for glucose, insulin. Insulin resistance was derived using the homeostasis model assessment method.
Results: As a group, BMI correlated well with IR (r = +0.55, p<0.001). Asian Indian m (M) and women (W) had equivalent BMI (kg/m^2) (M: 25.1 $\pm$ 4.2; W: 26.8 $\pm$ 5.0) to the Malays (M: 25.4 $\pm$ 4.0, W: 26.9 $\pm$ 5.4) but higher compared with the Chinese (M: 23.5 $\pm$ 3.6, W: 22.4 $\pm$ 3.8). Notwithstanding, Asian Indian men and women were more insulin resistant (M: 3.0 $\pm$ 3.2, W: 3.0 $\pm$ 3.1) compared to both Malay (M: 2.2 $\pm$ 2.5, W: 2.2 $\pm$ 1.8) and Chinese (M: 1.8 $\pm$ 1.8, W: 1.7 $\pm$ 1.7), all p<0.001. Each unit gain in BMI was associated with the largest delta rise in IR in the Chinese population followed by Indians and Malays (p for interaction<0.001). This relationship was more marked in women.
Conclusion: Much of the literature on IR in Asian ethnic groups has focused on the increased IR in Asian Indians which occurs at relatively low BMI. Our study confirms these findings. Unexpectedly, our data suggests that the Chinese population may be at even greater risk of increased insulin resistance with weight gain but are currently protected by the relatively low BMI. If this is true, it makes the prevention of obesity in this ethnic group, as China undergoes rapid socio-economic development even more important than it already is.
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312

Obesity is associated with poorer clinical outcomes following insulin initiation for patients with type 2 diabetes

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Background and aims: Little information is available on the association between BMI at time of insulin initiation with attainment of targets for glycaemic control in type 2 diabetic (T2DM) patients. This study was designed to describe HbA1c and weight post the first prescription for insulin.
Materials and methods: A cohort of patients with diagnostic codes for T2DM were selected from the General Practice Research Database (GPRD) for the period January 1st 2002- March 31st 2008. No patient had been prescribed insulin in the 6 months prior to the first insulin prescription (index date). Additionally, patients had to have ≥ 2 further prescriptions for insulin in the 12 months post the index date as well as ≥ 2 BMI and ≥2 HbA1c recordings (one index, one post index). Patients also had to have a minimum of 12 months data post the index date. The cohort was stratified into BMI categories at the time of insulin initiation: normal (18.5-24.9 Kg/m^2); overweight (25.0-29.9 Kg/m^2); obese (30.0-34.9 Kg/m^2); clinically obese (35.0-39.0 Kg/m^2); morbidly obese (≥40 Kg/m^2). At 6-monthly intervals (up to 24 months) information on HbA1c, BMI and weight was extracted for those patients with data at that point. A sensitivity analysis was conducted using the subset of patients who had data at every time point. A multivariate repeated-measures linear model was fitted assessing weight change from point of insulin initiation to 12 months. Adjustment was made for age, gender, region, baseline HbA1c, oral therapy and insulin type.
Results: 3783 patients fulfilled the inclusion criteria (normal weight, n=672; overweight, n=1259; obese, n=1070; clinically obese, n=480; morbidly obese, n=302). The median HbA1c value at the time of insulin initiation was 9.6% (interquartile range IQR 8.6-11.0). The greatest reductions in HbA1c were seen in the first 6 months of insulin use in all BMI categories. The improvement in HbA1c appeared to be greatest in those with lower BMI: median observed HbA1c at initiation and 6 months was 9.7% and 7.9% in normal weight patients and 9.6% and 8.2% in the clinically obese , respectively. A minority of patients achieved target HbA1c (≤7.5%) at any of the 6-monthly timepoints up to 24 months (for patients with a HbA1c at 24 months, the proportion achieving target was: normal 41%; overweight 34%; obese 30%; clinically obese 26%; morbidly obese). Sensitivity analysis supported these findings. The greatest weight gain was observed in the first six months and

appeared highest in normal weight patients (median 3.59 kg [IQR] 0.5-7.0) reducing to 2.00 kg (IQR-1.3-4.1) in clinically obese and 0.8 kg (IQR -2.4-4.5) in morbidly obese . Multivariate models showed that after adjustment for confounders, normal weight patients made the greatest gains; 5.07kg (95% CI 3.35, 6.79) as did those with HbA1c ≥ 12.1%; 5.55kg (95% CI 3.81, 7.28).
Conclusion: Obesity appears to be associated with a poorer response to insulin illustrated by higher HbA1c values and lower achievement of target. Patients with lower BMI have higher weight gains post insulin exposure than the obese, which may have clinical and public health implications, which require further investigation.
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313

High prevalence of obesity in the Spanish working population. Results from ICARIA study
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Background and aims: Obesity is one of the prevailing processes of major socio-health impact based on its high prevalence, its progressive growth and its relation to multiple associated diseases. The epidemiological data available in Spain are based on local or autonomic studies, as well as on health surveys with antropometric data stated by the pollsters. The aim of this study is to describe the prevalence of obesity in the Spanish working population, as well as its evolution in the recent years.
Materials and methods: Information obtained from medical screenings on companies carried out by IBERMUTUAMUR from May 3, 2004 through November 30, 2007 in all of the autonomous communities, which included 1,441,267 subjects. 356,030 subjects were excluded due to lack of complete medical records to calculate the BMI, with a final sample of 1,085,237 subjects, 74% men and 26% women, between 18-29 years: 30%; 30-39 years: 33,1%; 40-49 years: 22,5%; 50-59 years: 11,8%; >60 years: 2,6%. The weight and the size were obtained by direct measurement. Overweight was defined by BMI of 25-29,9, obesity by BMI>30, and morbid obesity by BMI>40kg/m^2
Results: The prevalence of obesity was 15.9% (18.5% in men and 8.7% in women). The prevalence of overweight was 38.5% (44.6% in men and 22% in women). A progressive increase was observed in the prevalence of obesity with the age: 18-29 years: 9.5%, 30-39 years: 14.6%, 40-49 years: 19.9%, 50-59 years: 25.4%, >60 years: 26.6%. For overweight, the prevalence was, according with the age groups: 28.5%, 39%, 44.3%, 48.9% and 51.4%, respectively. Progressive increase in obesity was observed during the 4-year study period (2004-2007), in men (17.7%, 18.1%, 18.7%, 19.1%), in women (7.7%, 8.4%, 9%, and 9.3%) and in the total (15%, 15.4%, 16.1%, 16.4%). The maximum prevalence in autonomous communities was observed in Cantabria (21.1%), in the Canary Islands (18.6%) and in Andalucía (18.1%), and the minimum in La Rioja (11.1%), in Navarra (11.7%) and in Cataluña (12.1%). Morbid obesity was observed in 0.5% of the subjects.
Conclusion: The prevalence of obesity and overweight in the Spanish working population are very high and constantly increasing. There are significant differences in the geographical distribution of obesity in Spain. These findings emphasize once more the need to boost specific obesity prevention and treatment (programs). The medical screening on companies is an excellent and efficient way to obtain information about the epidemiology of obesity and other prevailing processes, particularly if the studies are based on the active working population.

314

Predictors of drug choice in newly diagnosed type 2 diabetes
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Background and aims: Whilst, according to prescribing guidelines, type II diabetes is managed first by diet if appropriate, then by oral antidiabetic agents and with insulin only if insufficient diabetes control is achieved, in practice prescribing varies with some patients receiving insulin relatively early and others not receiving insulin despite poor control. This will impact on studies of differences in long-term diabetes-related morbidity associated with different treatments. We set out to establish what patient characteristics predict drug choice in type II diabetes.
Materials and methods: Using the UK General Practice Research Database (GPRD), all those newly diagnosed with type II diabetes between 1 January 1998 and 31 March 2007 were identified. The GPRD has been used extensively for epidemiological research in diabetes as well as other disease areas. Patient characteristics (sex, age at diagnosis, smoking, alcohol use, BMI, HbA1c at diagnosis, and comorbidity) were established as was prescribing of antidiabetic agents. Multivariate survival analysis of drug choice associated with any of these patient characteristics was carried out using Cox regression in Stata and hazard ratios (HR) with 95% confidence intervals (CI_{95}) were calculated.
Results: Out of 80,308 newly diagnosed study participants, 38,946 (48.5%) went on to receive drug treatment for their type II diabetes. Those with HbA1c values >10.0 were 25 times more likely to receive medication than those with normal control (HR 25.3; CI_{95} 19.3-33.1); women were less likely to receive drug treatment than men (HR 0.82; CI_{95} 0.78-0.85). The proportion initiated on insulin as their first antidiabetic drug halved from 6.1% in 1998 to 2.9% in 2007 (p<0.05). Other than being female (HR 0.84; CI_{95} 0.76-0.92), having poor glycaemic control (HR 1.53; CI_{95} 1.06-2.21) and being underweight (HR 1.94; CI_{95} 1.53-2.45), there were no obvious predictors of insulin being preferred over oral products.
Conclusions: In the UK, women were less likely than men to receive medicines when diagnosed with type II diabetes. Amongst those receiving antidiabetic medicines, men were more likely than women to receive insulin as their first antidiabetic drug. Other predictors were unsurprising; they included high BMI and poor glycaemic control.
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315

Hospital insulin therapy is associated with a reduced rate of mortality
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Background and aims: Uncontrolled hospital hyperglycemia and hypoglycemia are both associated with increased morbidity and mortality. While insulin therapy is used to control blood glucose, it is associated with higher rates of hypoglycemia. We sought to determine the relationship between insulin treatment for hyperglycemia and mortality.
Materials and methods: We examined relationships between insulin therapy and mortality in adult hospitalized patients without end stage disease who spent at least one day in ICU, with >1 BG test. Data is from 20,273 hospital admissions at two US academic centers between June 2003 and September 2007; analyzed for a potential mediation effect of glucose (BG) control and hypoglycemia and adjusted for diabetes status and primary inpatient diagnosis.
Results: Mean age was 60 vs 55 yrs for insulin therapy (IT+) vs no-insulin therapy (IT-) group, with 42% female in each group and 48 vs 7% with diabetes, respectively. Mean (±SD) for first BG was 9.0 (±4.5) vs 7.0 (±2.4) mmol/L; for last BG was 7.7 (±3.0) vs 6.3 (±1.8) mmol/L; 41 vs 5% had hypoglycemia (<3.9 mmol/L) and 9 vs <1% severe hypoglycemia (<2.2 mmol/L) in IT+ vs IT-. Unadjusted mortality was 9.2 vs 5.6% for IT+ vs IT- (p<0.0001). After accounting for age, diabetes status, first BG and primary diagnosis, adjusted mortality rate (π) was estimated as 5.5 vs 4.0% for IT+ vs IT- (p<0.0001). Further adjustment for hypoglycemia, % low BG (<7.2 mmol/L), % high BG (>10.0 mmol/L), and mean BG, yielded 6.0 vs 5.1% (p=0.0099) mortality. A final model reversed the relationship between IT and mortality by adding 2-way interactions between IT, DM, hypoglycemia, first BG, and mean BG and demonstrated a protective benefit of IT (π = 6.4 vs 8.6% for IT+ vs IT- with difference (δ= -2.2%, p<0.01). Patients with diagnosed diabetes had 6.0% mortality relative to 9.1% (δ= -3.1%, p<0.01) in those without diabetes. Mortality was estimated to be 10.8% for patients experiencing hypoglycemia (<3.9 mmol/L) vs 5.0% (δ= 5.7%, p<0.01) for patients without hypoglycemia, regardless of insulin therapy. Estimated mortality in patients with hypoglycemia was 6.9% for IT+ and 16.4% for IT- in contrast to 5.9% for IT+ and 4.3% for IT- in patients without hypoglycemia, p<0.01.
Conclusion: This retrospective study found that although unadjusted mortality appears higher in IT+ patients, careful adjustments for relevant parameters demonstrate a protective effect of insulin therapy on hospital mortality. Hospital insulin therapy is associated with a reduced rate of mortality.

Hypoglycemia was found to be a potential contributing factor for hospital mortality with or without insulin intervention.

	Mortality Rate[a], %	Difference (Δ), %	*P*-value
Insulin therapy			
Yes (IT+)	6.4	-2.2	<0.01
No (IT-)	8.6		
Diabetes diagnosis:			
Yes	6.0	-3.1	<0.01
No	9.1		
No Hypoglycemia			
IT+	5.9	1.6	<0.01
IT-	4.3		
Hypoglycemia (< 3.9 mmol/L)			
Yes[b]	10.8	5.7	<0.01
No[b]	5.0		
Hypoglycemia			
IT+	6.9	-9.5	<0.01
IT-	16.4		

[a]Model adjusted for age, diabetes status, first BG and primary diagnosis with added 2-way interactions between IT, DM, hypoglycemia, first BG, and mean BG
[b]Independent of insulin therapy

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316

25-hydroxyvitamin D levels and association with all-cause mortality in patients with type 2 diabetes mellitus

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Background and aims: We recently demonstrated that low baseline 25-hydroxyvitamin D (25OHD) levels in a large mixed patient population followed for a median of 7.7 years were predictive of all-cause and cardiovascular mortality (Arch Intern Med 2008; 168: 1340-1349). The aim of the present secondary analysis was to analyze whether patients with and without type 2 diabetes mellitus (DM) show a difference in that association. Such an analytical approach seems justified since patients with DM typically present with various distinct clinical comorbidities.
Materials and methods: All in all 1036 patients with DM and 2221 non-diabetic patients (non-DM) were included in this analysis. In order to adjust for seasonal variation in 25OHD levels we formed month-specific percentiles for the overall cohort. Patients with 25OHD levels above the 90th percentile [mean 25OHD level 35 (DM) and 33 ng/mL (non-diabetics)] were taken as referent population and compared to the respective diabetic and non-diabetic patient cohorts. Cox proportional hazards regression models were used including age, sex, physical activity, smoking status, cystatin C, NT-proBNP, systolic and diastolic blood pressure, use of statins, ACE inhibitors and beta blockers as confounders.
Results: Important differences in baseline characteristics were found for age (61 vs 66 years; non-DM vs DM), BMI (27.2 vs 28.7) and HbA1c levels (5.8 vs 7.3) (all P<0.001). Mean 25OHD levels were 15.3 (DM) and 18.2 ng/mL (non-DM; P<0.001). In general, the increase in all-cause mortality with declining 25OHD levels was more prominent for the non-DM patient group. Whereas a statistical significant increase in all-cause mortality already became evident with 25OHD levels below the 70th percentile in non-DM patients, 25OHD levels in DM patients had to be below the 20th percentile to be significantly associated with mortality. The interquartile range for 25OHD levels varying with season of the year below which mortality significantly increased was 15 to 23 ng/mL for the non-DM and 7 to 10 ng/mL for the DM cohort. Patients with 25OHD levels ≤10th percentile had a multivariate-adjusted hazard ratio for all-cause mortality of 5.1 (CI; 2.8 to 9.1) for non-DM as opposed to 2.9 (1.5 to 5.6) for patients with DM, respectively.
Conclusion: In conclusion, for the same month adjusted decrease in 25OHD levels the relative increase in mortality was greater for patients without DM than for those with DM. Nevertheless, 20% of patients with DM had 25OHD levels that were accompanied by a two- to threefold increase in mortality. Our data suggest that one in five patients with DM has 25OHD levels that are associated with a marked rise in mortality.

PS 8 Replicated genes for diabetes and obesity

317

A low "genetic load" of risk variants for type 2 diabetes is associated to better beta cell function in patients with newly diagnosed type 2 diabetes

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Background and aims: Genetic variability at a number of single nucleotide polymorphisms (SNPs) has been convincingly associated to the risk of type 2 diabetes (DM2). Each SNP, however, per se contributes quantitatively little to overall risk and is often of questionable biological significance in affecting the determinants of glucose (G) regulation. Thus, we aimed at assessing the joint effect of DM2 associated SNPs on key metabolic phenotypes, i.e. beta-cell function and insulin sensitivity, of patients with newly diagnosed DM2.
Materials and methods: 388 (263 M/125 F) drug-naive and GADA-negative patients (age: median=59.0 yrs; I.Q. range: 52-65; BMI:29.4 kg/m^2; 26.5-33.0) with newly diagnosed DM2 were studied. Beta-cell function and insulin sensitivity were assessed by mathematical modeling of G/C-peptide curves during a 240' frequently sampled OGTT and by euglycemic insulin clamp, respectively. The beta-cell responses to the rate of increase of G (derivative or dynamic control: DC; units: [pmol.m^{-2}]/[mM.min^{-1}]) and to G concentration (proportional or static control, PC, presented as the insulin secretion rate at G concentrations of 5.5, 8.0, 11.0, 15.0 and 20.0 mM, respectively; units: pmol.min^{-1}.m^{-2}) are herein reported. Insulin sensitivity is presented as the M value in the last 60' of the clamp (units: μmol.min^{-1}.m^{-2}). The following SNPs (related gene) were assessed: rs7903146 (TCF7L2), rs1111875 (HHEX), rs7756992 (CDKAL1), rs679931 (CACNA1E), rs5219 (KCNJ11), rs13266634 (SLC30A8), rs4402960 (IGF2BP2), rs10010131 (WFS1) and rs4430796 (TCF2). A "genetic load" score was computed by summing the number of at risk alleles. Patients were divided in 3 groups: low (LGL: ≤7, n=92), intermediate (IGL: 8-10, n=222) and high (HGL: ≥11, n=74) "genetic load" score. Parametric and nonparametric statistical methods were used as appropriate.
Results: No significant differences in age, gender, BMI, and HbA1c were detected across LGL, IGL and HGL groups. However, fasting plasma G was lower (p<0.03) in the LGL (6.8±1.4 mM) than in the IGL (7.3±1.9 mM) and HGL (7.4±1.5 mM) groups. Insulin sensitivity (607±356; 611±355; 640±356, p=0.89) and the DC of beta-cell function (643±742, 592±643, 558±546, p=0.98) were both similar across the 3 groups. However, both IGL and HGL displayed significantly worse (p=0.01) PC of beta-cell function (157±60, 212±101, 332±177, 528±320, 778±523 and 152±56, 200±85, 326±167, 532±305, 797±498 in the IGL and HGL, respectively, at 5 G levels) than LGL (172±71, 247±117, 410±231, 648±411, 948±646).
Conclusion: A lower number of the DM2 risk alleles assessed in this study hallmarks newly diagnosed DM2 patients with better beta-cell function. Genotyping DM2 risk alleles in newly diagnosed patients may be of help in optimizing metabolic therapy and in refining metabolic prognosis.
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318

Study of the relationship between the accumulation of genes responsible for type 2 diabetes mellitus and clinical presentation

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Background and aims: Several studies in Japan have reported that 9 to 10 sigle-nucleotide polymoriphhisms (SNPs) were involved in the onset of type 2 diabetes mellitus(T2DM) among Japanese. However, T2DM is a multifactorial disease whose onset depends not only on genetic predisposition but also environmental factors, and it remains unclear to what extent genetic predisposition affects clinical presentation. It has been reported in large scale studies in Europa that the odds ratio of each polymorphism on diabetes onset is

low, ranging from 1.1 to 1.5. Consequently, it may be that the accumulation of several polymorphisms induces the onset of diabetes. In this study, the effect of several polymorphisms and their combination on the clinical presentation of T2DM was investigated.
Materials and methods: The subjects were 620 T2DM outpatients of our hospital and associated hospitals and 501 controls.10 SNPs in 10 candidate T2DM-susceptibility genes, HHEX (rs1111875), CDKAL1 (rs7756992), CDKN2A/B (rs10811661), SLC30A8 (rs13266634), KCNJ11 (rs5219), IGF2BP2 (rs4402960), PPARG (rs4402960), TCF7L2 (rs7903146), FTO (rs8050136) and KCNQ1 (rs2237892), were genotyped by TaqMan PCR assay. Clinical information, including present BMI, age at the diagnosis of diabetes, history of body weight, age at the maximum body weight, family history, disease period, FPG, HbA1c, CPI (=F-CPR*100/FPG) and treatment, was collected and compiled in the database of Japanese Diabetes Conplication and its Prospective study(JDCP).
Results: The associations between the number of risk alleles for each polymorphism with the clinical data were investigated. There were no significant differences between total number of risk alleles (0-2) for each gene and the age at diagnosis of diabetes. Of these 10 genes, only 4 genes, CDKAL1, CDKN2A/B, SLC30A8 and KCNQ1 exhibited a significant difference between the T2DM group and the control group, and the odds ratio for the 4 genes were 1.62, 2.18, 1.71 and 2.39, respectively. The mean total number of risk alleles for the 4 genes with a significant difference from the control group was 4.9 in the T2DM group and 3.5 in the control group ($p<0.01$). The association of the number of risk alleles in 4 genes with clinical data was investigated. In comparison of the clinical presentation between the groups with a total of three or fewer risk alleles in 4 genes and six or more, although the maximum BMI of the former group was 28.5, which was greater than that of the latter group (27.1), the age at diagnosis of diabetes was 51.5 years in the former group and 48.1 years in the latter group ($p<0.05$). Regarding the indices of insulin secretory capacity, serum CPR was 2.09 in the former group and 1.67 in the latter group, and CPI was 1.55 and 1.16 respectively, showing a significant difference ($p<0.01$).
Conclusion: It was suggested that the risk alleles for each individual polymorphism have little effect on the onset of diabetes, however, the accumulation of risk alleles is associated with decreased insulin secretion and the earlier onset of diabetes.

319

Can knowledge about the multiple common type 2 diabetes susceptibility gene variants be applied to discriminate between glucose tolerance and diabetes?

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Background and aims: The list of validated type 2 diabetes susceptibility variants has recently been expanded from three to 19. The identified variants are common and have low penetrance in the general population. The aim of this study was to investigate the combined effect of the 19 variants by applying receiver operating characteristics (ROC) to demonstrate the discriminatory value between glucose-tolerant individuals and type 2 diabetes patients in a cross-sectional population of Danes.
Materials and methods: The 19 variants were genotyped in 4,093 type 2 diabetic patients and 5,302 glucose-tolerant individuals.
Results: Single variant analyses demonstrated allelic odds ratios (OR) ranging from 1.04 (95%CI: 0.98,1.11) to 1.33 (95%CI: 1.22,1.45). When combining the 19 variants subgroups with extreme risk profiles we showed a 3-fold difference in risk of type 2 diabetes (lower 10% carriers with ≤15 risk alleles vs. upper 10% carriers with ≥22 risk alleles, OR 2.93 (95%CI: 2.38,3.62,p=1.6×10-25). We calculated the area under a ROC curve to estimate the discrimination rate between glucose-tolerant individuals and type 2 diabetes patients based on the 19 variants. We found an area under the ROC curve of 0.60. No evidence of epistasis could be demonstrated. Simulation studies of 50 variants with minor allele frequency (MAF)=0.25, OR=1.10, 50 variants with MAF=0.25, OR=1.15, 25 variants with MAF=0.02, OR=0.02, 25 variants with MAF=0.01, OR=2.0, and 25 variants with MAF=0.005, OR=3 in 10,000 controls and 10,000 cases showed an area under the curve of 0.83.
Conclusion: The 19 validated common diabetes gene variants enable detection of subgroups at a substantial increased risk of type 2 diabetes; however the discrimination between glucose tolerance and type 2 diabetes is still too inaccurate to have clinical relevance. In contrast, by applying simulation studies we show that rare variants with considerable higher OR are expected to push the area under the ROC curve towards a threshold acceptable for clinical utility.
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320

The titre of antiGAD influences diabetes-associated genes in LADA patients: results from the HUNT study

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Background and aims: Need for insulin treatment in Latent Autoimmune Diabetes in the Adult (LADA) patients associates with autoimmune activity as assessed from titres of antiGAD. Here we tested whether high titres of antiGAD increase the association of LADA with genes conferring risk of type 1 and low titres with genes conferring risk of type 2 diabetes.
Materials and methods: DNA samples collected from the HUNT study (1995-96) were used for analysis. We analysed 120 type 1 diabetic, 126 LADA, 1090 type 2 diabetic and 1503 age and gender matched non diabetic subjects for 60 SNPs located in loci known to be associated with type 1 or type 2 diabetes, including 14 tag-SNPs used for HLA haplotyping. For HLA-typing we genotyped recommended tag SNPs for different HLA-alleles, which combined in HLA haplotypes were known to be associated with type 1 diabetes. LADA patients were then dichotomised on the basis of antiGAD-levels below (low antiGAD) or above (high antiGAD) the median of antiGAD. Genotype distributions were tested by logistic regression analysis under additive, dominant and recessive models. Odds ratio (OR) and 95% confidence intervals (CI) were calculated. Phasing HLA haplotypes and testing for association within cases and controls were performed by PLINK software and SPSS (version 14.0). Adjustment for sex, age and BMI was applied when appropriate. Correction for multiple testing was done by max(T) permutation.
Results: The type 2 diabetes associated *FTO* and *TSPAN8* genes and the majority of the most strongly associated HLA haplotypes for type 1 diabetes, were found to be significantly associated with LADA in general. One distinct haplotype GCA for DRB1*0401-DQA1*0301-DQB1*0301 was found to be associated with higher risk only for LADA ($p=0.013$). After dichotomising for antiGAD the AA/AC genotypes of rs8050136, GG/GC of rs1861866 and TT/TC of rs9931491 in the *FTO*-gene and the CC/CT genotypes of rs7961581 in *TSPAN8* were associated only with low-antiGAD LADA ($p=0.004$, $p=0.006$, $p=0.002$ and $p=0.0004$, respectively). The same *FTO* and *TSPAN8* genotypes were also associated with type 2 diabetes ($p=0.005$, $p=0.047$, $p=0.004$ and $p=0.009$, respectively). No associations were found between *FTO* and *TSPAN8* and type 1 diabetes. The strongly associated type 2 diabetes gene *TCF7L2* (rs7903146) was not associated with LADA in general, nor in subgroups of antiGAD. The HLA haplotypes were, after dichotomising for antiGAD, found to be mainly associated with high antiGAD LADA patients, except the GCA-haplotype for DRB1*0401-DQA1*0301-DQB1*0301 which was associated with higher risk in low antiGAD LADA patients ($p=0.0003$). Interestingly, yet another distinct haplotype was found, the GCTA-haplotype for DRB1*0401-DQA1*0301-DQB1*0302, which was only associated with higher risk in low antiGAD LADA ($p=0.006$) and not with type 1 and type 2 diabetes.
Conclusion: These findings indicate that genetic heterogeneity in LADA is linked to a varying degree of autoimmune activity.

321

Explorative analyses of the population impact of type 2 diabetes associated SNPs on diabetes related traits - the HUNT study

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Background and aims: Several genome wide association studies have identified single nucleotide polymorphisms (SNPs) associated with type 2 diabetes (T2D). These SNPs could act through T2D related traits and conditions and have an impact in such in people without T2D. This study explores the potential impact of some of these SNPs on T2D related traits (BMI, lipids, non-fasting glucose, blood pressure and self reported cerebro- and cardiovascular ischemic disease) in a general, normoglycaemic population.
Materials and methods: T2D related traits were identified in the second Nord-Trøndelag Health Study (HUNT2 1995-97). Thirteen T2D susceptible SNPs were replicated in samples from HUNT2 using TaqMan allelic discrimination assay. Preliminary study population n=1402 (later to be increased to n>4000), persons without diabetes and with a non-fasting glucose below 7.8 mmol/l. Associations between T2D related SNPs and relevant traits were analysed with logistic and linear regression adjusted for sex, age and BMI in an additive model of inheritance. Correction for multiple testing was not performed in this explorative setting.
Results: A negative association was identified for diastolic hypertension (>90 mm Hg) with the gene regions THADA (rs7578597; OR=0.19, 95% CI: 0.04 - 0.82, p=2.7x10^{-2}), TSPAN8 (rs796181; OR=0.51, 95% CI: 0.30 - 0.97, p=1.3x10^{-2}) and TCF7L2 (rs7903146; OR=0.46, 95% CI: 0.25 - 0.84, p=1.1x10^{-2}). Likewise for myocardial infarction with BCL11A (rs10490072; OR=0.45, 95% CI: 0.21 - 0.96, p=3.8x10^{-2}), for BMI with CAMK1D (rs12779790; regression coefficient=-0.43, 95% CI: -0.82 - -0.04, p=2.9x10^{-2}), for total cholesterol with BCL11A (rs10490072; regression coefficient=-0.11, 95% CI: -0.16 - -0.01, p=3.4x10^{-3}) and for HDL-cholesterol with BCL11A (rs10490072; regression coefficient=-0.04, 95% CI: -0.07 - -0.01, p=2.3x10^{-2}) and FTO (rs8050136; regression coefficient= -0.04, 95% CI: -0.07 - -0.01, p=1.2x10^{-2}). A positive association was observed for microalbuminuria with FTO (rs8050136; OR=3.90, 95% CI: 1.43 - 10.59, p=7.7x10^{-3}), for cerebral stroke with FTO (rs8050136; OR=2.17, 95% CI: 1.06 - 4.44, p=3.4x10^{-2}) and for increased levels of non-fasting blood glucose (for glucose values < 7.8 mmol/l) with the gene regions NOTCH2 (rs10923931; regression coefficient=0.12, 95% CI: 0.03-0.21, p=1.0x10^{-2}), ADAM30 (rs2641348; regression coefficient=0.14, 95% CI: 0.04-0.23, p=3.9x10^{-3}) and JAZF1 (rs864745; regression coefficient=0.06, 95% CI: 0.00 - 0.12, p=4.4x10^{-2}).
Conclusion: In a normoglycaemic population, T2D related SNPs were negatively associated with diastolic hypertension, myocardial infarction, BMI and increasing levels of both total cholesterol and HDL-cholesterol. A positive association was observed for microalbuminuria, cerebral stroke and increased levels of non-fasting blood glucose (for glucose values < 7.8 mmol/l). More samples will be added to further explore the possible clinical relevance of these associations when studying the population effect of T2D associated SNPs.

322

Analyses of the combined discriminative value of 20 validated obesity susceptibility variants, separately and in combination with environmental factors

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Background: Within the last few years, genome-wide association studies have led to the identification of 20 genetic variants associating with obesity or related phenotypes. The variants are common and exhibit moderate effect sizes in the general population. The aim of this study is to investigate the combined effect of these variants and their ability to discriminate between normal weight and overweight/obese individuals in a cross-sectional population of Danes, by applying receiver operating characteristics (ROC).
Methods: The 20 variants were genotyped in 4 study groups: the population-based Inter99 study, the ADDITION Denmark screening study cohort, a population-based study group and a type 2 diabetic patient group both sampled at Steno Diabetes Center. The combined study population included 5,512 normal weight, 7,458 overweight and 5,044 obese individuals.
Results: Individual variant analyses demonstrated per allele odds ratios (OR) for the 20 variants ranging from 1.07 (95% CI 1.00-1.13) to 1.27 (95% CI 1.20-1.35). When combining the variants, individuals with extreme risk profile carrying more than 22 risk alleles (highest percentile) showed a significant increase in risk of both overweight 1.69 (1.41-2.03), $p = 8.2 \times 10^{-9}$ and obesity 2.14 (1.75-2.62), $p = 2.4 \times 10^{-14}$, compared to individuals with minimal risk profile carrying less than 14 risk alleles (lowest percentile). The area under ROC curve predicting overweight and obesity using data on the 20 SNPs was determined to 0.53 and 0.58, respectively. When combining SNP data with information regarding age, sex, diet, physical activity, smoking, education, employment and use of anti-obesity drugs, the discriminative ability measured by area under the ROC curve increased to 0.64 for overweight and 0.70 for obesity, which for obesity is higher than for environmental factors alone (AUC = 0.67).
Conclusion: The 20 newly identified obesity variants confer a significantly increased combined risk of both overweight and obesity in carriers of many risk alleles. The discriminative value of the 20 variants is still sparse and too inaccurate for clinical preventive purposes.
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323

Variants in *RAPGEF1*, *ENPP1*, *TP53*, *NRF1*, *SLC2A2*, *SLC2A4* and *FOXC2* do not associate with type 2 diabetes or related phenotypes in studies of 9,750 Danes

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Background and aims: In 2008 a comprehensive step-wise study of 222 candidate genes in type 2 diabetes reported association of variants in *RAPGEF1*, *ENPP1*, *TP53*, *NRF1*, *SLC2A2*, *SLC2A4* and *FOXC2* with type 2 diabetes in a study of 4,805 Finnish individuals. We aimed at validating these associations in a Danish case-control study and furthermore sought to evaluate the impact on diabetes-related intermediary traits of these variants in a population-based sample.
Materials and methods: We genotyped nine genetic variants in seven genes (*RAPGEF1* rs4740283, *ENPP1* rs2021966 and rs858341, *TP53* rs1042522, *NRF1* rs1882095, *SLC2A2* rs10513684 and rs5400, *SLC2A4* rs222852 and *FOXC2* rs4843165) in 5,976 middle-aged subjects from the population-based Inter99 cohort who all had undergone an OGTT. Furthermore these variants were genotyped in additional samples adding up to a case-control study comprising 4,974 glucose-tolerant participants and 3,586 type 2 diabetic cases.
Results: Based on the effect sizes and genetic models previously reported we had >95% statistical power for all variants to detect association with type 2 diabetes at a 5% significance level. In analyses adjusted for the effect of age, sex and BMI none of the nine variants associated with type 2 diabetes when applying the same genetic model as previously reported (OR for individual variants 1.01 - 1.26, *P*-values for individual variants: 0.07 - 0.8). However for all nine variants the estimate of association was in the same direction as previously described. In studies of diabetes-related quantitative traits in 5,883 participants without treated type 2 diabetes of the Inter99 cohort we observed nominal associations of *ENPP1* rs858341 with 2.8%, 3.1% and 2.1% increases in incremental area under the serum insulin, plasma glucose and serum C-peptide curves during an OGTT (95% CI 0.6-4.9%, *P*=0.01; 0.4-5.7%, *P*=0.02; and 0.7-3.4%, *P*=0.003, respectively). Furthermore, *TP53* rs1042522 associated nominally with a 0.034 mmol/l increase in fasting plasma glucose per allele (95% CI 0.0045-0.064 mmol/l, *P*=0.02).
Conclusion: Recently reported associations of variants in *RAPGEF1*, *ENPP1*, *TP53*, *NRF1*, *SLC2A2*, *SLC2A4* and *FOXC2* do not associate with type 2 dia-

betes in the Danish population, however we are not able to exclude modest effects of these loci. This study underlines difficulties in replicating genetic association studies and further large-scale collaborative analyses are needed to clarify the impact of these variants.
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324

A variant in the *KCNQ1* gene predicts future type 2 diabetes and mediates impaired insulin secretion
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Background and aims: Two independent genome wide association studies for type 2 diabetes (T2D) in Japanese have recently identified common variants in the *KCNQ1* gene being strongly associated with T2D. Here we studied whether variants in *KCNQ1* would influence BMI, insulin secretion and action and predict future T2D in subjects from Scandinavia.
Materials and methods: Risk of T2D conferred by *KCNQ1* rs2237895 was studied in 2,830 T2D cases and 3,550 controls from Sweden and prospectively in 16,061 individuals from the Malmö Preventive Project. Effect of genotype on insulin secretion/action was assessed cross-sectionally in 3,298 non-diabetic subjects from the PPP-Botnia Study and longitudinally in 2,328 non-diabetic subjects from the Botnia Prospective Study (BPS). KCNQ1 expression and glucose-stimulated insulin secretion was measured in human islets from 18 and 10, respectively, non-diabetic donors.
Results: The C-allele of *KCNQ1* rs2237895 was associated with increased risk of T2D in both the case-control (OR 1.23 [1.12-1.34], $p=5.6\times10^{-6}$) and the prospective (OR 1.14 [1.06-1.22], $p=4.8\times10^{-4}$) study. Furthermore, the C-allele was associated with decreased insulin secretion (CIR p=0.013; DI p=0.013) in the PPP-Botnia study and in the BPS at baseline (CIR $p=2.1\times10^{-4}$; DI p=0.0015) and during follow-up (CIR $p=3.8\times10^{-4}$; DI $p=8.3\times10^{-4}$). The CC-carriers showed a reduced glucose-stimulated insulin secretion from human islets (p=0.0058).
Conclusion: A common variant in the *KCNQ1* gene is associated with increased risk of future T2D in Scandinavians which can be explained by its influence on insulin secretion.
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325

Genetic variation in KCNQ1 associates with beta cell function and fasting glucose levels: a study of 3734 subjects comprising three Asian ethnicities
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Background and aims: The potassium voltage-gated channel, KQT-like subfamily, member 1 (*KCNQ1*) has been found through a genome-wide association study to be a strong candidate for conferring susceptibility to type 2 diabetes in East Asian and European populations. We sought to describe the potential association between polymorphisms at the *KCNQ1* locus with insulin resistance, β-cell function and other type 2 diabetes-related traits in a sample of Chinese, Malays and Asian-Indians living in Singapore.
Materials and methods: We examined the associations between 4 previously reported *KCNQ1* SNPs with type 2 diabetes-related traits in 3734 participants from the population-based NHS98 cohort (2520 Chinese, 693 Malay, 521 Asian-Indians). Insulin resistance was calculated from fasting insulin and glucose using the Homeostasis model assessment method, while pancreatic β-cell function was assessed using the corrected insulin response at 120 minutes (CIR_{120}).
Results: SNPs rs2237897, rs2237892, rs2283228 were significantly associated with type 2 diabetes (OR= 1.48, $p=3\times10^{-4}$; OR=1.38, p=0.002; OR=1.31, p=0.012 respectively). Within the Chinese population, the risk alleles for rs2237897, rs2237892, rs2283228 were significantly associated with higher fasting glucose levels (p=0.014, 0.011 and 0.034 respectively) and reduced CIR_{120} (p=0.007, 0.013, 0.014 respectively). A similar trend was observed among the Malay and Asian-Indian minority groups although this did not reach statistical significance due to limited sample sizes.
Conclusion: The increased risk for type 2 diabetes associated with *KCNQ1* is likely to be caused by a reduction in insulin secretion. Further studies will be useful to replicate these findings and to fully delineate the role of *KCNQ1* and its related pathways in disease pathogenesis.
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326

The type 2 diabetes-associated C-allele of rs2237895 *KCNQ1* associates with reduced insulin release following an oral glucose load
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Background and aims: Polymorphisms in the potassium channel, voltage-gated, KQT-like subfamily, member 1 (*KCNQ1*) gene have recently been reported to associate with type 2 diabetes. The primary aim of the present study was to investigate the putative impact of these *KCNQ1* polymorphisms (rs2283228, rs2237892, rs2237895, and rs2237897) on estimates of glucose-stimulated insulin release.
Materials and methods: Genotypes were examined for association with serum insulin levels following an oral glucose tolerance test (OGTT) in a population-based sample of 6,039 middle-aged and treatment-naïve individuals of whom 4,568 were glucose-tolerant. Insulin release indices estimated from the OGTT and the interplay between insulin sensitivity and insulin release were investigated using linear regression and Hotelling T2 analyses.
Results: Applying an additive genetic model the minor C-allele of rs2237895 was associated with reduced insulin levels 30 min (mean±SD: (CC) 277±160 vs. (AC) 280±164 vs. (AA) 299±200 pmol/l, p=0.008) after an oral glucose load, insulinogenic index (29.6±17.4 vs. 30.2±18.7vs. 32.2±22.1, p=0.007), incremental area under the insulin curve (20,477±12,491 vs. 20,503±12,386 vs. 21,810±14,685, p=0.02) among 4,568 glucose-tolerant individuals. Adjustment for the degree of insulin sensitivity had no effect on the measures of reduced insulin release. The rs2237895 risk genotype had a similar impact in the total sample of treatment-naïve individuals. No association with measures of insulin release were identified for rs2237892, rs2237897 or rs2283228.
Conclusion: In a population-based sample of 4,568 glucose-tolerant and middle-aged people, the type 2 diabetes-associated rs2237895 C-allele of *KCNQ1* was associated with reduced insulin release following an oral glucose load, suggesting that the increased risk of type 2 diabetes previously reported for this variant is likely to be mediated through an impaired beta cell function.
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327

***IRS-1* G972R and type 2 diabetes: a paradigm for the difficult ascertainment of the contribution to disease susceptibility of "low frequency-low risk" variants**
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Background and aims: Insulin receptor substrate-1 (IRS-1) plays a central role in insulin signalling and glucose homeostasis. A deleterious role on both

in vivo and *in vitro* insulin sensitivity and secretion has been repeatedly and unanimously reported for the relatively infrequent "loss of function" *IRS-1* G972R SNP (rs1801278, minor allele frequency=0.05). Despite this strong candidacy, results on its association with type 2 diabetes (T2D) have been conflicting, making the role of this SNP uncertain. To obtain further insights onto this topic, we performed a meta-analysis of all available case-control studies.

Materials and methods: We analyzed all case-control studies published as of January 2009 and we included also five unpublished case-control studies in which all study subjects were self-reported Whites: four sets from the Genetics of T2D in Italy and the United States (GENIUS T2D) Consortium and one set recruited in Chieti, Italy. Of the 35 available studies, only those in which both cases and controls were in HWE were meta-analyzed (n=30), comprising 11,857 cases and 10,758 controls.

Results: According to a dominant model (GR+RR compared to GG individuals) R972 variant tended to be associated with T2D, though failing to reach statistical significance (OR=1.09, 95%CI 0.96-1.24 p=0.195). Some evidence of heterogeneity was observed across studies (p=0.064). In a meta-regression analysis, neither the mean BMI of cases nor that of controls (available in 21 studies corresponding to 19,368 individuals) explained a significant proportion of such heterogeneity (2.1%, p=0.47 and 1%, p=0.77, respectively). By contrast, the mean age at T2D diagnosis explained 49% of this heterogeneity (p=0.03) in the 12 studies (8,967 individuals) in which this variable was available. When these studies were subdivided in tertiles of mean age at T2D diagnosis, summary ORs of T2D were 1.51 (95%CI 1.17-1.95), 1.24 (95%CI 0.96-1.59) and 0.88 (95%CI 0.68-1.14) in the youngest (39-44.9 yrs at diagnosis), intermediate (45-50.9 yrs) and oldest (51-58 yrs) tertile (p for trend of ORs=0.0017).

Conclusion: Our findings illustrate the difficulties of ascertaining the contribution of "low frequency-low risk" variants to T2D susceptibility. In the specific context of the R972 variant, 200,000 study subjects would be needed to have 80% power to identify a 10% increase in T2D risk at genome-wide significance levels. Under these circumstances, a strategy aimed at improving outcome definition and decreasing its heterogeneity may critically enhance our ability to detect genetic effects, thereby decreasing the required sample size. Our data suggest that focusing on early-onset diabetes, which is characterized by a stronger genetic background, may be part of such strategy.

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PS 9 Genetic variation in type 2 diabetes

328

Association study between LXRβ polymorphisms and type 2 diabetes mellitus

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Background and aims: Liver X receptors (LXRs) are nuclear receptors involved in the control of lipid and carbohydrate metabolism as well as inflammation. LXRs exist in two isoforms, LXRα and LXRβ, with similar protein structure and ligand binding, but somewhat different physiological functions. LXRβ shows a ubiquitously tissue expression. The role of LXRβ is still under investigation. However, the study of mice deficient for LXRβ has revealed important specific functions of LXRβ. LXRβ polymorphisms have so far been associated with Alzheimer's disease and obesity. We hypothesized that single nucleotide polymorphisms (SNPs) in the LXRβ gene are associated with some of the metabolic changes seen in patients with type 2 diabetes mellitus (T2DM).

Materials and methods: We used the following cohorts: *French individuals from the Lille and Toulouse MONICA studies (http://www.ktl.fi/monica), n=2315. *The Norwegian HUNT study (http://www.ntnu.no/dmf/hunt/english), n=2930. Genomic DNA was isolated from whole blood. SNPs were genotyped by the restriction fragment length polymorphism method or the iPLEX system from SEQUENOM for use with the MassARRAY platform. Statistical differences between groups were analyzed by χ^2 or ANOVA tests. *In silico* functional analysis: MatInspector by Genomatix.

Results: We sequenced the LXRβ gene in 86 individuals with metabolic syndrome and 10 control individuals, and identified 9 SNPs with a minor allele frequency above 5%. Then we genotyped 5 tag-SNPs in the two cohorts. We identified an association between a tag-SNP (rs17373080) representing a big haplotype block and the risk of T2DM (OR=1.77, p=0.01) and obesity (OR=1.26, p=0.05) in both men and women. *In silico* analyses showed that another SNP located in intron 4 -in linkage with rs17373080- would create a binding site for the hepatic nuclear factor 4 (HNF4) and nuclear factor 1 (NF1A, B, C and X) transcription factors. *In vitro* EMSA and cell transient transfections will be performed to examine the impact of this polymorphism on LXRβ expression.

Conclusion: Our results indicate that a putative functional polymorphism in the LXRβ gene is associated with T2DM and obesity.

329

Association between variants in the INSIG1 gene and the risk of type 2 diabetes and related traits

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Background and aims: Insulin-induced gene 1 (INSIG1) is a protein that blocks proteolytic activation of sterol regulatory element-binding proteins (SREBPs), transcription factors that activate genes regulating cholesterol and fatty acid metabolism and possibly genes involved in glucose homeostasis. In

the present study, we tested for association between six tagSNPs, capturing all common variation in the *INSIG1* gene and risk of type 2 diabetes, obesity and related traits in 11,168 individuals from six population-based and case-control studies.
Materials and methods: Six *INSIG1* tagSNPs were genotyped in participants from the Hertfordshire study, the MRC Fenland study, the MRC Ely study, the MONICA-Lille study, the Addition (Cambridge) study, and the Cambridgeshire case-control study. For each study, we tested for association between SNPs and [1] risk of diabetes and obesity using logistic regression, and [2] continuous traits (BMI, waist circumference, fasting glucose, glucose 120' OGTT, plasma cholesterol, HDL, LDL and triglyceride levels, systolic and diastolic blood pressure levels) using linear regression, assuming an additive model. Summary statistics of each study were meta-analyzed using inverse variance methods.
Results: The meta-analysis of the three case-control studies, including 1,711 cases and 2,992 controls, showed no compelling evidence of association between any of the six *INSIG1* SNPs and the risk of type 2 diabetes. Although minor alleles of rs10258075 and rs10271719 tended to be associated with a higher risk of type 2 diabetes (OR 1.40 [95%CI 1.07-1.83], p = 0.015 and OR 1.31 [95%CI 1.00 - 1.70], p = 0.046, respectively), these observations were not significant when accounting for multiple testing. We tested for the association between the six SNPs and the risk of obesity in the 3 population-based studies, yet none of *INSIG1* SNPs showed significant association. In agreement with the case-control analyses, none of the association between the *INSIG1* SNPs and any of the six metabolic traits showed convincing association. While a few nominally significant associations emerged, they did not survive correction for multiple testing.
Conclusion: Although our study was sufficiently powered to identify associations of small effect size, our results suggest that common genetic variation in *INSIG1* is unlikely to have a major impact on type 2 diabetes and obesity risk and related traits.
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330

***FOXO1* variation and relationships with type 2 diabetes and intermediary quantitative traits**
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Background and aims: The transcription factor Foxo1 is involved in liver glucose metabolism, pancreatic beta-cell proliferation, and differentiation of adipocytes and skeletal muscle cells making human *FOXO1* a candidate gene for abnormal glucose regulation. We investigated whether variants in *FOXO1* are associated with type 2 diabetes, obesity, or with alterations in insulin sensitivity or insulin release.
Materials and methods: We performed a screening for rare mutations with subsequent genotyping in family studies and large-scale tagging of frequent single-nucleotide polymorphisms. A mutation analysis was carried out in 61 Danish type 2 diabetic patients. We tagged *FOXO1* using HapMap data and genotyped five variants (rs7337995, rs1334241, rs7981045, rs2701880, and rs2755213) in 7960 individuals of which 6898 were characterised by an oral glucose tolerance test.
Results: The mutation analysis resulted in the identification of four variants; Ala511Val, -30C>T, 2074T>C, and 2600delA. In family studies the two rare Ala511Val and -30C>T variants did not co-segregate with type 2 diabetes. In case-control studies of type 2 diabetes and obesity only the rs7337995 variant A-allele showed association with obesity (p = 0.02), and in studies of fasting and post-oral glucose load measurements of plasma glucose and serum insulin and C-peptide only the rs1334241 polymorphism was related to modest alterations in serum C-peptide release. No relationships with estimates of insulin resistance were observed, and no haplotype association or SNP-SNP interaction was found for any trait.
Conclusion: We conclude that common variation of FOXO1 is not a major contributor to the pathogenesis of type 2 diabetes.

331

Association of *TCF7L2* gene haplotypes with diabetes type 2, gestational diabetes but not with polycystic ovary syndrome in Czech cohorts
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Background and aims: Polymorphisms in the human transcription factor 7-like 2 (*TCF7L2*), which plays a key role in the Wnt signaling pathway, are strongly associated with type 2 diabetes (T2D) in multiple populations. Risk genotypes in *TCF7L2* gene are associated with impaired insulin secretion and incretin effect and with hepatic insulin resistance. The aim of our study was to examine the role of *TCF7L2* gene variants in the pathogenesis of T2D, gestational diabetes (G) and polycystic ovary syndrome (PCOS) in Czech population.
Materials and methods: The study involved 1460 Czech Caucasian individuals: 347 patients with T2D (D: M/F 115/232); 261 gestational diabetics (G); 147 offspring of T2D (O: M/F 48/99); 329 women with PCOS defined according to ESHRE consensus (PCOS) and 376 controls (C: M/F 123/253). Oral glucose tolerance test (oGTT) with sampling for blood glucose, insulin, C-peptide, proinsulin and glucagon and insulin tolerance test (ITT) were performed (except of T2D patients). Moreover, anthropometric parameters, 29 biochemical parameters and hormones and oGTT- and ITT- derived indices were evaluated. The SNPs: rs7901695 (T>C); rs7903146 (C>T); rs12255372 (G>T) in the *TCF7L2* gene were assessed by ABI TaqMan SNP Genotyping Assays. Particular haplotypes were generated by PHASE software. Statistics (Chi-Square test, Mann-Whitney test and GLM Anova) was done using NCSS 2004.
Results: PHASE generated 6 haplotypes whereas four most frequent haplotype combinations TCG/TCG, CTT/TCG, CTT/CTT and CTG/TCG were studied. The frequency of the risk homozygous CTT/CTT haplotype combination was decreasing in the studied groups as follows: D 10.6, O 9.5, G 6.1, C 5.32 and PCOS 4.9 [%] and the distribution of CTT carriers did not differ in particular groups with respect to BMI. The CTT haplotype showed a signif. association with T2D with OR [95%CI] 1.57 [1.23;2.01], p=0.0003, for CTT homozygotes the OR was 2.25 [1.28;3.98], p=0.006. Interestingly, the frequency of the fourth most often haplotype combination CTG/TCG was almost twice higher in gestational diabetics compared with all other studied groups (7.7% vs 2.9-4.26%) and it was associated with gestational diabetes risk, OR 2.59 [1.12;6.01], p=0.036. In cohorts we did not observe the influence of risk *TCF7L2* haplotype on fasting and stimulated insulin, proinsulin, C-peptide and glucagon secretion and on any other screened parameter except of polyunsaturated fatty acids where the % of fasting PUFA was significantly decreased in CTT carriers vs. non-carriers in control women (p=0.0009), esp. due to lower proportion of (n-6) PUFA. The same trend was apparent in gestational diabetics (p=0.06). After pooling of all women (except of T2D patients), the signif. decreased levels of oGTT-stimulated insulin were apparent but the insulinogenic index as well as disposition indices did not differ between CTT carriers and non-carriers. The most significant association remained % of fasting PUFA, even after adjustments for age and BMI (p=0.00008, power 0.98) or HOMAR (p=0.0001, power 0.97).
Conclusion: The strong association of *TCF7L2* haplotypes with T2D and gestational diabetes was confirmed. The most risk haplotype combination for T2D is CTT/CTT but surprisingly, CTG/TCG for gestational diabetes. In women, the CTT carriership was associated with lower % of fasting PUFA. The association with polycystic ovary syndome was not found.
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332

Evaluation of chloride channel-3 as a potential target for TCF7L2-dependent impairment of insulin secretion
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Background and aims: The transcription factor TCF7L2 is the most important type 2-diabetes gene discovered to date. The splicing pattern of the transcription factor is complex and varies among tissues. We have recently demonstrated that human (and rat) islets primarily express a TCF7L2 mRNA species that contains exon 4. Here we have investigated the correlation between expression of the exon 4-containing TCF7L2 splice variant and expression of ion channel target genes in human islets of Langerhans.
Materials and methods: The percentage of the exon4-containing-TCF7L2 transcripts was measured with QPCR using absolute quantification. Statis-

tic analyses were performed using non-parametric Spearsman correlation (SPSS 16.0). Candidate genes were analysed using Gene Set Analysis Tool kit (http://bioinfo.vanderbilt.edu/). Using rat clonal INS-1 cells, ClC-3 was silenced using the EGFP-containing $P^{RNA\text{-}H1.1}$ vector (GenScript Corp, Piscataway, NJ, U.S.A) with 76 basepair shRNA insert with the CTCCGGAATTCCAGAGATTAA target sequence in rat ClC-3. Expression and knockdown was verified by immunoblotting using an anti-ClC-3 antibody (kindly provided by Prof. T. Jentsch). Insulin granules were purified using a Phogrin-EGFP adenovirus (BD Adeno-X expression system 1, Clontech, CA, U.S.A). INS-1 cells were homogenized and EGFP-positive particles were collected using a Becton Dickinson Cell Sorter. Secretion in ClC3-silenced cells was quantified as the ratio of secreted hGH over total hGH content/well, in cells coexpressing hGH and the shRNA.

Results: The binding motif (A-C/G-A/T-T-C-A-A-A-G) of TCF7L2 was investigated in the promoter region of all annotated genes and regions in the human genome using a bio-informatics approach (Pearl). All together 2759 annotated genes were found to contain the TCF7L2 binding motif. Out of these 2759 genes, the expression of 205 genes correlated significantly (P=0.05) with exon4-containing-TCF7L2 expression. Using pathway analysis, the Chloride Channel 3 (CLCN3) was identified as a potential target and further evaluated. ClC3 protein expression was first verified by immunoblotting, and then silenced by RNA interference. A 65% down-regulation in ClC3 protein expression coincided with a 27% decrease (P<0.05) in regulated secretion. Cell fractionation experiments demonstrated that ClC-3 was primarily associated with intracellular membranes. A purified insulin granules preparation was enriched in insulin by a factor of ~20 over the homogenate when normalized to protein content. Interestingly, the granule fraction also expressed significantly higher amounts of ClC-3 than the total cell homogenate, whilst not expressing markers of plasma membrane, lysosomes, SLMV, early or recycling endosomes, or mitochondria.

Conclusion: The splicing pattern of TCF7L2 affects expression of the chloride channel ClC-3, which in turn has the capacity to regulate insulin secretion. This action is likely to involve a direct action on the ion composition of the insulin granule interior.

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333

Studies of circulating plasma levels of glucose, insulin, glucagon and incretins following a meal challenge in carriers of high and low risk *TCF7L2* genotypes

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Background and aims: The rs7903146 T-allele of transcription factor 7-like 2 (*TCF7L2*) has in several populations shown association with type 2 diabetes. The biological mechanism behind this association is, however, only partly elucidated. Some studies have indicated that the effect of the *TCF7L2* T-allele might be mediated via decreased incretin secretion and/or action. We aimed to study circulating levels of glucose, proinsulin, insulin, glucagon, glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2) and gastric inhibitory polypeptide (GIP) among individuals carrying the high-risk rs7903146 TT-genotype and low-risk GG-genotype, respectively, following a standardised meal challenge.

Materials and methods: A meal challenge was performed in 31 glucose-tolerant men (age: 54 ± 7 years and BMI: 26 ± 3 kg/m^2) with rs7903146 TT-genotype and in 31 glucose-tolerant age- and BMI-matched men with GG-genotype (age: 53 ± 6 years and BMI: 26 ± 3 kg/m^2). Plasma levels of proinsulin, insulin, C-peptide, glucose, glucagon, GLP-1, GLP-2 and GIP were obtained 0, 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 180, 210, 240 min after ingestion of a standardised breakfast meal.

Results: Carriers of the high risk TT-genotype had a significantly lower level of fasting plasma glucose (5.44 ± 0.36 mmol/l vs 5.76 ± 0.41 mmol/l, p=0.002). In contrast, levels of plasma GIP were significantly higher among TT-genotype carriers 135 min to 180 min during the meal challenge (67.41 ± 31.20 pmol/l vs. 50.57 ± 21.04 pmol/l, p=0.03). We did not observe any differences in fasting or stimulated levels of proinsulin, insulin, GLP-1, GLP-2 or glucagon. Analyses estimating prehepatic insulin secretion rates, beta-cell responsiveness to glucose and incretin secretion are presently being performed.

Conclusion: No significant difference in post-prandial levels of glucose, insulin, proinsulin, GLP-1, GLP-2 and glucagon was observed. Surprisingly, we found a significantly lower level of fasting plasma glucose among TT-genotype carriers and higher levels of post-prandial plasma GIP.

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334

Direct molecular phenotyping in crosses and congenic strains of the diabetic Goto-Kakizaki rat identifies novel diabetes candidate genes

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Background and aims: Insulin resistance and altered insulin secretion are cardinal features of type 2 diabetes (T2D). The Goto-Kakizaki (GK) rat is an inbred model of spontaneous T2D extensively used to identify genetic loci causing pathophysiological components of the disease. The aim of the study is to characterise functional candidate genes for obesity and diabetes through molecular phenotype analysis derived by genome wide gene transcription profiling in panels of F2 hybrids and congenic strains of the GK and non-diabetic Brown Norway (BN) rats.

Materials and methods: Illumina BeadChips were used to determine the abundance of over 20,000 transcripts in fat biopsies of GKxBN F2 hybrids (n=138) and in liver, skeletal muscle and fat biopsies of rats of 11 BN.GK congenic strains containing GK alleles across various segments of chromosome 1 on a BN genetic background. R/qtl was used for expression quantitative trait locus (eQTL) mapping. Transcript and protein levels were further tested by qRT-PCR and Western Blotting. The biological role of variants identified in functional and positional candidate genes was tested by chemiluminescent assays in transfected cell lines. Insulin secretion tests were performed in incubated islets.

Results: Data from genome-wide gene expression of fat biopsies in the F2 cross identified 585 statistically significant eQTLs (LOD>9), mostly regulated in *cis*, that co-localise to a large extent with QTLs for diabetes/obesity related variables and ^{1}H-NMR metabonomic traits. Analysis of tissue-specific expression profiles in chromosome 1 congenic strains indicated that the vast majority of differentially expressed transcripts were found in fat and liver, with a large proportion of the latter (74%) being liver-specific. Replicated eQTLs in F2 hybrids and congenics which co-segregate with the insulin secretion QTL, include genes localised in a 2Mb congenic locus in chromosome 1 associated with altered insulin secretion in vivo and vitro. Potentially functional variants were found in differentially expressed genes at the locus, including in a glutamate transporter (*Slc1a1)* that may account for the insulin secretion QTL.

Conclusion: Combining eQTL mapping in F2 crosses and congenics provides a novel strategy to identify positional and functional candidate genes for diabetes and obesity QTLs.

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PS 10 Genetic variation for metabolic traits related to type 2 diabetes and obesity

335

Circulating HMW adiponectin isoform is heritable and shares a common genetic background with insulin resistance in non diabetic white Caucasians from Italy: evidences from a family-based study

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Background and aims: Reduced circulating adiponectin levels contribute to the etiology of insulin-resistance. Adiponectin circulates in three different isoforms: high (HMW), medium (MMW), and low (LMW) molecular weight. The genetics of adiponectin isoforms is mostly unknown.
Our aim was to investigate whether and to which extent circulating adiponectin isoforms are heritable and whether they share common genetic backgrounds with insulin resistance-related traits.
Materials and methods: In a family based sample of 640 non diabetic White Caucasians from Italy, serum adiponectin isoforms concentrations were measured by ELISA. Three SNPs in the *ADIPOQ* gene previously reported to affect total adiponectin levels (rs17300539, rs1501299 and rs677395) were genotyped. The heritability of adiponectin isoform levels was assessed by variance component analysis. A linear mixed effects model was used to test association between SNPs and adiponectin isoforms. Bivariate analyses were conducted to study genetic correlations between adiponectin isoforms levels and other insulin resistance-related traits.
Results: All isoforms were highly heritable (h^2=0.60-0.80, p=$1x10^{-13}$-$1x10^{-23}$). SNPs rs17300539, rs1501299 and rs6773957 explained a significant proportion of HMW variance (2-9%, p=$1x10^{-3}$-$1x10^{-5}$). In a multiple-SNP model, only rs17300539 and rs1501299 remained associated with HMW adiponectin (p=$3x10^{-4}$ and $2.0x10^{-2}$). Significant genetic correlations (p=$1x10^{-2}$-$1x10^{-5}$) were observed between HMW adiponectin and fasting insulin, $HOMA_{IR}$, HDL-cholesterol and the metabolic syndrome score. Only rs1501299 partly accounted for these genetic correlations.
Conclusion: Circulating levels of adiponectin isoforms are highly heritable. The genetic control of HMW adiponectin is shared in part with insulin resistance-related traits and involves, but is not limited to the *ADIPOQ* locus.
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336

Angiopoietin-like protein 3 polymorphism is associated with dyslipidaemia in Korean type 2 diabetic patients

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Background and aims: Patients with type 2 diabetes mellitus frequently have central obesity and several forms of dyslipidemia. Angiopoietin-like protien 3 (ANGPTL3) has metabolic effects within adipose tissue, such as inhibition of lipoprotein lipase activity and stimulation of lipolysis. These effects were convincingly demonstrated in mice. These observations may indicate that Angptl3 might regulate lipid metabolism and distribution of body fat. We hypothesized that genetic variation within the ANGPTL3 gene contributes to metabolic traits, such dyslipidemia and obesity in patients with type 2 diabetes mellitus. We therefore genotyped for the 2 single nucleotide polymorphisms (SNPs) rs17123725 (C/T) and rs12023629 (A/G) of ANGPTL3, and investigated that the polymorphism is associated with metabolic traits in type 2 diabetic patients.
Materials and methods: One hundred and thirty-six type 2 diabetic patients (M: 65, F: 71; mean age: 59.80 ± 12.23 years) and forty-one control subjects (M: 18, F: 23; mean age: 58.49 ± 12.29 years) were recruited. All subjects underwent assessment for diabetes duration, the degree of obesity, and measured fasting plasma glucose, fasting immunoreactive insulin, HbA_{1C}, fasting plasma C-peptide, fasting free fatty acid, high sensitivity C-reactive protein and lipid profiles. The polymorphisms of ANGPTL3 were genotyped by direct sequencing of the corresponding polymerase chain reaction product.
Results: The genetic frequency of rs17123725 (CT) hetero type was not significantly different between type 2 diabetic patients and control subjects (11.8% vs. 9.8%, P= 0.722). Type 2 diabetic patients with rs17123725 (C/T) polymorphism had significantly the use of HMG-CoA inhibitors and fibric acids than those with rs17123725 (TT) wild type (62.5% vs. 32.8%, P <0.05). Age, body mass index, waist circumference, HbA1C, total cholesterol level, HDL-cholesterol level, LDL-cholesterol and Triglyceride level were not different in two groups. After adjusted for the use of HMG-CoA inhibitors and fibric acids, this polymorphism was independently associated with triglyceride. We analyzed type 2 diabetic patients according to rs17123725 (CT) hetero type polymorphism, excluding the patients with the use of HMG-CoA inhibitors and fibric acids. Type 2 diabetic patients with rs17123725 (CT) hetero type polymorphism had significantly higher level of triglyceride than patients with rs17123725 (TT) wild type (208.3 ± 114.6 vs. 136.1 ± 81.3 mg/dL, P < 0.05). All the genotyping of rs12023629 of ANGPTL3 revealed rs12023629 (A/G).
Conclusion: This study suggested that the rs17123725 (C/T) polymorphism might be associated with dyslipidemia in Korean type 2 diabetic patients.

337

Screening of 336 single nucleotide polymorphisms (SNPs) in 85 obesity-related genes revealed that *MKKS* and *MYO9A* variants are associated with metabolic syndrome

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Background and aims: Genetic factors are important in the development of metabolic syndrome. However, the genetic background of metabolic syndrome remains unclear. We screened single nucleotide polymorphisms (SNPs) in 85 obesity-related genes to determine which may be associated with metabolic syndrome.
Materials and methods: Patients with metabolic syndrome (n=1080) and control individuals without metabolic syndrome (control-1; n=1524) were recruited for this study. Control-2 (n=528) was selected from control-1 who had no risk of the metabolic syndrome. A total of 336 SNPs in 85 genes selected from the JSNP database were genotyped. Discriminant scores were estimated by linear discriminant analysis using age, gender, BMI, fasting plasma glucose, triglycerides, HDL cholesterol, systolic and diastolic blood pressure, and treatments of diabetes, dyslipidemia, and hypertension as variables. The differences in discriminant scores between two genotype groups (either dominant or recessive model) were analyzed by the Mann-Whitney U test using case and control-1. We conducted case-control association analyses using case and control-2.
Results: Three SNPs in the *MKKS* gene were significantly related to metabolic syndrome by case-control association study using case and control-2; rs1545 (odds ratio [OR] adjusted for age and gender, 1.45; 95% confidence interval [CI], 1.21-1.74; *P*=0.000043 [additive model]); rs1547 (OR, 1.45; 95% CI, 1.21-1.74; *P*=0.000041); and rs2294901 (OR, 1.46; 95% CI, 1.22-1.75; *P*=0.000033). We selected five tag SNPs (rs2294901, rs221667, rs6133922, rs6077785 and rs6108572). They were in one linkage disequilibrium (LD) block and rs6133922 (*P*=0.00042), rs6108572 (*P*=0.000013), and rs6077785 (*P*=0.000019) were significantly associated with metabolic syndrome. The discriminant score for the AA homozygotes at SNP (rs2306576) in *MYO9A* gene was significantly higher than those for the AG heterozygotes and the GG homozygotes (*P*=0.000077).
Conclusion: Genetic variations in the *MKKS* and *MYO9A* genes may influence the risk of metabolic syndrome.
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338

Genetic variants across regulatory pathways of lipid and glucose metabolism: interaction effects with BMI on glucose levels

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Background and aims: Much of the genetic variation in glucose levels remains to be discovered. Especially, research on gene-environment interactions is scarce. Overweight is the main environmental risk factor for hyperglycemia. Transcriptional regulation is important for both weight maintenance and glucose control. We therefore analyzed 349 single nucleotide polymorphisms (SNP's), occurring in transcriptional pathways of glucose and lipid metabolism, and their interaction effects with body mass index (BMI) on glucose levels.
Materials and methods: 349 SNP's were measured in 3244 participants of the Doetichem cohort. Non-fasting glucose levels and BMI were measured at baseline and after 6 years. SNP x BMI interactions were analyzed by mixed models and adjusted for age, sex and time since last meal. False Discovery Ratio (FDR) < 0.2, was used to adjust for multiple testing.
Results: Two SNP's, rs8192678 (P-value = 0.0002; FDR=0.07) and rs3755863 (P=0.0012; FDR=0.17), showed a significant interaction with BMI. These SNP's are both located in the PPARGC1A gene. In subjects with a BMI <=25 kg/m^2, rs8192678 (P=0.02) and rs3755863 (P=0.03) were significantly associated with glucose levels. No effect was observed in subjects with BMI >25 kg/m^2.
Conclusion: Using a pathway based approach, we found a significant association between the PPARGC1A (rs8192678; rs3755863) x BMI interaction and glucose levels. The association between glucose and PPARGC1A was only present in lean subjects.This suggests that the effect of the PPARGC1A gene, which is involved both in fatty acid oxidation and glucose metabolism, is modified by BMI.

339

The association of FTO gene haplotypes with OGTT stimulated metabolic parameters in lean women

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Background and aims: Several single nucleotide polymorphisms (SNPs) in the fat mass and obesity associated gene (*FTO*) are related to the susceptibility to diabetes mellitus type 2 (DM2) through an influence on obesity that plays an important role also in the pathogenesis of polycystic ovary syndrome (PCOS). Nevertheless, the mechanism by which the FTO influences obesity related traits is still unknown. The aim of our study is to compare the *FTO* gene haplotypes frequencies in the groups of patients with PCOS and healthy controls without family history of DM2 and PCOS; and to evaluate the associations of the haplotypes with detailed anthropometric data, parametres of glucose and lipid metabolism, selected hormones and adipokines.
Materials and methods: The SNPs: rs1421085 (T/C); rs1121980 (G/A); rs17817449 (T/G) and rs9939609 (T/A) in the *FTO* gene were assesed by ABI TaqMan SNP Genotyping Assays; haplotypes were generated using programme PHASE. Study cohorts: 294 patients with PCOS defined according to ESHRE consensus (age 27.7±6.36 years; BMI 26.8±6.55 kg/m^2) and 234 healthy women (age 29.8±10.67 years; BMI 23.4±4.41 kg/m^2) were biochemically (29 parameters) and anthropometrically (measurements were evaluated using programme Antropo) carrefully characterized including 3h OGTT and derived indices. For statistical evaluation (Chi-square test, Mann-Whitney test, ANOVA), NCSS 2004 software was used.
Results: Two major haplotypes TGTT; CAGA (covering more than 92 % of all possible combinations) did not differ in frequency distributions between groups. The association of the carriership of the risk haplotype CAGA with BMI was stronger in PCOS patients (p=0.003) than in controls (p=0.018). In the PCOS group the frequency of the minor haplotype CAGA significantly increased from the normal weight via the overweight to obese patients (p=0.008). To evaluate the influence of CAGA risk haplotype on metabolic parameters, we focused on lean subjects (BMI < 25 kg/m^2). After pooling of lean PCOS and control women, significantly increased late phase OGTT stimulated levels of glucose (120min p=0.014; 150min p=0.021), C-peptide (120min p=0.031; 150min p=0.005; 180min p=0.022) and insulin (150min p=0.027; 180min p=0.007) were detected in CAGA carriers vs. non-carriers. The lean CAGA carriers had decreased several disposition indices (beta-cell function x insulin sensitivity) (p=0.011 - p=0.043) and also significantly higher levels of fasting and OGTT stimulated growth hormone (0min p=0.020; 60min p=0.001).
Conclusion: Our study suggests that FTO influences not only BMI and obesity related traits but in lean persons it could also affect metabolic parameters such as OGTT stimulated glucose, insulin, C-peptide and surprisingly growth hormone levels.
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340

Impact of variation near MC4R on whole body fat distribution, liver fat and weight loss

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Background and aims: Polymorphisms near the melanocortin-4 receptor (MC4R) gene locus are associated with body weight. Recent studies have shown that they influence insulin sensitivity and incidence of the metabolic syndrome. Thus, we hypothesized that the candidate SNP rs17782313 near MC4R additionally influences body fat distribution and its change during lifestyle intervention.
Materials and methods: To test this, 343 subjects were genotyped for SNP rs17782313, T/C. Body composition was assessed using magnetic resonance technique. Subjects were characterized by an oral glucose tolerance test. 242 subjects participated in a 9 month lifestyle intervention.
Results: The C allele was associated with a higher BMI (p=0.0013), but had no impact on glucose tolerance or insulin sensitivity (all p>0.10). There was an effect of the SNP on total fat (p=0.022) and non-visceral fat (p=0.017), but not on liver fat and visceral fat (all p>0.33). During lifestyle intervention, SNP rs17782313 has no impact on changes in body weight or fat distribution.
Conclusion: Despite an association with BMI and non visceral adipose tissue, the SNP rs17782313 did not influence visceral adipose tissue. Thus, this candidate SNP for human obesity may preferentially affect the accumulation of subcutaneous adipose tissue. Furthermore, the variation near MC4R has no effect on success of weight loss during lifestyle intervention.
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341

Insulin-stimulated glucose uptake in subcutaneous fat and liver in patients with the mitochondrial m.3243A>G mutation

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Background and aims: Insulin resistance in adipose tissue and excess liver fat have been associated with mitochondrial dysfunction in metabolic syndrome and lipodystrophy. The aim of this study was to assess the effect of mitochondrial DNA point mutation m.3243A>G, the most common cause of mitochondrial diabetes, on adipose tissue and liver metabolism.
Materials and methods: Fifteen patients with m.3243A>G, eight with no glucose lowering medication (group 1) and seven patients with diabetes (group 2) were studied. In addition, 12 patients with metabolic syndrome and no glucose lowering medication and 20 healthy controls were examined. Regional glucose uptake was measured using positron emission tomography and 2- [^{18}F] fluoro-2-deoxyglucose during euglycemic hyperinsulinemia. Liver fat was assessed with proton magnetic resonance spectroscopy.
Results: Glucose uptake in subcutaneous fat was lower and free fatty acid levels were higher during hyperinsulinemia in groups 1 and 2 and in patients with metabolic syndrome as compared to the controls. (5 ± 1 and 6 ± 1 and 6 ± 1 vs 12 ± 1; μmol•min^{-1}•kg^{-1} and 0.09 ± 0.02, 0.16 ± 0.03, 0.12 ± 0.02 vs 0.05 ± 0.01; mmol•l^{-1}, Mean ± SE). Insulin-stimulated glucose uptake in the liver in groups 1 and 2 and in patients with metabolic syndrome did not signifi-

cantly differ from that in the controls (22 ± 3 and 17 ± 2 and 14 ± 1 vs 18 ± 1; μmol•min^{-1}•kg^{-1}). Liver fat content was not significantly elevated in groups 1 and 2 but was significantly higher in patients with the metabolic syndrome as compared to controls (4.7 ± 3 and 2.5 ± 1 and 8.5 ± 2 vs 1.2 ± 1; %).
Conclusion: Our results suggest that the patients with the m.3243A>G mutation are insulin resistant in adipose tissue with no concurrent metabolic changes in liver.
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PS 11 The role of genomics and metabolomics in drug response

342

DPP-IV inhibitors are efficient adjunct therapy in HNF-1α MODY patients
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Background and aims: The pharmacogenetic effect of marked sulphonylurea (SU) sensitivity in HNF-1α MODY has been well documented and, as such, SU remains the first line therapy in the treatment of MODY3 patients. Over time, however, this treatment becomes insufficient in a substantial number of these subjects. Dipeptidyl-peptidase (DPP)-IV inhibitors are a new class of therapeutic agents that enhance insulin secretion, a major pathophysiological defect in HNF-1α MODY. For the first time, we report two patients with HNF-1α gene mutations who failed to respond to SU monotherapy but showed marked response to DPP-IV inhibitors.
Aim of the study: To assess the efficacy of DPP-IV inhibitors in HNF-1α MODY patients who failed to respond to SU monotherapy and safety.
Materials and results: Case 1- a 39 years old female patient with a ArgR171X HNF-1α mutation and a 7 year history of diabetes treated with 80 mg of gliclazide and 1000 mg of metformin. At entry into the study, her initial HbA1c and mean glucose levels on Continuous Glucose Monitoring System (CGMS) were 7.2% and 162 mg/dL, respectively. Sitagliptine, a DPP-IV inhibitor, at a dose of 100 mg/day was added to the patient's previous treatment regimen. Case 2- a 62 years old female patient with a IVS7nt-6G>A mutation and a 41 year history of diabetes treated with 240 mg/day of gliclazide and 6 IU of insulin/day. Her initial HbA1c was 8.8%, and her average glycemia on CGMS record reached 172 mg/dL. In addition to her previous regimen, we began combined therapy that included 50 mg of the DPP-IV inhibitor vildagliptine twice daily. At the re-examination 3 months after the initiation of DPP-IV therapy, HbA1c levels of both patients had fallen to 6.3%. Similarly, significant improvement in glycemic control on CGMS was observed as the average glycemia decreased to 114 mg/dL and 134 mg/dL in Case 1 and Case 2, respectively. No episodes of hypoglycemia below 60 mg/dL were recorded and no other side effects were reported during the 3-month treatment period. Importantly, we saw a substantial rise in insulin level increments during Intravenous Glucose Tolerance Tests performed at the beginning and at the end of the observation in both patients (by 9.8 and by 13.4 μIU/ml in Case 1 and Case 2, respectively).
Conclusion: In summary, DPP-IV inhibitors seem to be an effective and safe component of combined therapy in HNF-1α MODY patients. We postulate that a future randomized controlled trial should determine their efficacy as an alternative to SU monotherapy in this monogenic diabetes.
Supported by: CEED3

343

Serum cystatin C level in diabetic HNF-1α gene mutation carriers
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Background and aims: Cystatin C is a low molecular weight protein produced at a constant rate in all nucleated cells. It is freely filtered in the renal glomeruli, reabsorbed and catabolised in the proximal tubules. Cystatin C was shown to be an excellent marker of glomelular filtration rate (GFR) in various group of patients, including diabetic subjects. The hepatocyte nuclear factor (HNF-1α) gene mutations cause one of subtypes of MODY. The phenotype is not only an early-onset diabetes, but also renal tubulopathy and impaired synthesis of certain proteins. Our aim was to measure cystatin C serum level in diabetic HNF-1α gene mutation carriers and investigate whether it is altered in this monogenic form of diabetes, in order to evaluate its clinical applicability as a GFR marker.

Materials and methods: We examined 25 HNF-1α MODY patients (mean age at examination 33.6 yrs, mean diabetes duration 8.7 years) and 98 individuals with type 1 diabetes (T1DM) (33.9 yrs and 14.2 yrs, respectively). In all study individuals serum cystatin C level was measured by immunonephelometric method.
Results: The mean cystatin C level was 0.77±0.22 mg/L in HNF-1α MODY and 0.87±0.22 in T1DM, while mean serum creatinine level was 73.0±21.1 umol/L and 68.2±20.1 umol/L, respectively. After adjusting for sex, age, diabetes duration, BMI and HbA1c in linear regression model, there was no significant difference in creatinine levels between the groups, while cystatin C serum concentration was 0.11 mg/L higher in T1DM cohort (p=0.013). We estimated patients' GFR with creatinine-based methods (Cockroft Gault, MDRD and Jelliffe formulas) and cystatin C-based methods (Tan, MacIsaac and Stevens equations). Across all used approaches, there was a consistent, about 15-20 ml/min/1.73m^2 greater difference between creatinine based and cystatin C based GFR estimates in MODY cohort compared to T1DM.
Conclusion: In summary, we present some evidence that cystatin C serum level in HNF-1α MODY might be altered by factors associated with the mutations in this transcription factor. This fact may limit its clinical applicability as a GFR marker in the examined monogenic form of diabetes.
Supported by: CEED3

344

A novel compound heterozygote mutation of transferrin receptor 2 gene exhibits severe diabetes in a Japanese patient with haemochromatosis
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Hereditary hemochromatosis (HH) is a group of disorders characterized by progressive iron overload, which may lead to severe clinical pathological conditions including diabetes and liver cirrhosis. Here we report a novel compound heterozygote mutation in transferrin receptor 2 (TFR2) gene in a Japanese patient with HH who exhibits severe diabetes, liver fibrosis and pituitary hypogonadism. DNA was extracted from whole blood sample with informed consent. The coding regions and splicing sites of the HFE, TFR2, HJV and HAMP genes were analyzed by direct sequencing of PCR-amplified genomic DNA. In the TFR2 gene, a novel compound heterozygote mutation, 1100T>G (L367R) and 2008-9ACdel, were found in this patient with hemochromatosis at the age of 40 years. Abdominal computed tomography showed very high liver density and severely atrophic pancreas. Serum ferritin was 10191 ng/ml, and transferrin saturation rate was 94.2 % and serum hepcidin was 0.6 ng/ml which was extremely suppressed despite of remarkable iron overloading. Liver biopsy showed mild hepatic fibrosis with sever and diffuse iron deposition in both Kupfer cells and hepatocytes. His insulin secretion was severely impaired, requiring treatement with daily fifth insulin injections (44U/day). We previously reported 6 TfR-2 related Japanese HH cases from 4 families for (a homozygous AVAQ 594-597 deletion, L490R and A364T missense mutations, and V561X nonsense mutation in the TFR2 gene). Among 7 TfR2-related cases including the current case, all had liver diseases and 4 had diabetes. Clinical manifestations of these TfR2-HH cases varied widely from almost no symptoms to very severe pathological conditions. HH is thought to be rare in Orientals including Japanese because of the low prevalence of the C282Y mutation of the HFE gene in Asians. However, the current case and our previous results suggest that the TFR2 mutations may not be very rare in hemochromatosis in Japan. Further study is required to elucidate the frequency of TFR2-related HH in patients with diabetes and liver dysfunction in Japan.

345

Increased transferrin saturation and C282Y/C282Y genotype predict diabetes mellitus in two general population cohorts and a case-control study
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Background: Diabetes mellitus is a common late complication to hereditary hemochromatosis. Previous studies of the predictive value of transferrin saturation or hemochromatosis genotypes for diabetes mellitus are hampered by the weaknesses of cross-sectional or case-control study designs in limited study populations. In this study, we investigated for the first time the predictive value of increased transferrin saturation or C282Y/C282Y genotype in a large study of populations of inhabitants from larger Copenhagen by comparing hazard ratios for developing diabetes in the general population with odds ratios for diagnosed diabetes obtained from both a cross-sectional population and a case-control design.
Methods: We examined 9726 individuals from the Copenhagen City Heart Study, 30,000 individuals from the Copenhagen General Population Study, and 6120 patients with either type 1 or type 2 diabetes mellitus representing three study designs: a prospective study with 15 years of follow-up, a case-control study, and a cross-sectional study.
Results: Increasing levels of transferrin saturation above 50% were associated with increased risk of diabetes (p-trend=0.002). In the prospective study, transferrin saturation above 60% was associated with diabetes with a hazard ratio of 2.1(1.1-3.9). In the cross-sectional and case-control studies, odds ratios were 2.0(1.0-3.8) and 2.9(2.0-4.2), respectively. For women, the corresponding risks were 3.7(1.2-11), 4.0(1.1-14), and 4.1(1.8-8.9). The hazard ratio for developing need of any anti-diabetic medication in C282Y/C282Y individuals was overall 2.2(1.0-4.6) and in women 2.9(1.1-2.7); stratification by insulin and oral anti-diabetic medication revealed similar results in women. Kaplan-Meier curves for women indicated that the risks of diabetes mellitus in individuals with transferrin saturation above 60% or need of anti-diabetic medication in the C282Y/C282Y genotype were greater in postmenopausal women. Apart from the case-control study, risks conferred by iron overload or genotype in men were not significantly increased.
Conclusion: Transferrin saturation above 60% and C282Y/C282Y genotype predict diabetes mellitus overall and in women; the greater risk in postmenopausal women is explained in part by cessation of menstrual blood loss and estrogen deficiency promoting reactive oxygen species formation to which pancreatic beta-cells are particularly susceptible.

346

The G-75A polymorphism of the Apolipoprotein A1 gene influences the effect of statin therapy on HDL-cholesterol levels in South Asian patients with type 2 diabetes
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Background and aims: Background: Apolipoprotein A1 (*ApoA1*) is the main structural lipoprotein of the HDL-cholesterol (HDL-C) particle and as such plays an important role in the regulation of reverse cholesterol transport. Several polymorphisms of the *ApoA1* gene have been identified and the G-75A polymorphism has been associated with higher HDL-C levels and in some studies with a greater increase in HDL-C with statin treatment. The effect of the G-75A polymorphism on HDL-C and the influence of statin therapy on HDL-C levels in South Asian individuals with type 2 diabetes, however, have not previously been investigated. The aims of this study were firstly to determine the effect of the G-75A polymorphism on HDL-C levels prior to statin treatment and secondly to examine the effect of statin treatment on HDL-C according to *ApoA1* genotype.
Materials and methods: This was a retrospective study of ethnically distinct Punjabi patients with type 2 diabetes who had been recruited to the UK Asian Diabetes Study. Data from 180 patients (81 men, 99 women) was investigated.

All patients were statin-niave and were commenced on either atorvastatin or pravastatin. Follow-up data was collected after 2-years. Genotyping was performed by restriction fragment length polymorphism.
Results: Three was no significant difference in baseline HDL-C levels between individuals with the -75A allele compared to those homozygous for the -75G allele (1.24+/-0.29 vs. 1.18+/-0.26mmol/l, p=NS). For those patients with the -75A allele there was no significant change in HDL-C with statin therapy (1.24+/-0.29 vs. 1.22+/-0.32mmol/l, p=NS), whereas there was a significant increase in HDL-C in individuals homozygous for the -75G allele (1.18+/-0.26 vs. 1.30+/-0.29mmol/l, p<0.0005).
Conclusion: This study has demonstrated that the G-75A polymorphism of the ApoA1 gene influences the effect of statins on HDL-C levels in South Asian patients with type 2 diabetes. Whether the greater increase in HDL-C levels with statin treatment for individuals homozygous for the -75G allele results in a greater reduced risk of cardiovascular disease requires further investigation.

347

Efficacy and safety of sulphonylurea use in permanent neonatal diabetes due to KCNJ11 gene mutations - minimum 2-year follow-up

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Background and aims: The most frequent causes of permanent neonatal diabetes mellitus (PNDM) are mutations in the KCNJ11 gene encoding the Kir6.2 subunits of the beta-cell K-ATP channel. Recently, many patients with Kir6.2-related PNDM have been successfully transferred from insulin therapy to sulphonylurea (SU) treatment, resulting in an immediate improvement in glycemic control. The long-term impact of SU treatment in PNDM patients, however, has not been determined. We aimed to investigate efficacy and safety of SU treatment in patients with PNDM due to KCNJ11 gene mutations that were on SU over a period of minimum 2 years.
Materials and methods: We monitored glycemic control and the occurrence of potential side effects in 14 Kir6.2-related PNDM patients from Poland (mean age 14.0 years, range 5-50) that carried various KCNJ11 mutations and that were on SU over the period of at least 2 years.
Results: The average HbA1c level prior to the switch from insulin to SU was 7.69% (range 6.3-10.2). A rapid improvement in glycemic control was achieved, as the mean reduction of HbA1c levels during the first visit following the transfer to SU (approximately 3-6 months post-transfer) was 1.68%. This level of glycemic control was maintained over the entire period of observation (median 34 months, range 27-51) with an average HbA1c level of 6.0% (range 5.3-6.7%) at the last visit. Three of the 14 patients were lost to follow-up: one was not available for examination, while SU treatment was discontinued in two others as a result of a diagnosis of hepatitis C and for socioeconomic reasons, respectively. Of the 11 patients observed over the entire follow-up period, a rapid progression of retinal changes was observed in one patient, a 34 years old woman, with pre-existing proliferative diabetic retinopathy. No causal relationship between these changes and SU treatment could be proven. In all the others patients, no side effects were observed.
Conclusion: In summary, the switch from insulin therapy to SU treatment in PNDM related to KCNJ11 mutations was found to be an efficient therapeutic method over a period of at least 2 years. Although no side effects were associated with SU treatment, its role in the development of chronic disease complications requires further attention.
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348

The comparative metabolomics of the experimental rat and diabetic patient urinary composition

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Background and aims: Metabolomics has developed to an accepted and valuable diagnostic tool to distinguish healthy and diseased state individuals. The comparative metabolomics of the diabetic experimental animal and patient samples is important for translational research and early diagnosis of Type 2 diabetes mellitus (DM). Among commonly used experimental models of diabetes, Zucker (fa/fa) rats represent obesity related disease state, whereas Goto-Kakizaki (G-K) rats are characterized by impaired glucose tolerance. The aim of the study was to develop appropriate methodology for metabolomic studies of urine samples taken from healthy and diabetic individuals.
Materials and methods: In present study, we used metabolomic analysis strategy based on nuclearmagnetic resonance (NMR) method to compare metabolite profiles of urine samples excreted by male G-K and Zucker (fa/fa) rats with those of age matched Wistar rats, as well as Type 2 DM patients and healthy individuals. Animals (male rats, 12 weeks at the beginning of experiment): 30 G-K and 10 Wistar rats; 40 Zucker (fa/fa) and 10 Wistar rats. Samples: Averaged daily urine samples were collected at the age of 12,14,16 and 20 weeks. Patients: 43 randomly selected human urine samples of Type 2 DM patients, and 20 samples of healthy volunteers. Chenomx NMR software was used for data processing and metabolite identification. Principal Components Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) analyses were carried out by pls and caret packages within statistical language R using in-house routines. PLS-DA models were evaluated using bootstrap resampling and cross model validation methods.
Results: PLS-DA models showed good classification accuracy between Wistar - G-K and Wistar -Zucker (fa\fa) animal strains (accuracy > 0.90). Eligible classification accuracy was obtained also by evaluation PLS-DA models of human urine samples (accuracy > 0.75). The metabolomic signatures of analyzed urine samples from Type 2 DM showed metabolite patterns different from those of healthy individuals both in experimental animal and patient samples. The metabolite profile included changed levels of creatine, citrate, hippurate, phenylalanine and some unidentified metabolites in the urine samples of G-K rats compared to Wistar rats, as well as Zucker (fa/fa) compared to Wistar rats and in the urine samples of Type 2 DM patients compared to healthy volunteers. Increased concentrations of glucose, niacinamide and trigonelline were observed in Zucker (fa/fa) and clinical samples, but not in G-K rat samples. Changes in formate and lactate concentrations were observed in G-K animal samples, but not in Zucker (fa/fa) and Type 2 DM patient samples.
Conclusion: The metabolomic studies of urine samples from diabetic individuals provide further insights concerning experimental methodologies for data generation and processing, as well as possible markers for diabetes research. Even though several components of metabolic profiles in Zucker (fa/fa), G-K rats and Type 2 DM patient samples are similar, our results give evidence that both models of experimental diabetes and clinical samples are clearly different in respect to metabolomic studies, which might be useful for evaluation of drug effects and understanding of metabolic syndrome and diabetes related metabolic pattern fingerprints.
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349

Detection of plasma amino acids and metabolites by mass spectrometry and magnetic resonance spectroscopy: effects of insulin deprivation in type 1 diabetes

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Background and aims: Nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry (MS) provide a wealth of distinct and complementary information from biological specimens that can help understand how biochemical pathways that are altered in by insulin deficiency. We used both of these analytical tools to determine the impact of insulin deficiency on plasma metabolic profiles during insulin deprivation and insulin treatment in type 1 diabetic participants.

Materials and methods: Type 1 diabetic individuals (N=7) were studied during insulin treatment (glucose=5.3±0.1 mM) and following 8 hours insulin deprivation (16.7±0.7mM) and compared with non-diabetic controls. Plasma samples were analyzed by ^{1}H NMR and by liquid chromatography tandem mass spectrometry following derivatization with Waters Masstrak AAA solution.
Results: Plasma glucose, acetone, acetoacetate and beta-hydroxybutyrate (by NMR) and branched chain amino acids (by MS) were elevated (P<0.05) during insulin deprivation as expected. In contrast, the plasma concentrations of gluconeogenic amino acids (by MS) were lower or unchanged during insulin deprivation. Many amino acid metabolites such as alpha-amino-N-butyric acid, alpha-aminoadipoic acid, beta-amino butyric acid and cystathionine were elevated (P<0.05) during insulin deprivation. Plasma lactate, acetate and citrate (citric acid cycle) and allantoin (index of oxidative stress) (by NMR) were all elevated (<0.05) during insulin deprivation. Five amino acids (valine, isoleucine, alanine, tyrosine, and histidine) were measured by both methods and demonstrated similar absolute micromolar concentrations and effects of insulin deprivation. All changes in metabolic pathways were normalized by insulin treatment in comparison with non-diabetic participants.
Conclusion: The concentrations of five amino acids were similar when measured by both MS and NMR, underscoring the sensitivity and accuracy of both analytical techniques for these compounds. The MS-based approach for measuring plasma amino acids and its metabolites revealed not only an expected increase in branched chain amino acids during insulin deprivation but also an increase in concentrations of many individual amino acid metabolites, indicating insulin's specific effects on amiDetectionno acid metabolism. The observed decrease in many gluconeogenic amino acids is consistent with enhanced gluconeogenesis during insulin deprivation. Furthermore, NMR analyses revealed a unique metabolic fingerprint of insulin deprivation in diabetes, evidenced by significantly (P<0.05) elevated tricarboxylic acid cycle intermediates. It is likely that plasma citrate levels are elevated as a result of a downstream limitation in the tricarboxylic acid cycle or mitochondrial electron transport. These results support that insulin deficiency causes muscle mitochondrial dysfunction, and accumulation of citrate may have increased the acetyl CoA/CoA ratio, resulting in less acetylation of acetate and increased conversion of pyruvate to lactate. Very high allantoin in plasma is indicative of high oxidative stress during insulin deficiency. These results demonstrate the potential of MS and NMR analyses of plasma as a powerful tool to map altered metabolic pathways in diabetes and related metabolic disorders.
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350

The genetic association between *HK1* and increased HbA_{1c} but not other glycaemic control related traits reveals the link between anaemia and HbA_{1c}

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Background and aims: Recently, a genome-wide association study reported an association between glycated hemoglobin (HbA1c) and rs7072268 within *Hexokinase1* (*HK1*). HK1 is ubiquitously expressed and contributes to catalyse the first step in the glycolytic pathway. Deficiency of HK1 in red blood cells (RBC) causes severe nonspherocytic hemolytic anemia in both humans and mice. Here we studied the contribution of rs7072268 to HbA1c, glucose homeostasis and type 2 diabetes (T2D) in several prospective European cohorts (overall ~14,000 individuals). In addition, we assessed the impact of rs7072268 on two RBC-related (hemoglobin and hematocrit).
Materials and methods: The impact of rs7072268 on HbA1c and the hematologic traits was first assessed in 4,663 non diabetic middle-aged French adults from the DESIR cohort and in 2,474 non diabetic obese Swiss adults. Other glucose homeostasis related phenotypes were studied in DESIR as well as in 5,346 individuals from the Northern Finland Birth cohort at 16 years of age; in 1,473 young French adults from the Haguenau study; and in 2,475 additional non diabetic French subjects. A T2D case-control study included 7,572 T2D French individuals and 5,562 controls. All genotyping was performed using a TaqMan assay.
Results: The rs7072268-T allele strongly associates with increased HbA1c (under an additive model, ß = $0.031\%_{HbA1c}$, overall p = $3.06x10^{-6}$). Paradoxically, we found no significant association with any other marker of glucose control (fasting glucose, 2h-post OGTT glucose levels, fructosamine levels). In contrast, the rs7072268-T allele decreases both hematocrit and hemoglobin levels (ß = $-0.166\%_{Hematocrit}$, p = $8.57x10^{-4}$ and ß = -0.056g/dl, p = $8.81x10^{-4}$, respectively). The T2D case-control analysis displayed no evidence for association after correction for multiple testing. However, a modest association was found with early-onset T2D only (under the best model [recessive], OR = $1.21\ [1.15\text{-}1.27]_{95\%CI}$, p = $1.70x10^{-3}$), and the rs7072268 genotype distribution was statistically different between early and late-onset diabetics (p = $5.20x10^{-3}$). In contrast, the rs7072268-T allele improves insulin sensitivity (Homa-IR) with no significant effect on insulin secretion Homa-B index after correction for multiple testing.
Conclusion: The *HK1* rs7072268-T allele strongly associates with increased HbA1c in European populations. Surprisingly, despite adequate study power, rs7072268 is not associated with other markers of glucose control. An explanation of this paradox may be its deleterious effect on RBC-related hemoglobin and hematocrit. HK1 may impair the relationship between serum glucose levels and HbA1c through its anaemic effect, which may have clinical implications for T2D diagnosis and management. The putative association with early-onset T2D is under investigation in large sample sets.

PS 12 Cytokines and ER stress in beta cells

351

Analysis of compartiment-specific caspase activation and oxidative damage through proinflammatory cytokines in insulin-producing cells

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Background and aims: Type 1 diabetes is an autoimmune disease characterised by selective destruction of pancreatic ß-cells. Proinflammatory cytokines, such as IL-1ß, IFN-γ and TNF-α, are key mediators of ß-cell death in autoimmune diabetes mainly by the induction of signalling pathways ultimately leading to apoptosis. In this process, reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated during cytokine-induced ß-cell destruction are involved as mediators of ß-cell death. In previous studies we have shown that overexpression of a mitochondrially located catalase (MitoCat) prevented the increase of caspase-9 activity after cytokine treatment. Therefore, the aim of this study was to characterise the influence of antioxidative enzymes and proinflammatory cytokines on the activation of ER-specific caspase-12 and additionally to investigate the interaction of cytokine-mediated ER-stress, mitochondrial apoptosis induction and oxidative DNA damage.
Materials and methods: Quantification of caspase-9 and caspase-12 activity in insulin-producing RINm5F control cells and in cells overexpressing cytosolic catalase (CytoCat) or mitochondrially located catalase (MitoCat) was performed after a 24 h exposure to IL-1ß or to a cytokine mixture (IL-1ß, TNF-α und IFN-γ) by flow cytometry. In order to examine the contribution of caspase-12 in cytokine-mediated ß-cell death, we additionally treated cells with a caspase-12 specific inhibitor. The cell viability was measured by the MTT assay and the oxidative DNA damage caused by hydroxyl radicals was quantified with 8-oxoguanine as a specific biomarker in a flow cytometry based assay after 72 h cytokine exposure.
Results: Exposure of insulin-producing RINm5F control cells to cytokines resulted in a significant activation of caspase-9 and ER-specific caspase-12. A comparable cytokine-mediated activation of caspase-12 was also observed in Cyto- and MitoCatalase overexpressing cells. However, the overexpression of MitoCatalase prevented the increase of caspase-9 activity after cytokine stimulation. Treatment of cells with a caspase-12 inhibitor led to a significant decrease of cytokine-mediated caspase-12 activation. Nevertheless, the inhibition of caspase-12 had no effect on caspase-9 activation and the cell viability was in the presence or absence of the caspase-12 inhibitor significantly decreased after cytokine incubation. Furthermore, the exposure of control and CytoCat cells to cytokines resulted in a significant induction of cytokine-reinforced iron catalysed hydroxyl radical mediated DNA damage, while the overexpression of MitoCat efficiently diminished the formation of 8-oxoguanine.
Conclusion: These results suggest that the mitochondrial apoptosis pathway is crucial for cytokine-mediated ß-cell death, whereas the inhibition of the ER-specific caspase-12 had no influence on cytokine-induced decrease of cell viability. Overexpression of mitochondrially located MitoCatalase could successfully suppress activation of caspase-9 and cytokine-induced hydroxyl radical mediated DNA damage, resulting in a greater resistance against proinflammatory cytokines. These data indicate that caspase-9 and not caspase-12 activation is crucial for apoptosis induction in insulin-producing cells and there is no crosstalk between the activation of these two compartment-specific caspases.

352

Inhibition of palmitate- and thapsigargin-induced GADD34 expression is associated with increased beta cell apoptosis

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Background and aims: Phosphorylation of eukaryotic translation initiation factor 2α (eIF2α) on its serine 51 residue reduces the rate of protein synthesis. The gene product of EIF2AK3, PERK, is a protein kinase residing in the endoplasmic reticulum (ER) membrane and is responsible for eIF2α phosphorylation under conditions of compromised ER function. Pancreatic β-cells are vulnerable to disturbances in PERK signalling. This is indicated by the diabetic phenotype associated with mutations in the genes PERK or eIF2α that abolish the ability to phosphorylate eIF2α. The phosphorylation status of eIF2α (p-eIF2α) is also controlled by dephosphorylation events catalyzed by protein phosphatase 1 and its regulatory subunit 15A, also known as GADD34. Expression of GADD34 is enhanced when p-eIF2α is increased and provides negative feedback loop. Pharmacological inhibition of eIF2α dephosphorylation by salubrinal has previously been shown to induce apoptosis by itself and to potentiate palmitate-induced apoptosis. However, since salubrinal increases expression of GADD34 the effects of this compound might depend on elevated levels of GADD34. The aim of the present study was to further investigate the relationship between GADD34 expression and pancreatic β-cell death induced by palmitate and thapsigargin.
Materials and methods: MIN6 cells were treated with a siRNA directed against GADD34. After siRNA treatment cells were exposed to 0.5 mM palmitate or 300 nM thapsigargin. After culture we assessed apoptosis induction by measuring DNA degradation. We also analyzed the phosphorylation status of PERK, eIF2α and protein levels of CHOP by Western blotting. Protein synthesis was assessed by measuring incorporation of ^{35}S-labeled serine and methionine. Cellular levels of mRNA were measured by quantitative PCR. Data is presented as means±sem and differences were evaluated by Students t-test.
Results: Treating MIN6 cells with thapsigargin activated PERK, as assessed by its phosphorylation. After 2 h of treatment, PERK phosphorylation had increased to 169±24 % ($p<0.05$) compared to untreated cells. Its activation increased steadily throughout the 22 h exposure period ending at 524±12 % of baseline level. In contrast, p-eIF2α peaked already after 4 h of treatment at 455±48 % ($p<0.05$) compared to untreated cells and retuned to basal levels after 18 h. This indicates a strong negative feedback. Indeed, in control cells thapsigargin caused 25±4 fold increase in GADD34 mRNA levels while the induction was only 3±0.6 fold in siRNA-treated cells. In concordance, the rate of protein synthesis paralleled p-eIF2α. Exposing cells to palmitate gave a similar, but somewhat delayed, response pattern. In cells with reduced levels of GADD34, p-eIF2α expression was enhanced by palmitate (60±15 %) and thapsigargin (45±10 %) treatment compared with negative siRNA-treated cells. Also, CHOP protein levels were increased by palmitate (50 %) and thapsigargin (35 %) treatment. Apoptosis, measured as DNA degradation, was enhanced in cells with reduced levels of GADD34 by palmitate (25±3 %) and thapsigargin (20±4 %) treatment compared with negative siRNA-treated cells.
Conclusion: In the present study, we show that inhibition of palmitate- and thapsigargin-induced expression of GADD34 is associated with increased pancreatic β-cell apoptosis. Hence, treating β-cells with a siRNA against GADD34 gives a phenotype similar to that obtained by salubrinal treatment.
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353

Inflammatory cytokines and ER stress converge on DP5/Hrk activation: a novel mechanism for pancreatic beta cell apoptosis

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Background and aims: Cytokines contribute for pancreatic beta cell apoptosis in type 1 diabetes (T1D) but the mechanisms leading to cell death remain to be clarified. Based on microarray analysis of insulin-producing INS-1E and primary beta cells we have identified upregulation of death protein 5 (DP5)/harakiri (Hrk) after cytokine treatment. DP5 belongs to the pro-apoptotic BH3-only protein family. We presently evaluated the role of DP5 for ER stress- and cytokine-induced beta cell dysfunction and death.
Materials and methods: INS-1E and primary FACS-purified beta cells were transfected with control or siRNAs targeting DP5 and treated with cytokines (IL-1β 10-50 U/ml+ IFN-γ 100 U/ml) or the ER stressor CPA (20 μM). The NO blocker L-NMA was used at 2.5 mM and the JNK inhibitor SP600125 at 10 μM. Real time RT-PCR, reporter analysis, immunofluorescence, Western blot, and viability assays (Hoechst/PI and caspase-3 activation) were performed to identify proteins involved in the mechanisms of DP5-mediated apoptosis.
Results: Cytokines induced DP5 expression in primary beta cells and INS-1E cells (3-25 fold increase in mRNA expression, n = 3-4, $P<0.05$). DP5 knockdown (KD) by RNA interference (>80% inhibition) prevented cytokine-induced cell death in INS-1E cells (14% apoptosis in cytokine-treated DP5 KD

cells vs. 29% apoptosis in cells treated with cytokines and control siRNA, n = 5, P<0.001). Similar findings were observed in primary beta cells. ER stress induced by CPA also increased DP5 expression and its inactivation by KD decreased apoptosis in primary beta cells (19% apoptosis in DP5 KD and CPA-treated vs. 30% apoptosis in CPA-treated control cells; n = 3; P<0,05). CPA-induced DP5 upregulation was prevented by chemical JNK/c-Jun inactivation. DP5 activation by cytokines also involves JNK/c-Jun phosphorylation and was antagonized by the anti-apoptotic protein JunB. Importantly, mitochondrial release of cytochrome *c* and ER stress are actually a consequence of potentiated DP5 activation via cytokine-mediated nitric oxide (NO) formation. Combined inhibition of JNK/c-Jun and NO formation abolished cytokine-induced DP5 mRNA upregulation and fully protected INS-1E cells from apoptosis (20% apoptosis in cytokine-treated cells vs. 6% apoptosis in JNK/NO inactivated cells treated with cytokines, n = 5, P<0.001).
Conclusion: Our findings demonstrate that DP5 is central for beta cell apoptosis following different stimuli, and that it can act up- and downstream of ER stress. These observations contribute to solve two important questions, namely the mechanism by which cytokines induce beta cell death and the nature of the downstream signals by which ER stress "convinces" differentiated secretory cells, such as beta cells, to trigger apoptosis. DP5 may be thus an interesting target for the prevention of beta cell demise in T1D.
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354

The role of endoplasmic reticulum stress induced by glucosamine in pancreatic beta cells dysfunction
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Background and aims: The endoplasmic reticulum (ER) represents the cellular compartment where newly synthesized proteins acquire their correct folding. Many factors can interfere during this process, leading to the accumulation of misfolded proteins into the ER. Such ER dysfunction is collectively termed "ER stress". To survive under ER stress conditions, cells activate a self-protective mechanism, termed the unfolded protein response (UPR). The ER dysfunction plays an important role in various diseases including Type 2 diabetes (T2D). It has been reported that hyperglycaemia (HG) causes the progressive deterioration of beta cells through a mechanism called glucose toxicity and that ER stress may be involved in the consequent pancreatic beta cell dysfunction. Glucosamine (GlcN), generated by the hexosamine pathway (HP) during HG, induces ER stress and causes disturbances similar to glucose toxicity. In this study, we sought to evaluate the role of ER stress induced by GlcN in cultured beta cells (INS-1E) and in isolated pancreatic mouse islets.
Materials and methods: Islets were isolated from male C57 mice by pancreatic digestion with collagenase. Total RNA was extracted from INS-1E cells or islets by the acid phenol method, and real-time PCR was performed to analyze the expression pattern of both beta-cell and UPR specific genes. Protein levels were measured by Western blotting; protein subcellular distribution and expression by immunofluorescence. Cell viability was assessed by MTT assays.
Results: MTT assays showed that 24 hours incubation with 10 mM GlcN did not affect INS-1E cells viability. In these conditions, both mRNA and protein levels of the ER stress marker BiP increased by 3.5- and 2.5-fold, in mouse islets and in INS-1E cells, respectively. Furthermore, GlcN determined also a 2-fold increase of Chop mRNA levels, another important marker of the UPR. In isolated islets, GlcN decreased by 70% the mRNA levels of both GLUT2 and glucokinase (GK) and by 80% the Insulin1 (Ins1) mRNA levels. Interestingly, similar results were obtained when INS-1E cells and islets were treated with Tunicamycin (Tun), a classical ER stress inducer that inhibits glycosilation. These effects were very likely exerted at the transcriptional level, as demonstrated by a parallel downregulation of the mRNA of the beta-cell specific transcription factors Pdx1 and NeuroD1. Furthermore, in INS-1E cells, confocal immunofluorescence studies showed a loss of the specific insulin signal constituted by secretory vescicles clustered in a sub-plasmamembrane location. Treatment of INS-1E cells with the chemical chaperon 4-Phenyl Butyric Acid (PBA 2.5 mM) was capable of partially prevent ER stress induced by GlcN and Tun, as demonstrated by a reduced induction of BiP mRNA levels. Furthermore, GLUT2 and Ins1 mRNA levels reduction was almost completely abolished. Oxidative stress appeared not to be involved in ER stress induction by GlcN, as treatment of INS-1E cells with the antioxidant N-acetyl-L-cysteine (NAC 1mM) did not modify the GlcN-induced increase of BiP mRNA levels.
Conclusion: The activation of the hexosamine pathway leads to alterations in the expression pattern of beta cell specific genes through the induction of ER stress implying that this mechanism could be responsible at least in part, for glucotoxicity-induced beta cell dysfunction.

355

Protection against stress-induced apoptosis by high density lipoproteins in pancreatic beta cells
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Background and aims: High Density Lipoproteins (HDL) have been reported to protect pancreatic beta against cytokine-, serum deprivation-, and oxidized LDL-induced apoptosis. We have recently determined that HDLs could also protect beta cells against ER (endoplasmic reticulum) stress-induced apoptosis in a very effective manner. As ER stress is one of the mechanism that induces beta-cells failure and death in the development of T2D and because the mechanisms of protection of HDLs are not well known in pancreatic beta-cells, we have started to assess by which mechanisms HDLs were protecting beta cells from ER stress.
Materials and methods: MIN6 cells were treated with 2 micro g/ml tunicamycin or 0.5 µM thapsigargin for 6 or 24 hours in the presence or in the absence of 1 mM HDL-cholesterol. ER stress markers (BiP protein expression levels, XBP1 splicing, ATF4 mRNA levels) and apoptosis were then assessed. The activation of PI3K-Akt pathway was also determined by Western blot analyses.
Results: By scoring pycnotic nucleus we confirmed that HDLs were protective against thapsigargin (TG) and tunicamycin (TM)-induced apoptosis. We then assessed the activation of the three ER stress pathways in presence of TG or TM with or without HDLs. ER markers induced by TG such as XBP-1 splicing or BIP expression were blocked by HDLs. ATF4 mRNA was partially reduced, suggesting that HDL could be able to act upstream of the ER, where TG is acting. However these markers, that are also induced by TM, were not blocked by HDLs even if the cells were partially protected, suggesting that in this case HDL could act more downstream of the ER response. Secondly, we looked at the involvement of the PI3K/Akt pathway by using the PI3K inhibitor LY294002 and a dominant negative form of Akt. PI3K inhibition was found to be deleterious for beta cells but HDL could partially reverse this toxic effect. In addition, when PI3K was inhibited, HDLs could still activate Akt. However, in the presence of a dominant-negative form of Akt, HDLs were still able to protect against TG- and TM-induced apoptosis in MIN6 cells, suggesting that the PI3K/Akt pathway might not be required for HDLs to protect beta cells.
Conclusion: These data demonstrate the potent protective effect of HDLs on beta cells experiencing ER stress. These results suggest that protocols aimed at increasing HDL blood levels could exert protective functions against development of diabetes. Further characterization of the intracellular signals modulated by HDLs and that favour beta cell survival is now crucially warranted.
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356

3 b hydroxysteroid-delta 24 redactase (DHCR24) protects pancreatic beta cells against endoplasmic reticulum (ER) stress induced apoptosis
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Background and aims: 3 beta-Hydroxysteroid-delta 24 reductase (DHCR24) is an endoplasmic reticulum-resident, multifunctional enzyme that possesses anti-apoptotic and cholesterol-synthesizing activities. We demonstrated that DHCR24 protects embryonic fibroblasts from apoptotic cell death induced by ER stress. We also proved that the protection was achieved by eliminating intracellular ROS produced by ER stress. Accumulating evidence suggests that endoplasmic reticulum (ER) stress involved in the pathogenesis of diabetes, contributing to pancreatic beta-cell loss and insulin resistance. The aim of the study was to investigate whether DHCR24 protect the apoptosis of pancreatic beta-cells induced by ER stress.

Materials and methods: MIN6 cells were cultured in the growth medium and infected by adenovirus expressing DHCR24 tagged with myc (Ad-DHCR24-myc) or transfected with siRNA targeting DHCR24 (siDHCR24). The Adenovirus expressing lacZ (Ad-lacZ) or non-targeting siRNA (siControl) were used as the controls. Tunicamycin (TM) was used as the ER-stress inducer. Western blotting was used to analyze the signaling cascade caused by ER stress. The survival of beta-cells were observed by the phase-contrast microscopy and calculated by dye-exclusion method using Trypan Blue. Apoptosis was determined by measuring the cleaved-caspase 3 by immuno-cytochemistry.
Results: Ad-DHCR24-myc infected MIN6 cells were resistant to TM-induced apoptosis, as compared to the Ad-LacZ infected cells. Western blot analysis revealed that expression of Bip/Grp78, a major ER chaperone, was increased stronger in Ad-DHCR24-myc cells. The sustained activation of ATF6 (Activating transcription factor-6) was also observed in Ad-DHCR24-myc infected MIN6, suggesting the upregulation of Bip induced sustained activation of ATF6, which might contribute to the anti-apoptotic function of DHCR24 upon the ER stress. When the mRNA of DHCR24 has been knocked down by the siDHCR24 in MIN6, the cells became much more susceptibile to ER stress, producing much more apoptotic cells, compared to the siControl-transfected cells.
Conclusion: This is the first demonstration that DHCR24 can protect pancreatic beta-cell from apoptotic cell death induced by ER-stress.
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357

Exendin-4 and cAMP protect pancreatic beta cells from lipotoxic ER stress via induction of antiapoptotic defense mechanisms
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Background and aims: Chronic exposure of pancreatic beta cells to saturated free fatty acids (FFA) causes endoplasmic reticulum (ER) stress and apoptosis, and may contribute to beta cell loss in type 2 diabetes. GLP-1 receptor activation has previously been shown to increase beta cell survival following exposure to synthetic ER stressors through ATF4-CHOP signaling and eIF2α dephosphorylation. We presently evaluated the molecular mechanisms involved in the protection by the GLP-1R agonist exendin-4 against the more physiologic ER stress and apoptosis induced by FFA in beta cells.
Materials and methods: INS-1E or FACS-purified primary rat beta cells were exposed to the FFA oleate or palmitate (0.5 mM, 1% BSA) with or without exendin-4 (10-50 nM) or the cAMP generator forskolin (20 μM). To selectively activate signaling in the ATF4-CHOP branch of the ER stress response, we used salubrinal (75 μM), an inhibitor of eIF2α dephosphorylation. Apoptosis was detected by Hoechst/propidium iodide staining, mRNA expression by real time RT-PCR and protein expression and eIF2α phosphorylation by Western blot. Protein knockdown was achieved by siRNA transfection.
Results: Palmitate-induced primary beta cell apoptosis was reduced from 23 ± 2 % to 14 ± 2 % and 10 ± 1 % by exendin-4 and forskolin respectively ($n = 4$; $p < 0.05$ vs palmitate) after 24 h. Exendin-4 and forskolin also prevented beta cell apoptosis induced by oleate (14 ± 2 % to 9 ± 2 % and 7 ± 1 %; $n = 4$; $p < 0.05$) or salubrinal (18 ± 1 % to 11 ± 1 % and 9 ± 1 %; $n = 4$; $p < 0.05$) after 24 h. Similar findings were observed in INS-1E cells (14 h). Using CHOP knockdown (93 % inhibition of CHOP protein compared to control siRNA treated with palmitate; $n = 3$; $p < 0.05$) we examined the involvement of ATF4-CHOP-GADD34 feedback signaling on forskolin-mediated INS-1E cell survival. The CHOP siRNA did not abrogate the protective effects of forskolin against palmitate (18 ± 3 % for control siRNA to 17 ± 3 % for CHOP siRNA; $n = 4$), excluding this pathway as the antiapoptotic mediator. We therefore examined the other branches of the ER stress response. Exendin-4 and forskolin induced XBP1s but its knockdown (74 % decrease in XBP1s protein; $n = 3$; $p < 0.05$) did not modify the antiapoptotic effect. Exendin-4 and forskolin potentiated the induction of the ER chaperone BiP by oleate (1.8- and 1.6- fold; $n = 4$; $p < 0.05$) or palmitate (1.7- and 2.1-fold; $n = 4$; $p < 0.05$). A 50 % decrease in BiP expression by siRNA resulted in a partial loss of the cAMP-mediated protection against palmitate (13 ± 1 % for control siRNA to 29 ± 3 % for BiP siRNA; $n = 4$; $p < 0.05$). The AP-1 subunit JunB, known to protect beta cells against synthetic ER stress, was also induced by forskolin (5-fold; $p < 0.05$). JunB knockdown abolished the cAMP protection against oleate, and it partially decreased the protection against palmitate (17 ± 2 % for control siRNA to 28 ± 2 % for JunB siRNA; $n = 4$; $p < 0.05$).
Conclusion: Exendin-4 and forskolin protect beta cells against lipotoxic ER stress and apoptosis by enhanced BiP and JunB expression. These findings identify two possible therapeutic targets to alleviate beta cell ER stress in type 2 diabetes.
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358

Protein levels of GRP78 and protein disulfide isomerases A3 and A6 are decreased during thapsigargin treatment of INS-1E cells
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Background and aims: Pancreatic β-cells are equipped with a developed endoplasmic reticulum (ER) in order to meet the high demand for synthesis of proteins destined for secretion, most notably insulin. To support the production, these cells express relatively large amounts of heat shock proteins with chaperone functions, protein disulfide isomerases (PDI) that catalyse formation of sulfide bridges and peptidylprolyl isomerases (PPI) which are responsible for isomerisation of proline peptide bonds. The ER is sensitive to alterations in homeostasis. For example, a reduction of ER Ca^{2+} levels elicits a signal response, the unfolded protein response (UPR), with the purpose to restore ER homeostasis by decreasing protein synthesis, increasing folding capacity and enhance protein degradation. In the present study we have focused on changes in protein levels of chaperones, PDIs and PPIs in INS-1E cells during thapsigargin (Th) treatment.
Materials and methods: INS-1E cells were treated with 200 nM Th for 0, 4, 16 or 24 h, cellular protein were isolated and subjected to iTRAQ labelling using 8 different isobaric tags. Protein identification and quantification was performed with LC/MS/MS. The phosphorylation status of eIF2α (p-eIF2α), protein levels of GRP78, CHOP, Bcl2 and cleaved caspase 3 were analysed by western blotting (wb). Protein synthesis was measured after incorporation of ^{35}S-labeled serine/methionine. Data is presented as means±sem. Differences were evaluated by Student's t-test.
Results: Quantitative iTRAQ proteomic analysis offers a way to identify and quantify 8 different samples in one homogenous assay. Two independent samples from each time point were combined in the analysis. From the positively identification of 91 unique proteins 8, 2 and 2 were classified as chaperones, PDIs or PPIs, respectively. Of the chaperones 4 localise to cytoplasm, 3 to mitochondria and 1 to ER. Heat shock protein 90β (cytoplasmic) and GRP78 (ER) decreased ($p<0.05$) with time. Other chaperones were unaffected. The PDIs, PDIA3 and PDIA6 (both localise to ER) decreased ($p<0.05$) with time ending at 73±6 % and 26±4 % of their baseline levels, respectively. Of the PPIs identified, PPIA (cytoplasmic) and PPIB (ER) levels were not affected by Th. The decreased level of GRP78 was unexpected since this chaperone is considered to be induced during UPR. The iTRAQ results could be verified in wb experiments. Treating cells with 30, 100, 200 or 300 nM Th dose-dependently decreased GRP78 levels with a maximal effect at 200 nM (45±4 %, $p<0.05$ *vs.* untreated cells). The time profile of Th-induced decrease in GRP78 was similar in the iTRAQ and wb analysis. Lowest levels, 35±2 % for iTRAQ and 51±4 % for wb, were seen after 16 h at 200 nM Th. In both dose and time series experiments, Th-induced decrease in GRP78 was paralleled by enhanced levels of p-eIF2α, CHOP and cleaved caspase 3 and decreased levels of Bcl2. We investigated if the lower levels of GRP78 after Th treatment were secondary to inhibition of protein synthesis. Exposing INS-1E cells to either 10 μM cycloheximide or 200 nM Th casued a similar inhibition of protein synthesis. However, cycloheximide only decreased GRP78 levels by 10±3 % ($p>0.05$) during a 24 h study period.
Conclusion: Disturbance of ER Ca^{2+} homeostasis leads to lower levels of GRP78, PDIA3 and PDIA6 in INS-1E cells which may contribute to the development of apoptosis.
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359

Role of activating transcription factor 3 in low glucose- and thapsigargin-induced apoptosis in cultured mouse islets

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Background and aims: The Integrated Stress Response (ISR) results from the phosphorylation of eukaryotic initiation factor 2 alpha by various types of stress, including endoplasmic reticulum (ER) stress and amino acid deprivation. It is characterized by increased expression of Activating Transcription Factor 4 (ATF4)-target genes such as *Atf3, Growth arrest- and DNA damage-inducible gene 153 (Gadd153, or Chop)* and *Gadd34*, and of oxidative stress-response genes like *c-Myc*, *Heme-oxygenase 1* (*Hmox1*) and *Metallothionein 1a* (*Mt1a*). We recently reported that, after prolonged culture in low, intermediate and high glucose concentrations, rat islet cell apoptosis follows an asymmetric V-shaped profile with a minimum in 10 mM, a large increase in 2 mM and a moderate increase in 30 mM glucose. Interestingly, these changes were preceded by parallel changes in the mRNA levels of ISR and oxidative stress-response genes, suggesting that both types of stress could play a role in islet cell apoptosis during culture in low and high glucose. The aim of this study was to investigate the role of the pro-apoptotic transcriptional repressor ATF3 in the stimulation of islet cell apoptosis under conditions associated with ISR activation.
Materials and methods: Islets isolated from *Atf3* knockout mice (*Atf3*$^{-/-}$) and their wild-type C57BL6/J littermates (WT) were cultured in serum-free RPMI medium containing 5g/l BSA and 2-10-30 mM glucose (G2-G10-G30) or G10 + 1 μM thapsigargin (TG) as ER stress positive control. Insulin was measured by RIA. Islet gene mRNA levels were determined by RTq-PCR. Islet cell histone-associated DNA fragments (commercial ELISA kit) and the percentage of TUNEL positive islet cells were measured as indicators of islet cell apoptosis. Results are means ± SEM for at least 3 experiments.
Results: After 1 week preculture in G10, the glucose stimulation of insulin secretion during further 18h culture and the insulin to DNA content ratio at the end of culture were similar in *Atf3*$^{-/-}$ and WT islets. As in rat islets, culture of WT mouse islets in G2 or G10 + TG vs G10 rapidly (18h) increased *Atf3*, *Gadd153, Gadd34, c-Myc, Hmox1* and *Mt1a* to *Tbp* mRNA ratios and later (3-7 days) induced islet cell apoptosis. In contrast, culture in G30 only slightly increased the mRNA levels of ISR and oxidative stress response genes as well as islet cell apoptosis. In *Atf3*$^{-/-}$ islets, the effects of TG and G30 vs G10 on apoptosis and gene mRNA levels were not different from those in WT islets. In contrast, the level of apoptosis induced by one week culture in G2 was significantly lower in *Atf3*$^{-/-}$ vs WT islets in one third of the experiments (mean [range] reduction for 6 experiments ~20% [0-60%], unpaired t-test P=0.4), irrespective of mouse gender and age. This slight reduction in islet cell apoptosis in G2 vs G10 was preceded by a larger increase in *Mt1a* mRNA levels in *Atf3*$^{-/-}$ vs WT islets (50 fold vs 28 fold, P<0.001 by two-way ANOVA + test of Bonferroni) without significant changes in *Hmox1, c-Myc, Gadd153* or *Gadd34* mRNA levels.
Conclusion: The increase in *Atf3* gene expression induced by culture in a low glucose concentration only slightly contributes to the stimulation of islet cell apoptosis by a mechanism that may involve repression of *Mt1a* gene expression.

360

Nitrooxidative stress as a mechanism of cytokine-mediated toxicity in insulin-producing cells

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Background and aims: Both nitrosative and oxidative stress contribute to the toxic action of proinflammatory cytokines in insulin-producing cells during T1DM development and it is generally assumed that mitochondria play a crucial role in cytokine-mediated beta cell death. However, the interactions between reactive nitrogen (RNS) and oxygen (ROS) species in cytokine-mediated toxicity remain unclear. Therefore it was the aim of this study to unravel the mechanism of this interaction.
Materials and methods: Insulin-producing RINm5F cells were stably transfected with the pcDNA3-hMnSOD or with hMitoCat. Cells were treated with IL-1β 600 U/ml or with a cytokine mixture (IL-1β 60 U/ml, TNFα 185 U/ml, IFNγ 14 U/ml) in the absence or presence of an iNOS blocker (5 mM L-nitroarginine) for 72 h. Cell viability was estimated by MTT test, overall oxidative stress by DCFDA oxidation, nitrite by the Griess assay and hydroxyl radical-mediated 8-oxyguanine formation by an OxyDNA kit. Hydrogen peroxide generation was measured by HyPer protein oxidation.
Results: In the control cells IL-1β caused a 40 % and a cytokine mixture a 70 % reduction in cell viability. This was significantly counteracted by iNOS blocker (IL-1β 78 % and Mix 70 % viable cells). MitoCat cells overexpressing catalase in the mitochondrium were protected against cytokine toxicity (IL-1β 80 %, Mix 71 % viable cells), while MnSOD overexpressing cells were more sensitive to cytokine toxicity (IL-1β 49 %, Mix 10 % viable cells). No further protection was seen in MitoCat cells after addition of the iNOS blocker, while a significant prevention of cytokine toxicity was observed in the MnSOD cells (IL-1β 65 %, Mix 48 %). DCFDA oxidation after exposure to cytokines in the absence or presence of iNOS blocker indicated that IL-1β toxicity is mostly related to NO and cytokine Mix toxicity to both NO and ROS formation. Indeed, in control cells exposed to IL-1β nitrite formation was the same as after cytokine Mix (IL-1β 3.9±0.7, Mix 4.7±0.5 pmol/μg protein), but the hydrogen peroxide (Con: 0.94±0.03, IL-1β: 1.07±0.03, Mix 1.20±0.04) and hydroxyl radical (Con; 100 %, IL-1β 227 %, Mix 391 %) generation were significantly lower after IL-1β than after cytokine Mix. Mitochondrial overexpression of catalase as compared to control cells diminished nitrite formation (IL-1β 0.6±0.2, Mix 1.9±0.3) and completely prevented hydrogen peroxide (Con: 0.94±0.02, IL-1β: 0.85±0.02, Mix 0.88±0.03) and hydroxyl radical (IL-1β 101 %, Mix 123 %) generation. In contrast, MnSOD cells showed significantly higher nitrite levels (IL-1 6.4±0.8, Mix 29±7), hydrogen peroxide (Con: 1.11±0.05, IL-1β: 1.50±0.03, Mix 3.05±0.33) and hydroxyl radical (IL-1β 661 %, Mix 904 %) generation.
Conclusion: The results revealed that IL-1β toxicity is NO-related, while a mixture of cytokines, simulating the situation in T1DM, stimulates the generation of both NO and ROS. This duality results in an interaction between NO and H_2O_2 leading to the formation of the highly toxic hydroxyl radical, which provides a unifying hypothesis for the mechanism underlying cytokine toxicity leading to beta cell death in T1DM. Optimal protection against cytokine toxicity requires a H_2O_2 inactivating enzyme in the mitochondria, allowing effective detoxification of H_2O_2, thereby preventing stress through hydroxyl radical formation in the presence of NO. In conclusion, the studies prove that proinflammatory cytokine-mediated beta cell death is due to nitrooxidative stress.

361

Cytokines regulate both transcriptional and alternative splicing networks in primary beta cells

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Background/rational: Cytokines contribute to insulitis and pancreatic beta cell death in Type 1 Diabetes. This effect is mediated by activation of complex gene networks that remain to be characterized. We presently utilized up-to-date array analysis to define the global pattern of genes, including spliced variants, modified by the cytokines IL-1β + IFNγ and TNFα + IFNγ in primary rat beta cells
Material and methods: FACS purified rat beta cells were exposed for 6 or 24h to IL-1β (50 U/ml) + IFNγ (100 U/mm) or TNFα (1000 U/ml) + IFNγ (100 U/ml) and then retrieved for array analysis using the GeneChip® Rat Genome 230 2.0 Array, containing > 31.000 probe sets. Three independent experiments were analyzed and gene expression considered as modified by cytokines when fold change was ≥ 1.5 and P ≤ 0.02 compared to controls. For identification of splice variants, cDNA from the three experiments were pooled and analysed using the exon array analysis GeneChip® Rat Exon 1.0 ST Array, containing ~ 1.000.000 probe sets. Most of the key results described below were confirmed by real time RT-PCR
Results: Nearly 16.000 transcripts were detected as present in control and cytokine-treated beta cells. TNF-α + IFNγ modified the expression of a higher number of genes compared to IL-1β + IFNγ at 6h (1.468 and 1.006 respectively), while at 24h this was inverted, with 2.531 and 1.929 genes modified by respectively IL-1β + IFNγ and TNF-α + IFNγ. TNF-α+IFNγ induced a higher expression of IRF1, IRF7, IL-15, IP-10 and CCL5, while IL-1β+IFNγ induced higher expression of CCL2 and CXCL1. Both treatments decreased the expression (20-80%; P ≤ 0.05, as confirmed by Real time RT-PCR) of genes involved in the maintenance of beta cell phenotype, including PDX-1, Mafa1, ins, isl1, foxo1, Glut2, PC1 and 2, NeuroD. Cytokines also decreased by > 80%

($P \leq 0.05$), as confirmed by Real time RT-PCR, the expression of receptors to the key growth/regeneration-inducing hormones GLP1, PRL and GH. Importantly, cytokines modified the expression of > 20 genes involved in RNA splicing. In line with this observation, exon array analysis showed cytokine-induced changes in alternative splicing of more than 40% of the cytokine-modified genes. This induction of alternative splicing was confirmed by PCR in three genes, namely iNOS, NF-κB2 and argininosuccinate synthetase.
Conclusion: The present study doubles the number of known genes modified by cytokines in primary rat beta cells, and suggests temporal and qualitative differences between the effects of TNF-α + IFN-γ and IL-1β + IFNγ in beta cells. Both IL-1β+IFNγ and TNF-α+IFNγ decrease the expression of genes related to beta cell function and growth/regeneration, suggesting that immune mediators of insulitis can "push back" newly developed beta cells into a de-differentiated state. Interestingly, cytokines modify alternative splicing in beta cells, indicating a new level of complexity in the beta cell responses to immune-mediated damage.
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PS 13 Signalling of beta cell damage

362

The effect of glucose variability on INS-1 cell apoptosis and the involvement of FOXO-SIRT system
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Background and aims: Glucose levels always change in some range in real life, and the degree of fluctuation would be more prominent in diabetes despite of meticulous control. Variable glucose levels have been reported to be more harmful than sustained high glucose in the vascular endothelial cells due to increased oxidative stress. Recent several studies reported that SIRT might regulate FOXO transcription factors under oxidative stress, and overexpression of SIRT could protect beta cells from cytokine induced apoptosis. But the effects of sustained and intermittent high glucose on the apoptosis and the changes of FOXO-SIRT system in pancreatic beta cells have not been studied so much. The purpose of our study is to clarify the effect of glucose variability on pancreatic beta cells, and the potential mechanisms related with FOXO-SIRT pathway.
Materials and methods: Cultured INS-1 cells were exposed to control (11mmol/l), SHG (sustained high glucose, 33mmol/l) or IHG (intermittent high glucose, 11 and 33mmol/l) alternating every 12 hours for 5 days. The degree of apoptosis was assessed by FACS and TUNEL assay. The levels of caspase-3, Mn-SOD, Bim and Bcl-2 were assessed via Western blot. The changes of FOXO-SIRT system were studied by Western blot and immuno-precipitation. The changes of glucose stimulated insulin secretion (GSIS) were also verified.
Results: INS-1 cells exposed to IHG for 5 days showed lower GSIS and the increased apoptosis than the cells in SHG. The changes of caspase-3 and Mn-SOD were compatible with the increased apoptosis in the cells exposed to IHG. The complex formations of SIRT and FOXO showed different patterns between IHG and SHG. The degree of acetylation of FOXO was lower in the cells exposed to SHG, but increased with SIRT inhibitor. Administration of SIRT inhibitor increased apoptosis, decreased caspase-3, MnSOD and Bcl-2 in the cells exposed to SHG.
Conclusion: IHG (Intermittent High Glucose) might be more harmful to the pancreatic beta cells than the SHG (Sustained High Glucose) causing increased apoptosis. FOXO-SIRT system might be closely involved in this process.

363

Transcriptome analysis of human type 2 diabetic islets
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Background and aims: Type 2 diabetes (T2D) is a multifactorial syndrome with beta-cell failure playing a major role. However, information on islets transcriptome in human T2D is scanty.
Materials and methods: We performed microarray analysis, followed by quantitative RT-PCR, of a few selected genes on six T2D (age: 71±9 yrs; gender: 3M/3F; BMI: 26.0±2.2 Kg/m^2) and seven non-diabetic (ND, age: 58±17 yrs; gender: 4M/3F; BMI: 24.8±2.5 Kg/m^2) isolated islet preparations. Standard RNA was hybridized on the Affymetrix platform (HG U133A). Gene expression intensities were normalized with RMA and differential expression was assessed by fitting a linear model on each gene using the limma package from bioconductor. Quantitative RT-PCR was performed using primers and probes from Applied Biosystems.
Results: When T2D islets were compared with ND samples, the expression of 5130 transcripts and variants resulted significantly ($p<0.01$) different; of these, 2927 were upregulated and 2203 downregulated. Changes included several genes involved in beta-cell function and turnover. By Gene Ontology and KEGG analysis it was observed that the differently expressed genes influence 81 processes and 13 pathways, including cell survival and secretion. Such findings were mostly confirmed by the Gene Set Enrichment Analysis, performed to evaluate how the overall gene expression profile could influence

intracellular processes. This analysis showed that out of 1412 gene sets studied, 144 were positively enriched and 234 negatively enriched in T2D islets. The expression of genes involved in oxidative phosphorylation and citrate cycle resulted consistently changed in T2D samples, and when quantitative RT-PCR analysis was performed, a significant reduction in the expression of pyruvate and succinate dehydrogenase complexes was found.
Conclusion: In conclusion, T2D islets show several alterations in the overall gene expression pattern; whereas it remains to understand what alterations may be cause or consequence of the diabetic condition, it is suggested that mitochondrial alterations might play an important role in T2D islet cell dysfunction.
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364

The role of iron in IL-1β induced NFκB activity and iNOS expression in pancreatic beta cells
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Background and aims: Interleukin (IL)-1β mediated selective destruction of the pancreatic β-cells is believed to be a central element in the pathogenesis of type 1 diabetes. β-cell sensitivity to IL-1β is acquired during β-cell maturation and dependent on expression of the transcription factor pancreatic duodenal homeobox-1 (Pdx-1). Microarray analysis of Pdx-1 overexpressing compared to control cells revealed a gene cluster involved in nuclear factor κB (NFκB) activation, reactive oxygen species (ROS) formation, iron influx via the divalent metal transporter 1 (DMT1) and inducible nitric oxide synthase (iNOS) as important in the maturation-acquired sensitivity to IL-1β. This lead to the hypothesis that IL-1β induced NFκB activity and iNOS expression are iron dependent in that iron catalysed ROS contributes to sustained NFκB activity.
Materials and methods: The iron chelator desferoxamine (DFO) was used to reduce IL-1β induced iron uptake in the INS-1 cell line. The effect of iron chelation on NFκB and iNOS promoter activity was studied using the dual Luciferase assay. Protein levels of iNOS and IκB was examined using Western blotting and apoptosis shown with the Roche death detection kit. Newborn rat islets were used to confirm effects of DFO on total cell death and nitric oxide (NO) levels.
Results: In INS-1, DFO (25 μM) reduced IL-1β (160 pg/ml) induced apoptosis by 25% ($p<0.05$), NFκB activation by 30% ($p<0.05$), and decreased iNOS at the protein level by 50% ($p<0.01$), whereas iNOS promoter activity does not seem to be affected by DFO. Cytokine induced total cell death was significantly reduced 30-40% with DFO doses from 10-100 μM in rat islets. Concentration of NO in the media of rat islets was significantly reduced with 100 or 200 μM of DFO ($p<0.05$).
Conclusion: Lowering the iron levels in INS-1 cells by DFO reduced IL-1β induced apoptosis by inhibiting NFκB activation as well as lowering iNOS expression at the protein level. Cytokine induced cell death and NO release was also reduced in rat islets following DFO treatment. Iron chelation may be a therapeutic option in preventing inflammatory β-cell destruction in islet transplantation.

365

The orphan receptor GPR30 and pancreatic islet hormone secretion
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Background and aims: The role of the newly discovered estrogen receptor, GPR30 in islet physiology is unclear. We examined GPR30 expression in relation to hormone secretion and possible antiapoptotic effects in isolated mouse islets using the synthetic GPR30 ligand G1.
Materials and methods: GPR30 expression was analyzed by confocal microscopy, Western blot in islets from female mice and RT-PCR in islets from both female and male mice. Hormone secretion and cAMP content in islets were determined with RIA and apoptosis with the Annexin-V method.
Results: Confocal microscopy revealed GPR30 expression in insulin, glucagon and somatostatin cells. GPR30 mRNA and protein expression was markedly higher in female *vs* male islets ($p<0.01$). Dose-response studies of G1 *vs* 17β-estradiol in isolated islets at 12 mM glucose showed an almost identical pattern in increasing insulin and inhibiting glucagon and somatostatin secretion. The 17β-estradiol genomic receptor (ERα and ERβ) antagonist ICI-182,780 (Fulvestrant) did not influence the amplifying effect of G1 or 17β-estradiol on cAMP content ($p<0.001$) or insulin secretion from islets. Cytokine-induced (IL-1β 100ng/ml, TNFα and INFγ 125ng/ml) apoptosis in islets, cultured for 24 h at 5 mM glucose, was almost abolished by G1 or 17β-estradiol treatment ($p<0.001$). Addition of ICI-182,780 did not affect this beneficial effect of G1 or 17β-estradiol.
Conclusion: GPR30 is expressed in islet endocrine cells. The synthetic GPR30 ligand G1 mimics the nongenomic effects of 17β-estradiol on hormone secretion, cAMP content in islets and its antiapoptotic effects. G1 or analogs thereof might be new potential candidate in therapeutic strategy of type 2 diabetes in women.
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366

Effect of angiotensin II on the insulin signalling pathway in NIT-1 cells
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Background and aims: Angiotensin II (Ang II) can cause insulin resistance in peripheral insulin target tissues such as muscle, fat and liver. The mechanism is to damage the insulin signaling pathway in these cells by reducing the tyrosine phosphorylation levels of insulin receptor (InR) / insulin receptor substrate -1 (IRS-1) and increasing serine phosphorylation levels. Reactive oxygen species (ROS) play important roles on the damage of insulin signaling pathway by Ang II. Whether the effect of Ang II on insulin signaling pathway in islet β-cell is not known. In the present study, the effect of Ang II on insulin signaling pathway was evaluated in mouse insulin-secreting cells NIT-1 cells and the role of ROS involved was studied.
Materials and methods: NIT-1 cells were cultured in DMEM with 10% fetal bovine serum, and then divided into six groups acted as control group, Ang II group, insulin group (Ins), Ang II + Ins group (AI), Ang II + Ins + saralasin group (AIS), Ang II + Ins + DPI group (AID). Expression of InR-β tyrosine phosphorylation (InR-β-Tyr), IRS-1 serine phosphorylation(IRS-1-Ser) and IRS-1 tyrosine phosphorylation (IRS-1-Tyr), P47 were evaluated by Western blot. H_2O_2 were detected by FCM.
Results: Expression of InR-β-Tyr and IRS-1-Tyr was higher in Ins group compared to control group, whereas expression of IRS-1-Ser was not significantly different between Ins group and control group. Level of InR-β-Tyr and IRS-1-Tyr was not significantly different between Ang II group control group,but the level of IRS-1-Ser significant increased in Ang II group compared with control group. After AngII treatment, InR-β-Tyr and IRS-1-Tyr induced by insulin were lower compared with Ins group (InR-β-Tyr 1.32 vs 1.87; IRS-1-Tyr 0.67 vs 1.17, $P<0.05$). With presence of saralasin, InR-β-Tyr and IRS-1-Tyr in AIS group were higher than in AngII group (InR-β-Tyr 1.67 vs 0.96, IRS-1-Tyr 1.03 vs 0.59, $P<0.05$). The level of H_2O_2 and P47 in AII group significant increased compared with control group (H_2O_2 37.46 vs 27.55, P47 1.55 vs 0.87, $P<0.05$). With presence of DPI, level of InR-β-Tyr and IRS-1-Tyr increased in AID group compared with AngII group (InR-β-Tyr 1.61 vs 0.96, IRS-1-Tyr 1.01 vs 0.59, $P<0.05$) and the level of IRS-1-Ser changed adversely (IRS-1-Ser 0.70 vs 1.17, $P<0.05$).
Conclusion: In conclusion, Ang II can significantly increase the level of IRS-1 serine phosphorylation and inhibit insulin-stimulated InR-β tyrosine phosphorylation in NIT-1 cells. oxidative stress induced by NADPH oxidase may play an important role in the effect of Ang IIin NIT-1 cells.

367

Apoptosis in INS-1E cells after ZnT-3 and ZnT-8 knock-out
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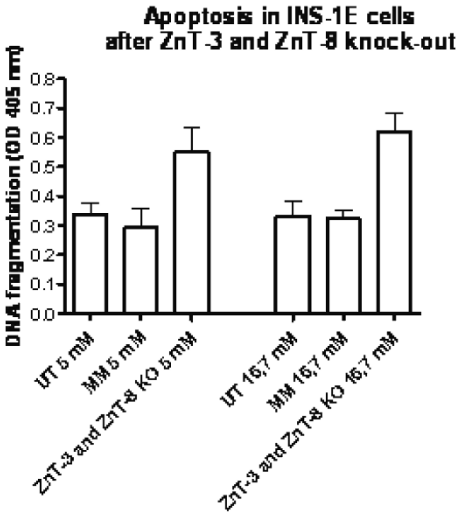
Background and aims: Insulin secreting β-cells contain large amounts of zinc. Intracellular metabolism of Zn^{2+} has to be subject to strict regulation due to its toxic potential at high concentrations. Transporters of the Slc30A-family (ZnT-1 to 10) regulate zinc efflux from the cytoplasm. Rising evidence connect ZnTs and diabetes. It has been shown that ZnT-8 is an antigen associated with the development of Type 1 diabetes. Furthermore, mutations in the same gene are associated with Type 2 diabetes. ZnT-3 KO mice have

decreased glucose tolerance which we have previously shown to be at least partly due to altered β-cell function. In this study we used siRNA-mediated knock-out to investigate the connection between the expression of these two zinc-transporters and INS-1E cell viability.
Materials and methods: 100 nM siRNA concentrations were used to perform single ZnT-3 and ZnT-8 knock-out in INS-1E cells. To avoid non-specific siRNA effects a total concentration of 100 nM was also used for the combined knock-out of ZnT-3 and ZnT-8. Knock-out of the two zinc-transporters at mRNA level was detected using Q-PCR. Following transfection and 48 hours of recovery the knock-out and control samples were stimulated with two different concentrations of glucose (5.0 and 16.7 mM) for 24 hours. Levels of apoptosis were then measured using an ELISA kit based on an enzyme-immunoassay-principle.
Results: A reduction of ZnT-3 expression to 30% ($p<0.01$) resulted in significantly increased apoptosis following stimulation with 5.0 mM glucose ($p<0.05$) and non-significantly at 16.7 mM glucose ($p=0.07$) when compared to mock-transfected controls. Knock-out of ZnT-8 to a level of 50% ($p<0.05$) resulted in increased apoptosis compared to controls only following stimulation with 16.7 mM glucose ($p<0.05$). Following combined knock-out we were able to reduce expression of ZnT-3 and ZnT-8 to 35 and 70%, respectively. The result was a significantly increased level of apoptosis following stimulation with both 5.0 and 16.7 mM glucose ($p<0.05$) (figure below).
Conclusion: The influence of ZnT-3 and ZnT-8 on INS-1E cell viability has until now not been described. Our results indicate that the presence of these two zinc-transporters, both separately and in combination is important for cell viability.



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368

Systemic osteoprotegerin delivery induces pancreatic islet structural and functional alterations in non-diabetic mice
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Background and aims: Osteoprotegerin (OPG), a secreted glycoprotein and member of the TNF receptor superfamily, is a soluble receptor activator of nuclear factor-κB (RANK) ligand (RANKL), and TNF-related apoptosis inducing ligand (TRAIL). Diabetic patients are characterized by elevated OPG, which is associated with subclinical atherosclerosis in both type 1 and type 2 diabetes. Recently, our group has demonstrated that OPG delivery leads to significant acceleration of atherosclerosis in diabetic apolipoprotein E-null mice. Although clinical studies have suggested that systemic levels of OPG are elevated in diabetes, the role of OPG in the endocrine pancreas has not been investigated. Therefore, the aim of this study was to examine the effects of OPG administration on pancreatic islet structure and function in mice.
Materials and methods: Three groups of C57B1/6J mice (n=20 for each group) were obtained at 10 weeks of age and studied for 12 weeks: 1. C57B1/6J mice treated with recombinant human OPG injected every 21 days intraperitoneally, 2. C57B1/6J mice treated with the vehicle alone, and 3. Untreated C57B1/6J mice. Tail cuff systolic blood pressure, fasting plasma glucose and insulin levels, pancreatic β-cell mass and density, β-cell apoptosis, peri- and intraislet fibrosis and macrophage infiltration, pancreatic gene expression of VCAM-1, MCP-1, CTGF and ACE were evaluated.
Results: Repeated intraperitoneal injections of recombinant human OPG in C57B1/6J mice significantly decreased β-cell mass (-28%; $p<0,005$) and density (-20%; $p<0,001$) and increased β-cell apoptosis (+38%; $p<0,001$), intraislet fibrosis (+24%; $p<0,001$) and peri- and intraislet macrophage infiltration (+44% and +66% respectively; $p< 0,001$) compared to untreated mice. OPG administration was also associated with hyperglycemia and reduced plasma insulin levels. In addition, OPG induced pancreatic gene overexpression of VCAM-1, MCP-1, CTGF and ACE.
Conclusion: The present study shows that OPG delivery leads to significant pancreatic islet structural and functional alterations, thus suggesting a possible role for OPG in the pathogenesis of diabetes.

369

PTPN2 and MDA5, two candidate genes for type 1 diabetes, modify beta cell responses to double-stranded RNA
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Background and aims: Beta cell destruction in early type 1 diabetes (T1D) is at least in part the consequence of a "dialog" between beta cells and the immune system. This dialog may be affected by the individual's genetic background. We presently evaluated whether modulation of two candidate genes for T1D, namely PTPN2 and MDA5, affects beta cell responses to double-stranded RNA (dsRNA), a by-product of viral replication.
Materials and methods: Candidate genes to be tested were selected by comparison between known candidate genes for T1D and genes expressed in pancreatic beta cells, as identified in previous array analysis from our group. This search identified two genes, PTNP2 and MDA5. Next, INS-1E and primary FACS-purified rat beta cells were transfected with control or siRNAs targeting PTPN2 or MDA5 and then exposed to intracellular polyinosinic-polycytidylic acid (PIC), a synthetic dsRNA. Two different PICs, one with 100-2,000 bp and the other with >2,000 bp, were used. Real time RT-PCR, Western Blot, and viability assays (Hoechst/PI) were performed to characterize beta cell gene/protein expression and viability.
Results: PIC induced an increase in PTPN2 (>2-fold) and in MDA5 (>28-fold) mRNA expression in INS-1E cells ($n = 3$-4; $P<0.05$), which was inhibited by >80% using specific siRNAs ($n = 3$-4; $P<0.05$). Similar findings were observed in primary beta cells. PIC induced 20.0±1.7% and 31.5±3.2% cell death after 24 h, as compared to 4.0±0.5% and 4.3±0.8% cell death in respectively control INS-1E or primary beta cells ($P<0.05$). Cell death was increased by 83.9% and 23.3% ($n = 4$-6; $P<0.05$) by the siRNA targeting PTPN2 in respectively INS-1E and primary beta cells, while the siRNA against MDA5 failed to modify dsRNA-induced apoptosis. On the other hand, the siRNA targeting MDA5 decreased PIC-induced expression of mRNAs coding for interferon beta (13.5-fold), CXCL10 (4.9-fold), interleukin 15 (2.1-fold), CCL2 (42.9-fold) and CCL5 (6.9-fold) ($n = 4$; $P<0.05$), while the siRNA targeting PTPN2 produced only minor changes in CXCL10 (1.2-fold) and interleukin 15 (1.6-fold) ($n = 3$), without changing expression of interferon beta, CCL2 and CCL5 in INS-1E cells. In preliminary experiments ($n = 2$-4), similar results were obtained in primary beta cells.
Conclusion: The present findings indicate that changes in PTPN2 and MDA5 expression modify beta cell responses to dsRNA. While MDA5 modulates the pro-inflammatory signals induced by dsRNA, PTPN2 seems to function as a defence mechanism against the pro-apoptotic signals generated by dsRNA. This suggests that PTPN2 and MDA5, two candidate genes for type 1 diabetes, modulate beta cell apoptosis and/or insulitis independently of their presumed regulatory roles in the immune system.
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370

XOMA 052, a monoclonal antibody that regulates interleukin-1 beta (IL-1 beta) activity: an example of a new class of regulatory antibody drugs that may confer a unique advantage in the treatment of type 2 diabetes mellitus

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Background and aims: IL-1 beta exerts biphasic effects on pancreatic beta cell function; it is beneficial at low concentrations and pathological at high concentrations. At low concentrations IL-1 beta acts as a stimulatory, growth, and survival factor for beta cells, leading to increased insulin secretion, increased proliferation, and reduced apoptosis. In type-II diabetes, increased secretion of IL-1 beta by beta cells results in macrophage infiltration which is hypothesized to contribute to loss of beta cell function and mass. Here we describe XOMA 052, an ultra-high affinity (0.3 pM) antibody that regulates, rather than blocks, IL-1 beta activity.
Materials and methods: XOMA 052 activity was characterized in vitro by analysis of binding kinetics for IL-1 beta and its receptors using surface plasmon resonance and kinetic exclusion assays. Neutralization of IL-1 beta mediated cytokine induction and proliferation by XOMA 052 was evaluated in multiple cell-based functional assays, including cell lines and whole blood assays.
Results: We demonstrate that XOMA 052 reduces the affinity of IL-1 beta for the soluble form of its signaling receptor, IL-1 Receptor I (RI) approximately ten-fold, and further reduces binding by IL-1 Receptor Accessory Protein (RAcP) extracellular domain another two-fold. Such a reduction in affinity would predict that binding of IL-1 beta by XOMA 052 will result in less efficient formation of the complex responsible for initiation of IL-1 beta-stimulated signal transduction, and thus a less sensitive dose-response to IL-1 beta stimulation. We have shown in multiple cell-based functional assays that XOMA 052 causes such a shift in the dose-response curve for IL-1 beta-stimulated responses. We have shown that XOMA 052 does not reduce the binding affinity of IL-1 beta to soluble IL-1 Receptor II (RII), which neutralizes IL-1 beta, and in its membrane bound form may facilitate clearance of IL-1 beta. We hypothesize that XOMA 052 may allow for low levels of beneficial IL-1 beta signaling while attenuating pathologically high levels and permitting the normal physiological mechanisms to clear IL-1 beta.
Conclusion: XOMA 052 regulates, rather than blocks IL-1 beta activity through its differential effects on the cytokine's binding to receptors responsible for signaling, neutralization, and clearance of IL-1 beta. This may be the first example of a new class of therapeutic antibodies that uses a novel strategy to regulate rather than shut down a targeted pathway to mitigate disease pathology while facilitating homeostatic regulation.

PS 14 Oxidative stress and ROS in beta cells

371

The role of mimitin in insulin-producing cells

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Background and aims: Proinflammatory cytokines, especially IL-1 are main mediators of pancreatic beta cell destruction and death in T1DM. Cytokines affect the expression of many genes and lead to both oxidative and nitrosative stress. Beta cell mitochondria are the central place of cytokine-induced beta cell death. Mimitin is a mitochondrial protein which has recently been discovered in human glioblastoma cells and was shown to be involved in cell proliferation. The expression, regulation and function of mimitin in insulin-producing cells is unknown. The aim of the study was to evaluate the role of mimitin in insulin-producing cells.
Materials and methods: Insulin-producing INS1E cells were were incubated with IL-1 600 U/ml or with a cytokine mixture (IL-1 60 U/ml, TNFα 185 U/ml und IFNγ 14 U/ml) in the absence or presence of an iNOS blocker (L-nitro-Arginine, 5 mM) for 6, 12 or 24 h. Thereafter RNA was isolated. Mimitin gene expression was analyzed by Real-Time PCR and corrected to beta actin levels. To study the localization of mimitin, cells were transiently transfected with pmaxFP™-green-Mimitin and incubated with a MitoTracker® Deep Red FM, and then analyzed by means of fluorescence microscopy. The pcDNA3-human mimitin vector was used to overexpress the protein in INS1E cells. The INS1E-hMimitin cells were incubated with cytokines for 72 h. Cell viability was measured by MTT assay and cell proliferation by BrdU ELISA. Insulin secretion was measured by RIA.
Results: Insulin-producing cells express mimitin on a very low level and the protein is expressed in the mitochondria. Cytokines downregulate mimitin expression after shorter incubations (6 h IL-1 87 %, cytokine mix 48 %; 12 h IL-1 72 %, cytokine mix 62 %, vs. untreated 100 %), but upregulate it after longer time periods (24 h IL-1 175 %, cytokine mix 147 %). Interestingly, the cytokine effects depend on NO, because the iNOS blocker counteracted cytokine-induced changes in mimitin expression (6 h IL-1 81 %, cytokine mix 85 %; 12 h IL-1 75 %, cytokine mix 107 %, 24 h IL-1 96 %, cytokine mix 78 %). INS1E-hMimitin cells show approximately 400-fold higher mimitin expression than untransfected cells. Overexpression of mimitin protected insulin-producing cells against cytokine toxicity (MTT 72 h: INS1E IL-1 57 %, cytokine mix 16 % vs. INS1E-hMimitin IL-1 85 %, cytokine mix 25 %). INS1E cells overexpressing mimitin exhibited a significantly higher basal proliferation rate compared to untransfected cells (160 % vs. control cells) and the suppressive effects of cytokines towards cell proliferation were successfully counteracted by mimitin overexpression (72 h: INS1E: IL-1 66%, cytokine mix 35%, INS1E-hMimitin IL-1 94%, cytokine mix 68 %). Basal insulin secretion was in INS1E 0.47±0.03 ng/ml/μg DNA and in INS1E-hMimitin 0.44±0.04. Glucose (30 mM) and KCl (25 mM) induced stronger insulin secretion in INS1E-hMimitin cells than in control cells (INS1E: 30 mM Glc 255%, KCl 430 %, INS1E-hMimitin 404 %, KCl 678 %). Mimitin overexpression counteracted completely IL-1 reduction in glucose- or KCl-induced insulin secretion, and the protective effect was weaker in the case of the cytokine mixture.
Conclusion: Mimitin is a novel, mitochondrial protein, its expression is regulated by cytokines and NO. Mimitin prevents cytokine-induced death of insulin-producing cells and stimulates cell proliferation. Importantly, mimitin counteracts also deleterious effects of cytokines on glucose-induced insulin secretion.

372

Modulation of K_{ATP} channels increases the activity of antioxidant enzyme systems and protects beta cells against ROS-induced cell damage

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Background and aims: We observed that K_{ATP} channel-deficient islet cells (SUR1-KO) were less sensitive to H_2O_2-induced alterations in membrane potential, $[Ca^{2+}]_c$ and insulin secretion compared to wildtype (WT) islets. In

addition SUR1-KO islet cells were protected against ROS-induced apoptosis. The aim of the present study was to elucidate whether similar effects could be evoked by modulation of K_{ATP} channel activity by sulfonylureas and to identify the underlying mechanisms.
Materials and methods: Apoptotic beta cells were identified by TUNEL and NucView™ (determination of active caspase3) labeling, respectively. Activity of catalase (Cat), superoxide dismutase (SOD) and glutathione peroxidase (GPx) was measured by suited enzyme assays.
Results: One hour incubation of WT islet cells with H_2O_2 or SNOC increased apoptotic cell death 3.0±0.4-fold (10 μM H_2O_2, n=3, P<0.01), 4.0±0.3-fold (25 μM H_2O_2, n=5, P<0.01) and 2.7±0.4-fold (100 μM SNOC, n=3, P<0.01), respectively. In contrast, the fraction of apoptotic cells was not affected by 10 μM H_2O_2 (n=3) or by 100 μM of the NO donor SNOC (n=3) in SUR1-KO islet cells and the effect of 25 μM H_2O_2 was much less pronounced vs WT (n=3). Protection against H_2O_2-induced loss of cell viability could also be achieved in WT islet cells by preincubation (4 hours) with tolbutamide (25 μM) or gliclazide (10 μM) (n=3). Next we tested whether KO or inhibition of K_{ATP} channels affected antioxidant enzyme systems. In SUR1-KO islets we observed an approximately 2-fold increase in the activity of SOD (n=4, P<0.05), Cat (n=3, P<0.001) and GPx (n=5, P<0.05), respectively, compared to WT islets. Importantly, treatment of WT islet cells with tolbutamide (100 μM) or gliclazide (10 μM) for four hours also elevated the activity of SOD (19±1 U/mg protein under control conditions vs. 34±3 U/mg protein after gliclazide treatment, n=3; P<0.01) and Cat (15±2 mU/mg protein vs. 26±2 mU/mg protein, n=3, P<0.05). The sulfonylurea-induced activation of SOD critically depended on $[Ca^{2+}]_c$ and was diminished by blocking Ca^{2+} influx through L-type Ca^{2+} channels (n=3).
Conclusion: Regarding ROS-induced cell damage the protective effects of K_{ATP} channel ablation is mimicked by pharmacological reduction of K_{ATP} channel activity. Both manoeuvres induced an up-regulation of antioxidant enzymes. The data indicate that modulation of K_{ATP} channel activity might provide a useful strategy to improve the resistance of pancreatic beta cells during periods of increased oxidative stress.
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373

Endogenous production of hydrogen sulfide by beta cells protects against oxidative stress-mediated cell death
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Background and aims: ß-cells produce hydrogen sulfide from L-cysteine via the enzyme cystathionine gamma-lyase (CSE). Hydrogen sulfide production in ß-cells has inhibitory effects on insulin secretion and as CSE expression increases in diabetes may participate in loss of ß-cell function. However, hydrogen sulfide is protective in a variety of disease states including those mediated by oxidative stress, the mechanisms involved range from induction of cytoprotective enzymes to direct interaction with reactive oxygen species. Hyperglycaemia increases cellular production of methylglyoxal leading to oxidative stress. Methylglyoxal levels are increased in Type I and II diabetics and may be involved in development of both diabetic complications and ß-cell dysfunction. The aim of this study was to examine whether endogenous ß-cell production of hydrogen sulfide protects against oxidative stress-mediated cell death induced by methylglyoxal.
Materials and methods: The BRIN-BD11 ß-cell line was exposed to methylglyoxal (MGO, 1 or 2 mM) or hydrogen peroxide (0.3 or 1 mM) for 4h either alone or in combination with 0.3, 1 or 3 mM L-cysteine or a hydrogen sulfide donor, sodium hydrogen sulfide. Cell viability was determined using the MTT assay and cell death assessed by propidium iodide and Hoechst staining. Inhibitors of CSE, DL-propargylgylcine (PAG, 3 mM), and glutathione synthesis, buthionine sulfoximine (BSO, 1 mM), were used in combination with L-cysteine.
Results: MGO exposure reduced ß-cell viability to 43±6% and 25±2% for 1 and 2 mM respectively (p<0.01). Simultaneous application of L-cysteine protected the ß-cells from MGO-mediated loss of cell viability; with 0.3, 1 and 3 mM L-cysteine in combination with 1 mM MGO increasing viability to 86±3%, 97±2% and 102±2% and in combination with 2 mM MGO to 59±8%, 63±7% and 86±3% (p<0.01 vs. MGO alone). This protective effect of 0.3 and 1 mM L-cysteine was blocked by 3 mM PAG (45±2% and 48±1% with 1 mM MGO, 28±1% and 27±4% with 2 mM MGO). BSO had no effect on the protective effect of L-cysteine ruling out the conversion of L-cysteine to glutathione as the mechanism of protection. MGO increased ß-cell apoptosis from 3±1% to 16±2% and 21±3% for 1 and 2 mM MGO respectively (p<0.01). L-cysteine treatment again protected against this MGO-mediated increase in apoptosis with 1 mM L-cysteine reducing apoptosis to 5±2% and 8±3% when applied in combination with 1 or 2 mM MGO, an effect which was again blocked by PAG but not BSO. Simultaneous application of exogenous hydrogen sulfide dose dependently protected ß-cells from MGO-mediated loss of cell viability; with 0.3, 1 and 3 mM hydrogen sulfide in combination with 1 mM MGO increasing cell viability to 71±6%, 71±5% and 73±4% respectively and in combination with 2 mM MGO to 62±7%, 67±7% and 71±5% (p<0.01 vs. MGO alone). Hydrogen sulfide (3 mM) also reduced the levels of apoptosis observed with 1 or 2 mM MGO to 8±2% and 11±3% respectively (p<0.05 vs. MGO alone). Similar results were observed when hydrogen peroxide was used as the oxidative stress inducer.
Conclusion: (i) Endogenous production of hydrogen sulfide from L-cysteine or exogenously applied hydrogen sulfide, via a donor, protects ß-cells against methylglyoxal-mediated ß-cell death and therefore may act as an anti-oxidant defence system for ß-cells. (ii) Increased production of hydrogen sulfide by ß-cells in diabetes may be detrimental to cellular function in the short term but may act to protect the ß-cell from cell death in the long term.

374

Protective effect of glucokinase activation against hydrogen peroxide-induced cell death in a model of pancreatic beta cells
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Glucokinase (GK) functions as the glucose sensor in pancreatic β-cells to enhance insulin release. Recently, GK was found to form a complex with a pro apoptotic protein BAD in mitochondrial membrane, suggesting that GK is involved in apoptotic signaling pathway. We investigated the effect of a small molecule GK activator (Cpd B) on cell death in MIN6 cells, a mouse insulinoma cell line. Cpd B activates the recombinant GK with EC50 of 70 nM in the presence of 10 mM glucose. When incubated with MIN6 cells at 1 μM, Cpd B prevented hydrogen peroxide (H_2O_2)-induced acute cell death (% cell survival was 66±1.2 for the H2O2-treated group and 93±1.7 for Cpd B treated group, $p < 0.05$, n=3). Pyruvate, a metabolic intermediate of glycolysis, also protected MIN6 cells from H_2O_2-induced cell death. An increase of intracellular NADH level was observed by the treatment of Cpd B or pyruvate. In contrast, a sulfonylurea (SFU) agent glipizide did not show such protective effect, indicating that an autocrine effect of insulin does not account for the suppression of acute cell death. Furthermore, Cpd B suppressed apoptotic cell death caused by glucose depletion in cultured MIN6 cells. The anti-apoptotic effect of Cpd B was not affected by inhibitors of insulin release such as somatostatin and diazoxide, however, it was blocked by respiratory chain inhibitors. Investigation of glucose metabolism revealed that Cpd B but not SFU increased glucose metabolism, ATP content and mitochondrial membrane potential in the cells, suggesting that GK activation prevents apoptosis through preservation of mitochondrial oxidative phosphorylation activity and ATP production. Consistent with this finding, Cpd B remarkably increased the level of GK associated with mitochondria even under low glucose conditions. Taken together, activation of glucose metabolism pathway and increased GK association with mitochondria could account for the anti-apoptotic effect of Cpd B. GK activators may have a potential for protecting β-cells from deterioration typically associated with type 2 diabetes.

375

Role of oxidative stress in the alterations of pancreatic beta cell survival and function induced by prolonged culture in low glucose
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Background and aims: Prolonged exposure of rodent beta cells to extreme low glucose concentrations markedly reduces *preproinsulin* gene expression and glucose-stimulated insulin secretion (GSIS) while triggering apoptosis. The latter, which is prevented by antioxidants, likely results from an increase in oxidative stress. However, whether oxidative stress contributes to the reduction of GSIS under this condition is unknown. Here, we tested the effect

of the catalytic antioxidant Manganese (III)tetrakis (4-benzoic acid) porphyrin (MnTBAP) on the alterations of beta cell function and survival during prolonged culture in low glucose.
Materials and methods: Male Wistar rat islets were cultured for 1 week in RPMI medium containing 5g/l BSA and 5-10 mM glucose (G5-G10) with or without 50 μM MnTBAP. After culture, glucose-induced changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and insulin secretion were measured in perifused islets. DNA fragmentation was measured with a commercial ELISA and gene mRNA levels by RT-qPCR. Results are means ± SEM for at least 8 islets from 3 experiments. Statistical significance of differences between groups was assessed by unpaired t-test or 2-way ANOVA followed by a test of Bonferroni.
Results: After culture in G10, acute stimulation from 0.5 to 20 mM glucose induced a transient decrease in $[Ca^{2+}]_i$ followed by a rapid rise in $[Ca^{2+}]_i$ and insulin secretion that was further increased by depolarizing islets with 30 mM extracellular K^+ (K30). In contrast, after culture in G5, the insulin secretory response to both stimuli was markedly reduced (by 99%, P<0.001), owing to a lack of glucose-induced rise in $[Ca^{2+}]_i$ (*P*<0.001) without changes in the response to K30, and to an ~70% reduction in islet insulin/DNA content ratio (*P*<0.001). These functional alterations were associated with an ~87% decrease in *preproinsulin* mRNA levels (*P*<0.001), a marked upregulation of the mRNA levels of the pro-apoptotic factor *c-Myc* (~16-fold, *P*<0.001) and of the oxidative stress-response genes *Heme oxygenase 1* (*Hmox1*) (~3-fold, *P*<0.001) and *Metallothionein 1a (Mt1a)* (~67-fold, *P*<0.001), and a 7-fold increase in islet cell apoptosis (*P*<0.001). As expected, the porphyrin derivative MnTBAP increased *Hmox1* mRNA levels under all culture conditions and did not affect beta-cell survival and function during culture in G10. In contrast, MnTBAP significantly reduced islet oxidative stress during culture in G5, as shown by a 50-70% reduction in *c-Myc* and *Mt1a* mRNA levels (*P*<0.001) and a 50% decrease in islet cell apoptosis (*P*<0.01). Despite these beneficial effects, MnTBAP only improved the glucose-induced $[Ca^{2+}]_i$ rise 2.5-fold (*P*<0.05), GSIS 4-fold (*P*=0.083), the insulin to DNA content ratio 1.5-fold (*P*<0.05) and *preproinsulin* mRNA levels 1.5-fold (*P*=0.062) in islets cultured in G5. As a result, the reduction in beta cell glucose responsiveness was still inhibited by ~97% as compared with islets cultured in G10 with or without MnTBAP.
Conclusion: An ~50-70% reduction in oxidative stress and islet cell apoptosis during culture in 5 vs 10 mM glucose is not sufficient to improve the beta cell ability to respond to a subsequent glucose challenge. These results suggests that beta cell function is more sensitive than islet cell survival to moderate levels of oxidative stress. Alternatively, the loss of GSIS after culture in G5 is mostly independent from the induction of islet cell oxidative stress and apoptosis.

376

Generation of oxidative stress is responsible for the sustained activation of the transcriptional repressor ICER in beta cells

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Background and aims: Type 2 diabetes (T2D) develops in patients whose pancreatic β-cells fail to secrete enough insulin to compensate for insulin resistance. Environmental stressors such as chronic hyperglycaemia and elevated levels of free fatty acids contribute to β-cell failure by generating oxidative stress (OS) and promoting sustained activation of the inducible cAMP early repressor (ICER). Oxidised LDL (oxLDL) are also thought to participate in the pathogenesis of T2D. The ratio of the plasma circulating levels of oxLDL-cholesterol particles over native LDL are increased in diabetic patients. Independent studies have shown the adverse effects of oxLDL on insulin synthesis, secretion and on β-cell survival. In this study, we investigated whether ICER activation is responsible for the oxLDL-induced β-cell dysfunction and, since these modified lipoproteins induce OS, we sought for a potential link between induction of ICER and OS.
Materials and methods: Native LDL fractions were freshly prepared from plasma of human healthy donors by sequential centrifugation. In vitro oxidization of LDL by copper was adjusted to obtain oxidised LDL qualitatively similar to those observed in patients. The lipoprotein preparations were dialyzed against phosphate-buffered saline and culture medium to remove copper and salts. Mouse MIN6 insulin-secreting cells and isolated rat islets were exposed to 2 mM oxLDL-cholesterol or 150 μM hydrogen peroxide (H_2O_2) in the presence or absence of 1mM of the antioxidant N-acetylcysteine (NAC).
Results: Exposure of MIN6 cells and rat isolated islets to 2mM of oxLDL-cholesterol particles induced a drastic rise in the content and activity of ICER. In line with the induction of ICER, the expression of target genes important for synthesis and secretion of insulin such as the exocytotic genes (Rab3a, granuphilin, Noc2 and Rab27) and connexin 36 were diminished and nutrients-induced insulin secretion was impaired. Islet brain 1 (IB1) is a JNK scaffold protein that preserves β-cells against JNK-mediated apoptosis provoked by many pro-apoptotic stressors. IB1 has been recently identified as an ICER target gene and its expression is reduced in the cells cultured with oxLDL. Suppression of ICER by small interfering RNAs prevented the loss of expression of all its target genes evoked by oxLDL and improved β-cell function. Implication of OS in the activation of ICER was demonstrated by the up-regulation of the transcriptional repressor in β-cells treated with H_2O_2. Co-incubation of the cells with 1mM of the antioxidant NAC abrogated induction of ICER, and restored nutrients-induced insulin release.
Conclusion: The data show that persistent induction of ICER mediates the harmful effects of oxLDL and emphasize OS as the mechanism involved in this dysregulation.
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377

Cytoprotective effect of prostacyclin synthase overexpression in insulin-producing cells

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Background and aims: Proinflammatory cytokines play a crucial role in the pathogenesis of type 1 diabetes, a disease with an inflammatory background. One of the cytokine-regulated pathways is the arachidonic acid metabolism pathway, comprising the induction of cyclooxygenases and the production of different prostaglandins. Through this pathway cytokine toxicity is mediated in many cell types, including pancreatic beta cells. Interestingly, some cell types have been shown to be insensitive to such toxicity and this correlated with a high expression of prostacyclin synthase. Therefore it was the aim of this study to evaluate the role of prostacyclin and the molecular mechanisms underlying its effects in insulin-producing cells.
Materials and methods: Insulin-producing cells were stably transfected with the pcDNA3.1-hPGIS. Cells were treated with IL-1β 600 U/ml or with a cytokine mixture (IL-1β 60 U/ml, TNFα 185 U/ml, IFNγ 14 U/ml) for 72 h and thereafter cell viability was measured by MTT test, proliferation by a BrdU ELISA and caspase-3 by a chemiluminescent method. NFκB and iNOS promoter were analyzed by a SEAP-reporter gene assay. iNOS WBs were performed and nitrite was measured by Griess assay. Glucose oxidation was estimated by use of [U-^{14}C]glucose.
Results: The endogenous prostacyclin synthase expression in rat pancreatic islets and in insulin-producing RINm5F cells is extremely low (around 5 and 1 %, respectively, of that found in rat liver). Cytokines were toxic in control cells (MTT, IL-1β 67 %, Mix 40 % viable cells), but not in PGIS overexpressing cells (MTT, IL-1β 91%, Mix 85 % viable cells). Cytokines slowed down proliferation of control cells (IL-1β 88 %, Mix 55 %), but not in PGIS overexpressing cells (IL-1β 102 %, Mix 84 %). Similarly, caspase-3 was significantly activated by cytokines in control cells (IL-1β 10-fold, Mix 5-fold induction), but this induction was counteracted by PGIS overexpression (IL-1β 13 %, Mix 200 %). This effect was specific for cytokines, because camptothecin induced caspase-3 activation in the same manner in control and PGIS cells (6-fold). NFκB and iNOS promoter were highly activated by cytokines in the control cells (NFκB IL-1β 217 %, Mix 190 %; iNOS promoter IL-1β 422 %, Mix 531 %) and, again, this activation was significantly lower in PGIS cells (NFκB IL-1β 111 %, Mix 119 %; iNOS promoter IL-1β 163 %, Mix 153 %). Also the cytokine-stimulated iNOS protein induction was high in control cells and extremely low in PGIS cells. Nitrite levels were elevated in control cells treated with cytokines (IL-1β 3.9±0.7 vs. Mix 4.7±0.5 pmol/μg protein), but there was virtually no nitrite in cytokine-treated PGIS cells. Cytokines significantly decreased glucose oxidation in control cells (IL-1β 63 %, Mix 34 % vs. untreated 100 %), and, in contrast, induced it in PGIS cells (IL-1β 286 %, Mix 376 %).
Conclusion: Prostacyclin synthase overexpression had a large protective effect against cytokine toxicity in insulin-producing cells. This protective effect of PGIS against cytokine toxicity correlated with a decreased activation of the transcription factor NFκB and the iNOS promoter as well as a reduced iNOS protein expression and nitrite production. Moreover, cytokine-induced caspase-3 activation and reduction of glucose oxidation and cell proliferation were suppressed by PGIS overexpression Thus, prostacyclin synthase overex-

pression may represent a novel strategy for protection of pancreatic beta cells against inflammation-based destruction during T1DM development.

378

Intermittent hypoxia-specific expression of CCL2, CXCL9, and CXCL10 genes via NFkappaB activation in pancreatic beta cells

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Background and aims: Obstructive sleep apnea syndrome (OSAS) is a highly prevalent sleep disorder characterized by cyclic intermittent hypoxia. Metabolic syndrome characterized with obesity, hypertention, dyslipidemia, and hyperglycemia is prevalent in OSAS, which recently comes to be considered as an inflammatory systemic disease. Chemokines enhance the inflammatory response by recruiting immune-cells to sites of injury or inflammation. Although diabetes is frequently associated with OSAS, effects of intermittent hypoxia on chemokine gene expression in beta-cells are elusive.
Materials and methods: Rat insulinoma RINm5F cells were exposed to sustained hypoxia (1% O2) for 24 hours, 64 cycles of intermittent hypoxia (5 min hypoxia (1% O2)/10 min normoxia (21%O2)), or normoxia. Total RNA was prepared from sustained hypoxia-, intermittent hypoxia-, and normoxia-treated cells. Quantitative RT-PCR of CCL2, CCL20, CXCL2, CXCL9, CXCL10, and pancreatic derived factor (PANDER) was performed using sustained hypoxia-, intermittent hypoxia-, or normoxia-treated cell RNA as template. RINm5F cells were transiently transfected with reporter plasmid consisting of a luciferase reporter gene under the control of consensus binding sites for stress-related transcription factors, nuclear factor kappa B (NFkappaB), hypoxia induced factor (HIF), interferon regulatory factor 1, heat shock transcription factor (HSF), serum response factor (SRF), activator protein 1 (AP-1), or cyclic AMP responsive element binding protein (CREB). After transfection, cells were exposed to sustained hypoxia, intermittent hypoxia, or normoxia for 24 hours. Transcriptional activation was measured by luciferase activity of the cell extract.
Results: Quantitative RT-PCR revealed that mRNAs of CXCL10 (1.9 fold), CCL2 (6.3 fold), and CXCL9 (12 fold) were increased by intermittent hypoxia. By reporter assay, we found that NFkappaB was activated by intermittent hypoxia (% increase over normoxia; 40.1±10.6%, $p<0.05$) but not by sustained hypoxia. HSF (32.4±6.0%, $p<0.005$), SRF (44.9±6.6%, $p<0.005$), AP-1 (73.6±9.6%, $p<0.0005$), and CREB (115.0±11.9%, $p=0.0001$) were activated by sustained hypoxia.
Conclusion: NFkappaB but not HIF, HSF, SRF, AP-1 nor CREB was activated by intermittent hypoxia in pancreatic beta-cells to induce expression of CCL2, CXCL9, and CXCL10 genes. These chemokines could directly or indirectly damage beta-cells to induce apoptosis. In fact, CD95 mRNA was significantly induced by intermittent hypoxia in beta-cells. These results indicate that NFkappaB activation is a key step of chemokine induction in pancreatic beta-cells by intermittent hypoxia and suggest possible mechanism of hypoxia-reperfusion injury of beta-cells induced by cycles of hypoxia- reoxygenation in OSAS patients.

379

Is autophagy the mechanism of dioxin-induced cell death of the beta cell line INS-1E?

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Background and aims: Several epidemiological studies have clearly demonstrated a positive correlation between dioxin exposure and type 2 diabetes incidence. In order to clarify the biological basis of this correlation we have previously demonstrated that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is highly toxic for INS-1E cells and that, even at very low doses, markedly impairs glucose-stimulated insulin secretion. The aim of this research has been to further investigate the mechanism of the acute TCDD citotoxicity in INS-1E cells.
Materials and methods: INS-1E cells were exposed for 1 hr to TCDD either alone or in the presence of different concentrations (0-300 μM) of epigallocatechin-3-gallate (EGCG), the main polyphenol component of green tea. We have then assayed: 1) cell survival by WST-1; 2) ultrastructural alterations by electron microscopy; 3) mitochondrial membrane potential by cytofluorimetry; 4) changes in intracellular calcium concentrations by fluo-3/AM-based fluorimetry; 5) glucose-stimulated insulin secretion.
Results: Our results show that EGCG was able to significantly increase, in a dose-related manner, the survival of INS-1E cells exposed for 1 h to 12.5 or 25 nM TCDD. Besides evidence of apoptosis or necrosis in many cells, one of the most interesting ultrastructural alterations induced by 1 h exposure to TCDD was a remarkable activation of autophagy, as indicated by the presence of several large autophagic vacuoles containing identifiable organelles, such as mitochondria or degraded membranes. Autophagy was further confirmed by monodansyl cadaverine fluorescence. In EGCG-protected cells, necrosis or apoptosis were significantly reduced and autophagic vacuoles were no longer found after 1 hr exposure to TCDD. Conversely, ultrastructural evidence of autophagy activation was found in both unprotected and EGCG-protected INS-1E cells after 10 and 30 min of exposure to TCDD. EGCG had no effect on TCDD-induced increase in intracellular calcium concentration, but significantly prevented TCDD-induced mitochondrial depolarization. Finally, EGCG was also able to fully prevent TCDD-induced impairment of glucose-stimulated insulin secretion.
Conclusion: Our study clearly shows that EGCG is highly effective in preventing TCDD toxicity in INS-1E cells, in terms of both cell survival and preservation of their functional properties. Moreover, our ultrastructural findings show that the protective effect of EGCG does not inhibit the early activation of autophagy induced by TCDD. Autophagy is a mechanism for bulk degradation of cytosolic proteins and organelles which has been claimed to be the hallmark of a new type of programmed cell death, the autophagic cell death. Our results rather indicate that in TCDD-induced cytotoxicity, autophagy could be interpreted as a pro-survival mechanism. The protective effect of EGCG could be likely related to its ability to preserve mitochondrial function in INS-1E cells, thus preventing apoptosis and impairment of glucose-stimulated insulin secretion.

380

Restoration of glucose stimulated insulin secretion by copper addition - a possible role of interleukin-1β and oxidative stress

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Background and aims: Cytokines are mediators of β-cell dysfunction in diabetes. When fed high-sucrose low-copper diet (HSD), Cohen diabetic sensitive (CDs) rats exhibit hyperglycemia while resistant (CDr) rats remain normoglycemic. Prevention of β-cell dysfunction was obtained in the CDs rats by addition of adequate (15ppm) copper (Cu) concentration to HSD (HSD-Cu). In the CDs-rat, hyperglycemia was associated with exocrine infiltration of macrophages secreting IL1-β which may promote the production of free radicals and a successive diminished glucose stimulated insulin secretion (GSIS).
Aim: To analyze the role of IL1-β, oxidative stress and Cu deficiency in the reduced GSIS of the CDs rats.
Materials and methods: Islets isolated from normoglycemic CDs and CDr rats were pre-incubated for 24h w/o IL1-β (1.25-10U/ml), w/o Cu-GHL (0.4-0.7umol/l) or L-NOARG (5mM). Islet insulin-secretion was evaluated (RIA) in KRB + 1.7 or 16.7 mmol/l glucose. In-vivo, OGTT (glucose, 3.5g/kg) and insulin tolerance test (ITT, insulin 0.25U/kg) were performed on CDs rats fed HSD-Cu or treated with aminoguanidine (50mg/kg, i.p.) for 15 days.
Results: In isolated islets, the initial GSIS of CDs was ~2fold lower than CDr (1103±178 vs. 2052±152pmol/l $P=0.001$, respectively). Low IL1- β (1.25U/ml) increased the GSIS of CDr (2581±84 pmol/l $p<0.05$) but had no effect on CDs. 2.5U/ml IL1-β markedly decreased GSIS of CDs but had no effect on CDr (620±97 vs. 2187±262pmo/l, $P<0.001$ respectively). Larger concentrations of IL1-β reduced GSIS of CDs and CDr but had a bigger effect on CDs. Cu completely restored the initial GSIS of CDs (2409±428pmol/l $p=0.02$) up-to the GSIS of CDr and prevented the reduction in GSIS induced by IL1-β in both CDs and CDr. Addition of iNOS inhibitor (L-NOARG) did not affect the reduced initial insulin secretion of CDs but prevented the reduction in GSIS induced by IL1-β in both CDs and CDr. In-vivo, CDs-HSD rats treated with the iNOS inhibitor aminoguanidine or fed HSD-Cu exhibited a reduction of the OGTT-AUC ($P=0.02$ and $P=0.001$ respectively) compared to vehicle treated CDs-HSD rats. Exogenous insulin produced a significant reduction in the ITT-AUC of aminoguanidine and HSD-Cu treated rats (2950±168 and 2549±303 mg/dl/min respectively) compared to vehicle treated rats (4533±531mg/dl/min, $p<0.05$).
Conclusion: In vitro, the initial lower capacity of the CDs-islets to secrete-insulin in response to glucose was restored by Cu but not by iNOS inhibi-

tion. CDs-islets are more sensitive to the deleterious effect of IL1-β compared to CDr. The IL1-β-induced reduction in insulin-secretion was reversed by both Cu and iNOS inhibition. In vivo, Cu and iNOS inhibitor significantly decreased the hyperglycemia of the CDs-HSD rats and increased their peripheral sensitivity to insulin. The ability of iNOS inhibitor to prevent the IL1-β induced reduction in insulin secretion provides a possible link between oxidative stress and β cell dysfunction. The fact that Cu protected the β-cells from the deleterious effect of IL1-β in a similar fashion as iNOS inhibitor suggest that the restoration of β cells function by Cu is at least partially obtained by reducing oxidative stress. Understanding the mechanisms regulating β-cell dysfunction is important for the development of novel disease modifying treatments.
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PS 15 Beta cell lipotoxicity

381

Inhibition of MiR-375 supresses lipoapoptosis in mouse insulin-secreting cell line NIT-1
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Background and aims: Free fatty acids (FFAs) -induced apoptosis of pancreatic β-cells, namely lipoapoptosis, has been regarded as a critical determinant in the pathogenesis of Type 2 diabetes (T2D). MiR-375 is a evolutionarily conserved and islet-specific microRNA that is validated as a regulator of insulin secretion. The mechanism underlying the action of miR-375 is due to the reduction in expression of its target gene, myothophin. The purpose of this study was to investigate the role of miR-375 in the lipoapoptosis of a pancreatic β-cell line, NIT-1.
Materials and methods: We transfected mouse β-cell line NIT-1 with miR-375 or antisense oligonuleotide(2′ -O-me-375) to enhance or specifically inhibit miR-375 function .As negative controls ,NC-miR375 and liposome were also transfected. The change in the percentage of apoptotic cells upon palmitate challenge (incubated with 500uM palmitate for 48h) was evaluated by Hochest33342 and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end-labeling (TUNEL) staining, respectively. Meanwhile,western blot for myothophin was performed.
Results: Results showed that overexpression of miR-375 was associated with enhanced pancreatic β-cell lipoapoptosis (TUNEL positive cells:from13.14±4.24%to 24.00±12.53%; hochest33342 stained apoptotic cells:from 14.90±3.52 % to 23.06±12.97%,$P < 0.001$). In contrast, inhibition of endogenous miR-375 function by 2′ -O-me-375 suppressed the lipoapoptosis(TUNEL positive cells:from 13.14±4.24% to 6.06±3.89 %, Hochest33342 stained apoptotic cells:from 14.90±3.52% to 7.86±3.48%,$P < 0.001$). We also found that the protein level of myotrophin is inversely correlated with the percentage of palmitate -induced apoptotic NIT-1 cell.
Conclusion: MiR-375 may serve as a regulator of pancreatic β-cell lipoapoptosis and a potential therapeutic target of Type 2 diabetes.

382

WITHDRAWN

383

Palmitate triggers distinct cellular redistribution of PKCdelta and counteracts its degradation in isulin secreting INS-1 cells
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Background and aims: Previous work demonstrated that transgenic mice over-expressing a kinase dead PKCdelta (PKCdeltaKN) in beta-cells are protected from high fat diet - induced impaired glucose tolerance. However, the importance of PKCdelta and the molecular mechanisms underlying high fat diet - induced beta-cell failure are not completely understood. Upon stimulation, PKCdelta translocates to cellular compartments such as plasma membrane, nuclei, mitochondria and endoplasmic reticulum. This translocation is a prerequisite for the regulation of diverse cellular processes by PKCdelta. The present study examines changes in PKCdelta signalling induced by saturated free fatty acids.
Materials and methods: To follow palmitate-mediated subcellular redistribution of PKCdelta, INS-1E cells were transfected with a wild type PKCdelta (PKCdeltaWT) or a kinase dead PKCdelta (PKCdeltaKN) using a retroviral infection system. The expression of PKCdelta was assessed by Western blotting and its subcellular distribution by immunocytochemistry. The percentage of apoptotic cells was quantified by TUNEL versus TOPRO3 DNA staining.
Results: Short term treatment (1-10 min) with palmitate (600 μM) triggered a transient nuclear accumulation of PKCdeltaWT followed by a cytoplasmic redistribution of the kinase. After long term treatment (1 d) with palmitate cytosolic clustering of PKCdelta was observed without any visible degradation. More importantly, after depletion of PKCdelta by treating the cells with phorbol ester for 1 d (PMA, 100 nM) the addition of palmitate resulted in a

significant reappearance of cytosolic PKCdelta protein. In contrast to palmitate, PMA within the first min induced a rapid translocation of PKCdelta to the plasma membrane, followed by a transient nuclear accumulation and perinuclear localisation of the kinase. In INS-1 cells that over-expressed PKCdeltaKN, PKCdelta immunoreactivity accumulated in the nucleus after stimulation with palmitate or PMA without subsequent redistribution and any sign of degradation. Palmitate (600 μM for 2 d) treatment increased the percentage of apoptotic cells 2- to 3-fold in control INS-1 cells and in cells over-expressing PKCdeltaWT. This effect of palmitate was neither inhibited nor mimicked by the phorbol ester (PMA, 100 nM for 2 d).
Conclusion: The increased and sustained expression of PKCdelta in the presence of long term treatment with palmitate may explain that palmitate but not PMA stimulates apoptotic cell death. Nuclear extrusion and degradation of PKCdelta depends on intact kinase activity.
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384

Over-expression of kinase-dead PKCdelta induces cell cycle arrest in insulin secreting INS-1 cells
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Background and aims: The serine/threonine protein kinase C delta (PKCdelta) is a ubiquitously expressed enzyme involved in opposing cellular processes such as apoptosis, cell survival and proliferation. In pancreatic beta-cells PKCdelta was proposed to mediate the pro-apoptotic effect of saturated free fatty acids, but the underlying mechanism is still a matter of debate. Beta-cell dysfunction involves long term transcriptional changes. One transcription factor important for beta-cell survival during oxidative stress is FoxO1. The aim of the present study was to analyse the impact of PKCdelta upon changes in FoxO1 activation and beta-cell survival during palmitate exposure.
Materials and methods: INS-1 cells were transfected with PKCdeltaWT or PKCdeltaKN using a retroviral infection system. Clones that highly over-express the transgene were selected. Cell growth was estimated by cell counting. DNA content of nuclei and the amount of fractionated DNA were assessed after propidium iodide staining (Nicoletti method) with fluorescence activated cell sorting (FACS). The expression and specific phosphorylation of proteins were quantified by Western blotting and their subcellular distribution followed by immunocytochemistry.
Results: INS-1 cells that over-express PKCdeltaKN display a significant reduced growth rate compared to control and PKCdeltaWT over-expressing cells. Both populations of control INS-1 cells and cells over-expressing PKCdeltaWT consist of about 30 % of cells in G1/Go and 70 % of cells in G2. In contrast, the G1/Go DNA peak is missing in the population of cells over-expressing PKCdeltaKN. Almost all intact nuclei of these cells exhibit the double amount of DNA reflecting cell cycle arrest in G2. The PKCdeltaKN over-expressing cells contain reduced amount of FoxO1 that displays increased phosphorylation at Ser256 a prerequisite for nuclear extrusion and degradation of FoxO1. PKCdeltaWT over-expressing cells contain increased amount of FoxO1 with significantly reduced phosphorylation at Ser256. Palmitate induces FoxO1 translocation to the nucleus in control and PKCdeltaWT over-expressing cells while over-expression of PKCdeltaKN counteracts palmitate-induced nuclear accumulation of FoxO1.
Conclusion: The observation that over-expression of PKCdeltaKN reduces cell growth and induces cell cycle arrest at G2 suggests that PKCdelta is involved in the modulation of beta-cell growth. The changes in palmitate-mediated and PKCdelta-dependent FoxO1 distribution disclose a signalling pathway important for free fatty acid-induced beta-cell dysfunction. This mechanism may explain at least in part adaptive changes in beta-cell mass during obesity.
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385

Palmitate-induced dihydrosphingosine-1-phosphate production attenuates ceramide-dependent apoptosis of pancreatic beta cells
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Background and aims: Type 2 diabetes (TD2) is characterised by beta-cell dysfunction, which correlates with increases in plasma lipids and beta-cell apoptosis. Different mechanisms for the detrimental effects of chronic exposure to long chain fatty acids, in particular palmitate, have been suggested, including the de novo synthesis of ceramide, a bioactive sphingolipid. In mammals, ceramide species differ by the size and the degree of saturation of their carbon chain, suggesting that specific species might have different biological effects. To further our understanding of the role of sphingolipids in the control of palmitate-induced beta-cell apoptosis, a mass spectrometry approach has been carried out to generate a sphingolipidomic profile of palmitate-treated beta-cells.
Materials and methods: INS-1 cells were cultured with 0.4 mM palmitate, various concentrations of glucose in the presence or absence of either sphingosine-1-phosphate (S1P) or N,N-dimethylsphingosine (DMS) for 24h. In some cases, INS-1 cells were transfected with sphingosine kinase 1 (SphK1). INS-1 cell viability was measured by MTT assay. INS-1 cell apoptosis was determined by chromatin condensation assessed by DAPI staining and caspase 3/7 activity assay. SphK activity and ceramide levels were measured by in vitro kinase assay and in vitro DAG kinase assay, respectively. Sphingolipid metabolites were analyzed by mass spectrometry (LC-MS/MS).
Results: Treatment of INS-1 cells with palmitate in the presence of high glucose concentrations (HG) resulted in a 7-fold increase of caspase 3/7 activity within 24 h, indicative of apoptosis. LC-MS/MS analysis showed that palmitate increased ceramide levels, especially N-C16:0 and N-C18:0 ceramides. Although HG did not induce apoptosis after 24h, it did upregulate the production of ceramides. The increase of ceramides induced by palmitate plus HG was associated with an increase in dihydrosphingosine (DHS) and dihydroceramide levels, suggesting an enhancement of the de novo sphingolipid biosynthesis. Interestingly, palmitate plus HG stimulated DHS phosphorylation into dihydrosphingosine-1-phosphate (DHS1P). To determine the role of DHS1P in beta-cell lipotoxicity, we used DMS to inhibit the activity of SphK, the enzyme responsible for DHS1P production. We showed that DMS increased by 2-fold palmitate plus HG-induced caspase activity. Furthermore, SphK1 over-expression in beta-cells drastically increased DHS1P levels, partially inhibited caspase activation and decreased palmitate-induced ceramide accumulation. Finally, we showed that addition of S1P, a DHS1P analog, inhibited beta-cell apoptosis induced by palmitate plus HG.
Conclusion: Together, these results indicate that lipotoxicity is associated with the increased generation of ceramides that correlates with beta-cell apoptosis. Interestingly, palmitate also induces de novo biosynthesis of another sphingolipid, DHS1P, which seems to attenuate beta cell apoptosis. Therefore, up-regulation of DHS1P biosynthesis opens novel therapeutic strategy for preventing beta-cell apoptosis during TD2.
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386

Accumulation and oxidation of fatty acids modulate beta cell apoptosis but not the UPR
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Background and aims: Elevated levels of lipids are detrimental for beta-cell function and mass. Promoting lipid accumulation or oxidation has been connected with accentuation or attenuation of these effects on the β-cell, respectively. Different mechanisms of palmitate-induced beta-cell apoptosis have been proposed, where development of the unfolded protein response (UPR) has been studied intensely during recent times. The aim of the present study was to investigate if enhanced and reduced beta-cell apoptosis, observed in response to altered metabolism of lipids, involves modulation of the UPR. To address this issue beta-cells were exposed to AMPK-agonist AICAR or CPT1-inhibitior etomoxir.
Materials and methods: Insulin-secreting INS-1E cells were cultured for 3 or 24 hours in the presence of 0.5 mM palmitate complexed with 0.5% BSA and 1 mM AICAR or 0.2 mM etomoxir. Apoptosis was determined (cytoplasmic

oligonucleosomes and protein content) after 24 hours culture, whereas UPR markers phosphorylated (p) PERK, p-eIF2α, CHOP and BiP were measured (western blotting) in cells cultured for 3 and 24 hours.
Results: Apoptosis was induced 4-fold in cells cultured in the presence of palmitate compared to control. Presence of AICAR reduced apoptosis by 40%, whereas etomoxir accentuated apoptosis resulting in 15-fold induction in comparison to control. Protein content was reduced by 30% in the presence of palmitate. In the presence of AICAR the reduction was only 15%, whereas etomoxir reduced protein content by 50%. When UPR markers were examined, strong induction of PERK and eIF2α phosphorylation as well increased levels of BiP and CHOP were observed in the presence of palmitate. Expression levels of UPR markers were neither affected by AICAR nor etomoxir, however.
Conclusion: Development of the UPR is not dependent on the oxidation or accumulation of lipids in palmitate-exposed cells, which is in contrast to apoptosis.
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387

Serine/threonine protein phosphatase 5 is involved in the regulation of fatty acid-induced beta cell apoptosis
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Background and aims: In type 2 diabetes, where pancreatic β-cells are exposed to elevated glucose concentrations, a reduced β-cell mass is seen. Since type 2 diabetes is often linked to obesity, the β-cells are in conjunction with elevated glucose levels often also exposed to a hyperlipidemic environment, producing so-called glucolipotoxicity. Elevated levels of glucose and fatty acids are associated with increased production of reactive oxygen species that can cause cellular damage and initiate apoptosis. An important positive regulator of apoptosis is the mitogen-activated protein kinase (MAPK) signaling pathway. One mammalian MAPK is c-Jun N-terminal kinase (JNK), which can be activated by an apoptosis signal-regulating kinase. Serine/threonine protein phosphatase 5 (PP5) is a single polypeptide chain that are present both in the cytosol and the nucleus. It has been suggested that PP5 can act as a negative regulator of ASK1/JNK signalling. The aim of this study was to investigate the role of PP5 in the regulation of apoptosis signaling in MIN6 cells exposed to the fatty acid, palmitate.
Materials and methods: Clonal pancreatic MIN6 cells with reduced levels of PP5 were exposed to 0.5 mM palmitate together with 0.5 % BSA for 24 hours, or to 0.5 mM hydrogen peroxide for 15 min. Apoptosis induction was assessed by measuring the levels of cytoplasmic DNA-histone complexes. We also determined the protein phosphorylation levels of JNK, c-Jun, p38 and PP5 protein levels by western blotting. In order to reduce the PP5 expression level, MIN6 cells were treated with siRNA directed against PP5. We also analysed PP5 mRNA levels by quantitative PCR. Data is presented as mean±SEM.
Results: MIN6 cells treated with 0.5 mM palmitate for 24 hours showed a 5.8 ±0.2 fold induction of apoptosis compared to untreated cells. We next investigated the ability of palmitate to activate MAPK signalling by determining phosphorylation levels. This showed that both phosphorylated JNK levels increased by 135±19 % and the total c-Jun levels with 185±38 %, while the phosphorylation levels of p38 were not affected, compared to control after 24 hours of palmitate exposure. PP5 mRNA and protein levels were reduced by 60-70 %, 48 hours after siRNA treatment. The ability of palmitate to induce apoptosis was increased by 9.3±6.5 % in cells with reduced levels of PP5, compared to the control cells without reduced PP5 levels. MIN6 cells with reduced PP5 levels that were exposed to 0.5 mM hydrogen peroxide for 15 min, showed 78±25 % increased phosphorylation levels of JNK and phosphorylated c-Jun followed the same pattern, compared to the control cells without reduced levels of PP5.
Conclusion: The data presented here suggests that PP5 exerts an important role in the regulation of the apoptosis signalling cascade, since MIN6 cells treated with siRNA against PP5 showed increased levels of apoptosis after exposure to palmitate as compared to control cells. This effect, together with the finding that palmitate activates both JNK and c-Jun, suggest that enhancement of PP5 expression might be harnessed to advantage to reduce cellular damage induced by lipotoxicity and may prove valuable in efforts aimed at conferring β-cell protection against diabetic lipoapoptosis.
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388

Comparative analysis of *db* and *ob* gene-related pancreatic beta cell function; evidence for a compensatory mechanism of cell protection in *ob/ob* mice
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Background and aims: The *db* gene homozygous *db/db* mice develop diabetes with a marked obesity, severe insulin resistance, and limited capacity of insulin secretion. The *ob* gene homozygous *ob/ob* mice with a similar genetic background to *db/db* also develop severe obesity due to overeating, but hyperglycemia is not significant because of a compensatory hypersecretion of insulin. The molecular mechanism to explain a different phenotype for β cell function between 2 strains of mice has not clarified yet. In this study, we extensively analysed the β cell function and gene expression profile of pancreatic islets in *db/db*, *ob/ob*, and lean littermates *m/m* mice.
Materials and methods: Body weight (BW), fasted blood glucose (FBG), fasted insulin (FIRI), TG and FFA were measured at 8 and 12 weeks of age. The β cell mass and cell proliferation/apoptosis were assessed by histological analysis including PCNA and 4-HNE immunostaining of the islet tissue. Gene expressions specific for the core area of pancreatic islet were analyzed by Laser Capture Microdissection method and real time RT-PCR. Primer pairs encoding genes associated with pancreatic hormones, cell proliferation, apoptosis, cell cycle, and oxidative stress were prepared, and real-time RT-PCR with Sybr Green was applied.
Results: BW in *db/db* mice was significantly lower than those in *ob/ob* mice, but was significantly greater than in *m/m* mice. FBG in *db/db* mice was significantly greater than *ob/ob* and *m/m* mice (287.4±43.2, 93.1±3.5, 78.3±9.5 mg/dl at 12 weeks of age, $p<0.05$, respectively). FIRI in *db/db* and *ob/ob* mice were significantly higher than that in *m/m* mice, but no difference was observed between *db/db* and *ob/ob* mice. On the other hand, fed blood glucose (fBG) was significantly lower in *ob/ob* with significantly higher fIRI compared with *db/db* mice (fBG: 276.5±25.3, 470.4±40.3, $p<0.05$, fIRI: 31.3±3.9, 12.7±6.4, $p<0.05$). TG and FFA levels in *db/db* mice were significantly higher than those in *ob/ob* mice. The β cell mass was significantly larger in *ob/ob* than *db/db* or *m/m* mice, and the β cell ratio in *db/db* mice was reduced when compared to the other groups of mice at 12 weeks of age (cell mass: 3.48±0.59 in *ob/ob*, 2.30±0.42 in *db/db*, 0.62±0.10 mg in *m/m*, and β cell ratio: 87.2±0.26, 74.9±1.52, 80.5±2.8%, respectively). The β cell gene expression for insulinII were significantly increased in *db/db* and *ob/ob* mice. ERK1 gene related with cell proliferation was significantly up-regulated at 8 weeks of age both in *db/db* and *ob/ob* compared with *m/m* mice. At 12 weeks this gene expression was significantly down-regulated in *db/db* mice, whereas further up-regulated in *ob/ob* mice. The expression of CAD gene related with apoptosis promotion was already increased at 8 weeks and further accelerated at 12 weeks in *db/db*, but not in *ob/ob* mice. The gene expression of pdx-1 related with cell differentiation and SOD2 related with anti-oxidative stress was much less in *db/db* than in *m/m* mice at 12 weeks. In contrast, these genes were significantly up-regulated in *ob/ob* compared with *m/m* mice. Morphometric results for PCNA and 4-HNE corresponded with the data of gene expression analysis.
Conclusion: Our results strongly suggest that the *ob* gene, but not *db* gene, homozygote acquires a compensatory mechanism of β cell protection, resulting in suppression of cell apoptosis, and promotion of cell differentiation and proliferation probably through anti-oxidative stress mechanism.

389

OxLDL toxicity towards insulin secreting cells is mainly exerted extracellularly
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Background and aims: CD36 is known in many types of cells to scavenge oxidized LDL (oxLDL) and possibly native LDL (nLDL), thus increasing lipoprotein uptake. However, scavenging of oxLDL and/or nLDL has not been demonstrated in beta cells, nor whether putative scavenging affects beta cell functions.

Materials and methods: We evaluated by FACS the uptake of FITC-labeled oxLDL or nLDL, during 6h in a tet-on insulin secreting cell line (INS-1) over-expressing CD36. We quantified insulin secretion and content by RIA after 48 h of co-culture with oxLDL or nLDL. Viability of cells was evaluated by MTT assay.
Results: CD36 over-expression increased uptake of oxLDL 2.2±0.3 fold, p<0.01, n = 4, whereas over-expression did not affect uptake of nLDL, n = 4. Uptake was independent of ambient glucose concentrations (2.5, 10 or 21.5 mM). In acute experiments neither lipoproteins nor CD36 over-expression affected insulin secretion tested at 2.5 and 21.5 mmol/l glucose. In long-term experiments over-expression decreased glucose induced insulin secretion from 11.3±2 to 8.5±1 (% released of cellular insulin contents, p = 0.06, n=4) when cells were cultured with 20 μg/ml oxLDL for 48h. The corresponding decrease after culture with nLDL was from 7.8 ±1 to 6.3±1 %, p=0.063, n=4. Exposure to oxLDL (20 μg/ml) for 48h decreased insulin content similarly in control cells (-29 ± 7 %) and in CD36 over-expressing cells (-29 ± 6 %), p<0.02 for the oxLDL effect. An eqvimolar concentration of nLDL did not exert any significant effect (-2 ± 5% for control cells and -3±4% for CD36 over-expressing cells. Viability after 48h incubation with oxLDL were for control cells 101 ± 9 % with 20, 64 ±14 with 50 and 4±1 % with 100 μg/ml of oxLDL (expressed as % of no addition). Corresponding effects in CD36 over-expressing cells were 91±6, 64±12 and 3±1 %, n=3. Hence, CD36 over-expression did not potentiate the negative effect of oxLDL.
Conclusion: Over-expression of CD36 leads to a preferential uptake of ox-LDL which is independent of ambient glucose concentration. The increased uptake is not associated with aggravation of the inhibitory effects of oxLDL on insulin content and viability. These findings indicate that oxLDL toxicity towards insulin secreting cells is mainly exerted extracellularly.
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390

Deterioration of islets structure and function in Zucker diabetic fatty (ZDF) rats during disease progression is prevented by pioglitazone but not by metformin

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Background and aims: The ZDF rat is a well accepted model to demonstrate the efficacy of anti-diabetic drugs, however the data evaluating the effect on disease progression and β-cell protection are rather limited. Our previous study in male ZDF rats looking at the age-dependent changes has shown that deterioration of islet structure and function precedes alterations of the metabolic profile. The aim of this study was to characterize the effect of the commonly used anti-diabetic drugs Metformin (MET) and Pioglitazone (PIO) on disease progression and prevention of β-cell failure.
Materials and methods: Measurement of metabolic parameters under basal and glucose challenge (oGTT at 2g/kg) together with quantitative analysis of islet morphology and *in situ* pancreas perfusion were performed after 4 weeks of treatment with MET (300 mg/kg p.o.) or PIO (10 mg/kg) in 6-week old male (ZDF-6w) rats and compared to age-matched Vehicle (V) or ZDF-6w rats. Pancreata were stained for insulin, glucagon and dapi.
Results: Treatment of ZDF rats with PIO and MET for 3 weeks led to a similar improvement of glucose levels during an oGTT ($AUC_{glu\text{-}2h}$ PIO: 1460 , MET: 1393 and V:1736) while only PIO significantly prevented hyperinsulinemia ($AUC_{ins\text{-}2h}$ PIO: 500, p<0.001, MET: 950, n.s. vs. V: 980 ng/mL*min). These changes were accompanied by a significant increase in insulin sensitivity (Matsuda Index: PIO: 400, p<0.01, MET: 200, n.s. vs. V: 130). In addition, PIO significantly decreased fasting blood glucose levels (PIO: 5.1, p<0.001, MET: 5.9, n.s. and V: 7.4 mM), fasting TG (PIO: 2.4, p<0.05, MET: 8.3, p<0.01 vs. V: 4.5 mM) and postprandial response of TG during oGTT ($AUC_{tg\text{-}2h}$ PIO: 210, p<0.01, MET: 800, p<0.01 vs. V: 500 mM*min). Quantitative immunohistochemistry analysis showed that MET was not able to prevent either islet area expansion (+70%, p<0.0001, V: +96%, p<0.0001) and/or loss of insulin staining intensity (-25%, p<0.0001, V: -38%, p<0.0001) when compared with ZDF-6w. PIO, on the contrary, prevented loss of insulin staining intensity (PIO: -0.5%, n.s.) without changing the islet area when compared with ZDF-6w (PIO: +22%, n.s.). These results were further supported by the total pancreatic insulin content, which was preserved with PIO (+9%, n.s.), but markedly reduced with MET (-37%, p<0.01, V: -40%, p<0.01), compared to ZDF-6w. PIO prevented α-cell invasion into the islet core better than MET (PIO: +109%, MET: +282% and V: +429%, all p<0.001) compared to ZDF-6w. *In situ* pancreas perfusion showed that PIO significantly reduced basal insulin secretion when compared to V and MET (-84% and -59%, respectively). While the profiles of phase I and II of insulin secretion were similar in both treatment groups, PIO attenuated the off-response to a greater extent than MET ($AUC_{off\text{-}response}$ PIO: -20%, MET: 15%).
Conclusion: Our data have clearly show that MET and PIO have different effects on disease progression and β-cell failure in the ZDF rat model. In particular, 4-week treatment with MET led to an improvement of glucose tolerance and insulin sensitivity but failed to protect the islets from the deterioration of their structure and function. On the contrary, PIO treatment protected the β-cell from exhaustion occurring during disease progression, which translated into a better metabolic profile and organ function.

PS 16 Experimental type 2 diabetes

391

Predicted targets of co-expressed microRNAs in pancreatic islets of GK rats are enriched for genes involved in regulation of beta cell mass and glucose-stimulated insulin secretion

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Background and aims: The Goto-Kakizaki (GK) rat is a well-characterized animal model of non-obese spontaneous type 2 diabetes (T2D) with reduction in beta cell mass and impaired glucose-stimulated insulin secretion as main associated phenotypes. While mRNA expression and functional analyses of gene products implicated in the GK rat disease progression are abundant, the contribution of microRNAs (miRNAs) in eliciting the GK phenotype has not been fully explored. Previous studies with cultured insulin-secreting cell lines identified specific miRNAs important for proper beta cell function. We aimed to investigate whether these miRNAs are differentially-expressed in the pancreatic islets of the GK rat. Moreover we aimed to detect other miRNAs that could be involved in the development of the disease phenotype and identify their potential targets via bioinformatic approaches.
Materials and methods: We utilized LNA (locked nucleic acid)-based arrays to profile the miRNA expression levels in the pancreatic islets of GK rat *vs.* control Wistar rat (N=5 per group, 60 days old, females). Co-expressing miRNAs were then used as input to the meta mir-target inference (MAMI) web application combining the results of five miRNA target prediction tools (miRanda, PicTar, TargetScanS, microT and miRTarget). Gene enrichment analysis was performed to identify Gene Ontology (GO) categories associated with collective targets of co-expressed miRNAs (Fisher Exact p-value <0.005).
Results: We identified two distinct clusters consisting of 46 up-regulated and 32 down-regulated miRNAs in the GK rat using Significant Analysis of Microarrays (SAM). Enrichment for GO categories of the combined 2073 predicted targets of nine most upregulated miRNAs in GK rat yielded functional annotation clusters highly representative of genes in developmental process, cell differentiation, regulation of apoptosis among other GO terms potentially involved in the maintenance of beta cell mass. Interestingly, we found in the cell differentiation category the gene myotrophin, previously shown to be targeted by miR-375. Significant enrichment was also found for the GO category: vesicle-mediated transport, which include genes with central roles in glucose-stimulated insulin secretion such as RAB27A, RAB3A and granuphilin. We found in our arrays one of the most upregulated miRNAs in GK rat as being mir-124, known to regulate the expression of Rab27A and Rab3A. In addition we also observed increased expression of previously known negative regulators of insulin secretion such as mir-9 and mir-375 in GK rats relative to controls.
Conclusion: The predicted collective targets of co-expressed microRNAs upregulated in the GK rat were significantly enriched for genes involved in the maintenance of pancreatic beta cell mass and glucose-stimulated insulin secretion. The relatively higher expression of mir-9, mir-375 and mir-124 we observed indicates negative regulatory effects of these miRNAs in insulin secretion in the GK rat pancreatic islets, similar to previous reports in cultured insulin-secreting cell lines. Our bioinformatic analysis yielded testable hypotheses to support the notion that the GK diabetic phenotype could be caused by impaired miRNA regulation of targets involved in the maintenance of beta cell mass and insulin secretion.
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392

Increased VEGF expression in beta cells leads to islet inflammation and diabetes

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Background and aims: Islets of Langerhans are abundantly vascularised and this vascularization is key to islet function. It regulates insulin access to systemic circulation, and the arrival of nutrients and secretagogs to endocrine cells. Furthermore, it has been recently described that islet endothelial cells directly regulate ß-cell proliferation and insulin gene expression. VEGF (Vascular Endothelial Growth Factor) is considered the main pro-angiogeneic factor and it can induce islet vessel formation and mantainance. Thus, VEGF may play an important role in controling insulin secretion and ß-cell mass.
Materials and methods: The effect of VEGF in islet vascularization and endocrine pancreas function was examined in transgenic mice overexpressing VEGF specifically in ß-cells and in animals treated with adeno-associated viral vectors carrying a RIP/VEGF gene (AAV/VEGF).
Results: Transgenic mice showed disorganised islets with increased vascularization. However, ß-cell mass, blood glucose levels, insulinemia and insulin secretion in vivo was not different between two-month-old transgenic mice and controls. Nevertheless, transgenic mice developed glucose intolerance overt diabetes with age. Moreover, these animals showed endocrine pancreas inflammation, with abundant macrophage infiltration and high levels of cytokine expression. To confirm these results and to avoid undesirable effects of the expression of the transgenic during embryonic development we used AAV vectors to transduce the islets of healthy adult mice. The AAV-mediated expression of VEGF in ß-cells in vivo also led to islet inflammation and glucose intolerance, suggesting a detrimental role of VEGF in pancreatic islet function.
Conclusion: Long-term VEGF overexpression in ß-cells may induce endocrine pancreas inflammation leading to islet dysfunction and diabetes.
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393

IGF-II expression in beta cells induces susceptibility to develop diabetes

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Background and aims: Both type 1 and type 2 diabetes are characterized by a deficit in ß-cell mass and increased ß-cell apoptosis. Replacing missing insulin-producing ß-cells to treat diabetes is a major challenge for regenerative medicine. A better understanding of the mechanisms involved in both ß-cell death and ß-cell regeneration in adult life is needed to develop means to increase ß-cell mass and revert diabetes. IGF-II induces ß-cell proliferation and differentiation in vitro and is involved in the regulation of islet growth and differentiation. We have shown that expression of IGF-II in ß-cells of transgenic mice (RIP-I/IGF-II) leads to increased ß-cell mass, hyperinsulinemia and altered glucose and insulin tolerance tests. Furthermore, polymorphic variants in locus IDDM2, which contains insulin and the growth factor IGF-II genes, have been described to be associated with type 1 diabetes risk. In addition, associations have been reported between IGF2BP2 (which encodes and IGF-II mRNA binding protein) and type 2 diabetes. These findings suggest a key role of IGF-II in diabetes susceptibility.
Materials and methods: IFN-ß/IGF-II double transgenic were obtained and phenotyped. Treatment with streptozotocin was performed in IGF-II transgenic mice. Expression profile analysis in islets from IGF-II transgenic mice was performed using GeneChip™ Affimetrix™ Technology.
Results: To determine whether IGF-II overexpression in ß cells could predispose transgenic mice to develop overt diabetes, we tested the susceptibility of RIP/IGF-II mice in front of several stimuli. Transgenic and control mice were treated with very low doses of streptozotocin (STZ) and after this treatment only transgenic mice became diabetic, indicating that IGF-II overexpression in ß cell increased sensitivity to STZ damage. Furthermore, IGF-II transgenic mice were crossed with RIP/IFN-ß transgenic mice, which show an important infiltration in their islets, mimicking an autoimmune process, a common feature in the diabetic pathology. We obtained IFN-ß/IGF-II double transgenic mice, which spontaneously developed diabetes during the first two months of age. All these findings suggest that an increase of IGF-II in ß cell may be key in the initiation of the diabetic process. Data from gene expression profile in islets from RIP/IGF-II showed a reduction in Glut2 and insulin gene expression in ß cells, probably due to a reduction in the transcription factors that regulate their expression, such as Pdx1 and HNF3ß. Transgenic islets also showed hyperexpression of molecules involved in the immune response, such as MHC class I and II, although no lymphocyte infiltration was observed. In addition, transgenic islets presented alteration of expression of genes involved in β-cell endoplasmic reticulum (ER) stress, characteristic of diabetic process.
Conclusion: These results indicate that local expression of IGF-II in ß-cells may increase susceptibility to develop diabetes by inducing metabolic and molecular alterations.
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394

Diet-induced gene expression of isolated pancreatic islets from a polygenic mouse model for the metabolic syndrome

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Background and aims: NZL mice are related with the New Zealand Obese (NZO) strain and develop juvenile-onset obesity and maturity-onset hyperglycemia. Exposure of these mice to a high-fat diet (HFD) results in rapid destruction of the pancreatic beta cells leading to frank diabetes. A carbohydrate free HF diet (CHFD), which still leads to the development of severe obesity and severe insulin resistance, prevents this destruction of beta cells. In order to investigate the contribution of dietary carbohydrates to pancreatic beta cell failure, we isolated pancreatic islets with laser capture microdissection and performed genome-wide expression analyses.
Materials and methods: Cryosections of pancreata from 8 week old, CHFD and HFD fed NZL-mice were briefly stained with Cresyl-Violet. Islet cells were isolated with laser capture microdissection (LCM). The RNA was purified and labelled cRNA was generated without an amplification step. The cRNAs were hybridised to Affymetrix Mouse 430 2.0 GeneChips. Differential expression of selected genes was validated by TaqMan-Realtime-PCR.
Results: By comparison of gene expression levels from isolated islets and total pancreas, we identified 230 transcripts that were highly enriched (>10-fold) in islets cells, many of those genes have an unknown function. In the early, pre-diabetic state of the animals, 786 islet transcripts were differentially regulated (>2-fold) between the two diets. Pathway analysis identified oxidative phosphorylation (OXPHOS) as the predominant gene set that was significantly up-regulated in response to the diabetogenic HFD. Moreover, HFD induces early changes in gene expression associated with increased cell cycle progression, proliferation, and differentiation of islet cells such as *Cdkn1b*,*Tmem27*, and *Pax6*. Regulators of the cellular redox state such as *Cat, Gpx1, Prdx4* and *Txnip* were also substantially increased in HFD islet, indicating that glucose-induced oxidative stress is relevant for disease progression. Furthermore, we identified 24 novel human candidate genes (e.g. *DACH1, CACNA1D, CHD2, CLIP2, IGFBP2*) for type 2 diabetes by correlating disease progression-associated genes in mouse islets with data from two publicly available genome-wide scans (DGI, WTCCC) for diabetes.
Conclusion: Our data clearly demonstrate the critical role of dietary carbohydrates in the pathophysiology of high fat-diet induced diabetes. LCM allows isolation of authentic pancreatic islet cells from their native tissue environment for genome-wide expression profiling. In islets, the diabetes-protective effect of a carbohydrate-free high-fat diet correlates with a reduced expression of genes for OXPHOS and antioxidants, implicating a major contribution of reactive oxygen species to diet-induced beta-cell failure.
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395

Attenuation of Ca^{2+} microdomains and diet-induced diabetes in mice

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Background and aims: Diet-induced diabetes in C57/Bl6 mice shows striking similarity to the evolution of diabetes in humans. The compensatory mechanisms put in place by the β- cell to overcome the increased demand in insulin are initially effective. However, by 15 weeks on high fat diet, β- cell function worsens together with glucose tolerance. Our aim was to pinpoint the cellular mechanisms leading to impaired glucose induced insulin secretion in the long run.
Materials and methods: Insulin release mesurements were combined with capacitance measurements and optical imaging of secretion by Total Internal Reflection Microscopy (TIRF) microscopy. Cytoplasmic $[Ca^{2+}]_i$ was measured by microfluorimetry. Intracellular ATP levels were determined by the luciferin/luciferase method. Membrane currents and potentials were recorded by the patch-clamp method. All results are based on at least 6 separate measurements from at least 3 different mice in each group.
Results: β-cell metabolism, electrical activity and $[Ca^{2+}]_i$ were unaltered by high fat feeding. Instead, a rapid component of exocytosis during capacitance measurements was decreased by up to 50%. This inhibition was confined to the first 500 ms of depolarization. These experiments were corroborated by TIRF microscopy which showed a strong inhibition of rapid (during first 300 ms) in the high fat diet (HFD) group of recombinant mIAPP-Cherry release. Using the perfused pancreas preparation, we demonstrated that this effect correlated with strong (>50%) inhibition of both 1st and 2nd phase insulin secretion. The inhibitory effect of the HFD was particularly strong for glucose-induced insulin secretion and the effect was less pronounced for insulin release evoked by high extracellular K^+**.** Thus, increasing the influx of calcium rescues insulin secretion. We consequently tested whether Ca^{2+} microdomains at the plasma membrane were affected. Evanescent-field illumination of voltage-clamped cells infused with EGTA (10 mM) and Calcium Green 6F (10 μM) stimulated by a single 50-ms depolarization from -70 mV to 0 mV showed that HFD results in a significant decrease in the clustering of calcium signal within microdomains. All differences stated are significant at the 0.05 level (at least).
Conclusion: Our results implicate that a reduction in Ca^{2+} microdomains within β-cells plays a part in the aetiology of diet induced type 2 diabetes.
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396

Administration of a synthetic peptide demonstrating intrinsic kinase inhibitory properties increases residual pancreatic beta cell mass and improves glycaemic control in db/db mice

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Background and aims: Using proteomic analyses, we have previously identified a novel serum biomarker from the resistant strain of the Cohen diabetic rat model. Although the natural biological function of this marker (termed D3 peptide) is unknown, our studies indicate that it functions as a potent kinase inhibitor *in vitro*. In the present studies we assessed the efficacy of synthetic D3 peptide as a potential biotherapeutic using leptin receptor-deficient db/db mice as a model for type 2 diabetes.
Materials and methods: Seven week old db/db mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Animals were administered 1 mg of D3 peptide in PBS, or PBS alone via the i.p. route twice weekly for 28 days. For a further 7 days, dosing frequency was increased to once every 48 hours. Body weights and non-fasting blood glucose levels were determined on a weekly basis. Plasma levels of glucose, insulin, IL-6, resistin, adiponectin and lipid profiling were performed on day 28. Following termination of the study, histological examination was conducted on hepatic, pancreatic, and adipose tissues.
Results: Treatment of db/db mice with D3 peptide reduced non-fasting blood glucose levels compared to control animals for the duration of the study. On day 28, despite observing only a slight increase in plasma insulin levels in the peptide treated group ($p>0.05$), fructosamine levels were significantly reduced compared to untreated controls ($p<0.01$). No significant differences in plasma lipid profiles or additional disease indicators were noted at this time. By day 35, mean blood glucose levels were reduced by approximately 30 % in peptide treated animals ($p<0.005$). Although the mass of both hepatic and pancreatic mass was increased in animals receiving D3 peptide, histological analysis revealed no significant differences between the treatment groups for insulitis, islet inflammation, acinar inflammation, islet hyperplasia, liver degeneration or liver inflammation. However, inflammation scores in both mesenteric and gonadal adipose tissues were approximately double in D3 peptide treated animals ($p<0.001$ in both instances). Interestingly, a marked increase in residual pancreatic beta cell mass was observed in peptide treated animals compared to PBS controls (3.80 ± 0.4 mg vs. 1.45 ± 0.2 mg respectively, $p<0.0001$).
Conclusion: The present study demonstrates that D3 peptide is efficacious at significantly reducing blood glucose and fructosamine levels in the db/db model of type 2 diabetes. Based on the current data set, we hypothesize that the peptide mediates its effect by regulation of kinase-dependent pathways causing a reduction in beta cell death, or the induction of beta cell proliferation leading to the observed increase in residual beta cell mass. However, as peptide treatment failed to cause a significant increase in plasma insulin levels, the peptide may also be contributing to improved glycemic control by upregulating systemic glucose uptake, or improving insulin sensitivity. Additional studies are planned to further explore the efficacy of D3 peptide using additional diabetic animal models, and to define its mechanism of action.

397

Exposure of human islets to palmitate mimics the mild inflammatory response observed in islets from type 2 diabetic individuals

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Background and aims: Gluco- and lipotoxicity may contribute to β-cell dysfunction and apoptosis in type 2 diabetes (T2D). It has been proposed that increased circulating levels of IL-1β, TNF-α and IL-6 are independent risk factors for T2D, but the role of inflammation in the pathogenesis of T2D is controversial. Our aim was to analyze the expression of chemokines and cytokines in human islets from T2D patients and to evaluate *in vitro* the potential contribution of free fatty acids (FFAs) and high glucose to the putative inflammatory process.
Materials and methods: Chemokine and cytokine expression was studied by microarray analysis of laser capture microdissected β-cells from 10 non-diabetic and 10 T2D pancreata. Human islets were exposed to the FFAs oleate or palmitate (0.5 mM, 1%BSA) for 24 and 48h at 6 or 28 mM glucose. Chemokine and cytokine mRNA expression was measured by real time RT-PCR (n=6-9) and IL-6 and Gro-α protein secretion by ELISA (n=4-6). IL-1-receptor antagonist (IL-1ra) was used to block the IL-1R.
Results: The microarray analysis of human β-cells showed increased expression of several chemokines and cytokines in T2D compared to non-diabetic controls including: *IL-8* (2.2-fold induction vs control, p=0.015), *MCP-1* (3.7-fold, p=0.015), *eotaxin* (4.4-fold, p=0.063), *MCP-4* (3.4-fold, p=0.001), *Gro-α* (3.4-fold, p=0.09), *IL-6* (2.3-fold, p=0.48), *IL-1β* (1.9-fold, p=0.11). Exposure of human islets to palmitate, but not to oleate or high glucose, induced mRNA expression of *IL-8* (5.0±1.0-fold vs control after 24h, p<0.001; 7.4±1.8-fold after 48h, p<0.001), *MCP-1* (2.1±0.4 after 48h, p<0.001), *Gro-α* (7.6±2.9 after 24h, p<0.05; 7.1±2.4 after 48h, p<0.001), *IL-6* (4.7±0.8 after 24h, p<0.001; 6.5±0.9 after 48h, p<0.001), *IL-1β* (2.0±0.3 after 24h, p<0.01; 2.8±0.6 after 48h, p<0.05) and *TNF-α* (2.4±0.5 after 24h, p<0.01; 3.1±0.7 after 48h, p<0.01). mRNA induction by palmitate was similar at 6 and 28 mM glucose and was paralleled by protein secretion to the culture medium: IL-6 (2.0±0.3-fold vs control after 24h, p<0.05), Gro-α (2.7±1 after 48h, p<0.05). Multiple regression analysis of chemokine and cytokine mRNA expression suggested that IL-1β and TNF-α might regulate chemokine and cytokine expression. IL-1ra abolished palmitate-induced cytokine and chemokine expression at the mRNA and protein level but it did not prevent human islet cell death (control 5±0.7 % cell death; palmitate 13±1; palmitate + IL-1ra 14±2.1 after 3 days, n=4). Palmitate, but not oleate or high glucose, activated NF-κB in human islets, as evaluated by IκBα mRNA expression (2.4±0.6-fold induction vs control after 24h, p<0.05; 2.6±0.6 after 48h, p<0.01); this increase was abolished by IL-1ra. Immunofluorescence demonstrated that NF-κB nuclear translocation occurs in β- and non-β cells.
Conclusion: Islets from T2D patients have a mild increase in the expression of selected chemokines and cytokines. A similar mild inflammatory response is observed in human islets exposed in vitro to saturated FFAs, this response is at least in part dependent on IL-1β production; but it does not contribute to lipotoxicity. The mechanisms by which palmitate induces chemokines, and their potential role in β-cell dysfunction in T2D remain to be clarified.
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398

Kir6.2 accumulation in adult Sur1-KO islet cells triggers only moderate oxidative stress without an endoplasmic reticulum stress response

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Background and aims: The K_{ATP} channel subunits SUR1 and Kir6.2 contain a retention signal in their amino-acid sequence that prevents their cell surface addressing until they are correctly assembled in functional hetero-octamers. It is therefore likely that, when expressed in the absence of their partner, each subunit accumulate in the endoplasmic reticulum (ER), thereby inducing an ER stress with eventual oxidative stress. To test this hypothesis, we investigated whether the absence of SUR1 in pancreatic endocrine cells alters the localization of Kir6.2 and induces ER stress- and oxidative stress-responses.
Materials and methods: We used islets from wild type (WT) and *Sur1* knockout (*Sur1*$^{-/-}$) mice in which 1) the sub-cellular localization of Kir6.2 and calreticulin (an ER marker) were determined by immunofluorescence and immunogold, 2) the rough ER (RER) length and Kir6.2 gold particle density were measured by morphometry (results are means ± SEM) and 3) the mRNA levels of several ER stress-response genes and antioxidant enzymes were evaluated by real-time RT-PCR.
Results: In WT islet cells, Kir6.2 immunostaining was diffuse in the cytoplasm and markedly enhanced at the plasma membrane. In contrast, Kir6.2 was mainly localized around the nucleus of *Sur1*$^{-/-}$ islet cells without positive immunoreactivity at their plasma membrane. This staining pattern was similar to that of calreticulin, suggesting ER localization of Kir6.2. Moreover, calreticulin staining intensity was higher in *Sur1*$^{-/-}$ than WT islet cells, suggesting a greater RER density. At electron microscopy, Kir6.2 gold particles were mainly localized on secretory granules of WT islet cells while they were mostly observed on the RER in *Sur1*$^{-/-}$ endocrine cells, leading to a significant increase in Kir6.2 gold particle density in the RER (0.053 ± 0.014 vs 0.027± 0.009 particles/μm in *Sur1*$^{-/-}$ vs WT beta cells, P<0.01). *Sur1*$^{-/-}$ islet cells also showed an increase in the length of the RER (32.6 ± 4.2 vs 8.5 ± 1.6 μm/cell in *Sur1*$^{-/-}$ vs WT beta cells, P<0.001) but no dilation of its lumen. Despite these multiple signs of Kir6.2 retention in the RER, Xbp1 mRNA splicing and the mRNA levels of the ER stress-response genes Bip, Edem and Gadd153 were not different between WT and *Sur1*$^{-/-}$ islets. In contrast, the mRNA levels of the antioxidant enzymes Sod1, Sod2, Gpx2 and Catalase were significantly up-regulated in *Sur1*$^{-/-}$ vs WT islets by 1.5, 1.9, 2.6 and 2.2-fold respectively.
Conclusion: Our results suggest that, in *Sur1*$^{-/-}$ islet cells, sequestration of the majority of Kir6.2 subunits in the RER is associated with an increase of RER length and mild oxidative stress without activation of the classical ER stress response.
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399

Increased pancreatic islet GLP-1 content and release in mice with deletion of glucagon receptor

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Glucagon-like peptide (GLP)-1 is an incretin hormone released from the intestine L-cells after ingestion of a mixed meal, exerting various effects on pancreas insulin and glucagon release as well as on gastrointestinal motility. Prior studies showed that mice with a null mutation of the glucagon receptor (GCGR KO) have increased circulating GLP-1 levels. However, the sources of this elevated GLP-1 have not been clearly defined. To address this outstanding question, we performed immunohistochemistry (IHC) analysis of the pancreas and assessed GLP-1 content and release using islets from wild type (WT) and GCGR KO mice. We observed a significant increase of GLP-1 positive cells in the pancreatic islets of GCGR KO mice relative to the WT mice. Likewise, the hormonal contents of glucagon and active GLP-1 were significantly increased in islets isolated from KO mice compared with WT mice (by 5 and 90 folds respectively, n=6). When islets were statically cultured in glucose-containing culture medium, release of active GLP-1 and glucagon from the KO islets markedly increased when compared with that of WT islets. This elevated hormone release by KO islets appeared to be unaffected by changes of glucose concentration in the culture medium from 3 mM to 15 mM. Microarray and real-time RT-PCR analyses revealed elevated mRNA levels of proglucagon (15 fold), PC2 (1.94 fold), ISL-1 (1.82 fold) and MafB (2.31 fold) genes, but decreased mRNA levels for MafA (0.49 fold), PC1 (0.5 fold) expression in the GCGR KO islets. Our data suggest that elevated plasma GLP-1 in GCGR KO mice is derived at least in part by the increased pancreatic islet GLP-1 synthesis and release possibly resulted from the compensatory amplification of the preproglucagon gene due to the absence of glucagon receptor signaling. Antagonizing glucagon receptor may yield additional benefits through the increase in GLP-1 production in pancreatic islets.

400

Effect of glucose abnormalities and pancreatic islet amyloidosis on the cell composition of the islets of Langerhans in non-human primates

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Impaired insulin secretion is a major physiopathologic defect in type 2 diabetes mellitus (T2DM), and pancreatic islet amyloidosis (PIA) may play an important role in the development of beta cell dysfunction. We examined the relationship between beta and alpha cell volume versus with PIA and metabolic variables in non-human primates.
Methods: Forty baboons (matched by age, sex and weight) were divided into 5 groups based upon % islet volume occupied by PIA: 0, 1-25, 26-50, 51-75, >75%. Immunohistochemical staining for insulin, glucagon and somatostatin was performed in pancreatic sections to measure beta and alpha cell volume and size using a stereological approach (CAST-OLYMPUS). Plasma glucose, insulin, glucagon and FFA concentrations were determined before death in all baboons. ANOVA was used for comparisons between groups and Pearson Correlation coefficient to evaluate the relation between variables.
Results: Beta cell volume per islet indirectly correlated with FPG (R^2=-0.54, p<0.001), PIA (R^2=-0.76, p<0.001), Ln(FFA) (R^2= -0.24, p<0.001) and directly with HOMA-B (R^2= 0.61, p<0.001). Baboons with FPG >125mg/dl had 62% reduction in beta cell volume. Alpha cell volume and alpha/beta cell volume ratios were progressively elevated with increasing FPG and PIA (p<0.05). Baboons with FPG=95-125 mg/dl had a 21% increase in alpha cell volume, those with PIA >50% had a 30% increase in alpha cell volume and 44% decrease in delta cell volume. Individual beta and alpha cell size also increased accordingly to FPG and PIA (p<0.05). The sum of all islet components (amyloid and beta/alpha/delta cells) was 99.4±4.1% in baboons with FPG <80 mg/dl and PIA=0%, and it was slightly increased (13%) in baboons with PIA >25% and FPG >95mg/dl (p=NS), and this increase was around 50% when only the islet volume free of PIA was considered (p<0.05).
Conclusion: PIA and hyperglycemia are associated with reduced beta cell volume and function, and increased alpha cell volume and size. These histological abnormalities may explain the defect in insulin secretion and hyperglucagonemia in T2DM.

PS 17 Experimental insulin secretion

401

Melatonin receptor signalling is involved in insulin secretion in the INS-1 832/13 beta cell line

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Background and aims: Genome-wide-association studies (GWAS) have recently suggested that a receptor (MTNR1B) for the pineal hormone melatonin is involved in the pathogenesis of Type 2 Diabetes Mellitus (T2DM). Melatonin binds predominantly to its receptors MTNR1A and MTNR1B, which are G-protein coupled receptors and are proposed to exert an inhibitory effect on insulin secretion. It remains uncertain which receptor mediates this effect. Here, we investigated which receptor exerts an effect on glucose stimulated insulin secretion (GSIS) and to what extent. Also, this study aimed at providing information about whether endogenous melatonin is present in the pancreatic β-cell and if it interferes with insulin secretion.
Materials and methods: To address this issue we knocked down the two different melatonin receptors (MTNR1A and MTNR1B) in the INS-1 832/13 β-cell line by RNA interference. The transfected cells were then stimulated with glucose alone and in combination with the cAMP-raising agent forskolin, given the suggested cAMP-lowering effect of melatonin.
Results: Both MTNR1A and MTNR1B were successfully knocked down by ~81% (n = 3) on the mRNA level. Upon knock down of MTNR1A in INS-1 832/13 β-cells, there was a trend towards an inhibitory effect on GSIS, whereas knock down of MTNR1B did not affect GSIS. A similar tendency was observed when β-cells were stimulated with high glucose (16.7mM) in combination with 35 mM potassium and 250 μM diazoxide to evoke K_{ATP}-independent insulin secretion. Stimulation with high glucose in combination with 2.5 μM forskolin provoked a 3-fold increase in GSIS compared to stimulation by 16.7 mM glucose alone. In contrast, knock down of MTNR1A significantly inhibited GSIS under these conditions (91 ±10 vs. 186 ±51 ng/mg/h; p = 0.03, n = 3). Silencing of MTNR1B was not associated with any inhibition of GSIS under the same conditions. mRNA for the enzyme arylalkylamine-N-acetyl transferase (AANAT), active in melatonin synthesis, was expressed in INS-1 832/13 β-cells.
Conclusion: Our data show that blocking melatonin receptor signaling in INS-1 832/13 β-cells reduces a full insulin secretory response, particularly that potentiated by cAMP. A block of MTNR1A exerts the strongest effect. Melatonin is likely to be synthesized in these cells since we were able to demonstrate expression of AANAT, the rate-limiting biosynthetic enzyme. Our data suggest that melatonin receptor signaling is involved in control of insulin secretion. It is possible that endogenous melatonin may play a role in these processes.
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402

5-hydroxytryptamine exposure stimulates insulin secretion in clonal beta cells but not in islets of Langerhans

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Background and aims: Receptors 5-HT1a (5-hydroxy-tryptophane receptor 1a) and 5-HT1a (5-hydroxy-tryptophane receptor 2b) are G-protein coupled receptors activated by 5-Hydroxytryptamine, also known as serotonin. 5-HT1a couples negatively to adenylate cyclase while 5-HT2b activates Gαq. This means that the receptors act by two distinct intracellular pathways and activate a cascade reaction, resulting in different cellular events. Both receptors have previously been found in islets of Langerhans from rodents. In addition, serotonin has been shown to be present in islets from several different animal species. As both receptors and amines are present, serotonin may potentially regulate hormone secretion from islets of Langerhans. We have therefore investigated the effect of a serotonin analogue (α-methyl serotonin) on insulin secretion in a clonal β-cell line and in islets of Langerhans from mouse and humans.
Materials and methods: We used RT PCR and sequence specific primers to detected expression of the receptors in the β-cell line (INS 832/13) and

in islets of Langerhans. Immuno-histochemical analysis was used to detect the receptors at the protein level in rodent islet and human islets. Islets from mouse was isolated by standard collagenase digestion and incubated with glucose and α-methyl serotonin at 37 ºC for 1h and assayed for insulin secretion. Insulin was measured either by radio-immuno assay or with insulin ELISA depending on the species of the islets of Langerhans.
Results: 5-HT2b mRNA was found in the 832/13 cells. We also found both 5-HT1a and 5-HT2b to be expressed in rodent and human islets, at both mRNA and protein level. Interestingly, 5-HT receptors in islets were localized in two different cell types in the islets. In rodent islets, 5-HT2b was predominantly expressed in β-cells while 5-HT1a was more abundant in the α-cells. In human, islets the situation was reversed. Stimulation with α-methyl serotonin provoked a significant increase in insulin secretion at stimulatory concentrations of glucose when the clonal β-cells were incubated with the analogue for 2h. In mouse and human islets, however, no difference between the control and α-methyl serotonin treated islets were detected at either concentrations of glucose.
Conclusion: Our results from the clonal cell line suggest a role for serotonin and its receptors in regulation of insulin secretion. Because both 5-HT1a and 5-HT2b are expressed in rodent and human islets and activate two very distinct cellular pathways, these receptors may in fact modulate secretion from both α and β-cells. This dual regulation of both insulin and glucagon secretion could obscure the actual effect of α-methyl serotonin on insulin secretion. This issue needs to be further investigated because these receptors may serve as potential drug targets to stimulate islet hormone secretion.
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403

New paracrine role of pancreatic polypeptide in mouse and human pancreatic islets
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Background and aims: Pancreatic polypeptide (PP) is a hormone synthesized in pancreatic islet F-cells. PP is released in response to food ingestion and remains elevated for up to 6 h postprandially. It has recently received much attention for its metabolic role in humans, suppressing food intake and gastric emptying. For this reason, PP is nowadays in the spotlight for the development of treatments against obesity and diabetes in humans. We have explored the possible existence of additional paracrine roles of PP in the endocrine pancreas. Indeed, endocrine pancreatic hormones often exhibit paracrine roles in the regulation of islet secretion that are complementary to its extra-islet functions. Thus, insulin from beta cells negatively regulates glucagon secretion and somatostatin from delta cells negatively regulates both insulin and glucagon secretion. These paracrine regulations constitute a complex network necessary for a precise tuning of the endocrine pancreas in response to different metabolic states. Up to date, the possible intra-islet functions of PP are totally unknown.
Materials and methods: We have performed immunolocalization analysis of the PP receptor, NPY4R -also named as PP1R- in mouse and human pancreatic islets. Additionally, we have evaluated its role in the modification of pancreatic islet hormone secretion in vitro.
Results: Our data show the expression of PP receptor in both mouse and human models, were PP1R is exclusively expressed in glucagon-secreting alpha cells. In addition, our preliminary results indicate that these receptors are functional, and their activation by PP regulates glucagon secretion in isolated pancreatic islets.
Conclusion: Pancreatic polypeptide influences glucagon secretion by acting on PP1R present in pancreatic alpha cells in mouse and human islets. Our data reveal for the first time paracrine signalling of pancreatic polypeptide. Interestingly, it is known that glucagon modifies food intake in humans, probably by acting on central glucagon receptors. Our data indicate that some of the metabolic properties of PP may be mediated by changes in glucagon levels and suggest a possible functional parallelism on PP roles in CNS and endocrine pancreas.
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404

Kisspeptin the endogenous ligand for the novel beta cell GPCR, GPR54, inhibits insulin secretion
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Background and aim: Kisspeptins are a family of peptides that activates the G-protein coupled receptor GPR-54, which is a novel islet GPCR that couples to Gq and the PLC pathway. The KISS1 gene encodes for a hydrophobic 145 amino acid protein that can be cleaved into peptides of differing length e.g. kisspeptin 54 (KP54), kisspeptin 13 (KP13) and kisspeptin 10 (KP10). Both human and mouse islets express both the receptor, GPR54, and the gene encoding the ligand, KISS1. The function of kisspeptins in islet function and insulin secretion is however still unclear. We therefore explored the potential effect of kisspeptins on insulin secretion and expression of kisspeptin peptide in mouse islets.
Methods: Insulin secretion was measured in isolated islets after collagenase digestion of pancreata from C57BL/6J mice. The islets were divided in groups of three and incubated in HBSS with glucose concentrations of 2.8, 5.6, 8.3 and 11.1 mM, with or without 100 nM kisspeptin-13 for 60 min at 37 ºC. Insulin secretion was also measured during IVGTT where the mice were given an intravenous injection of with 0.35 g glucose with or without 3 nmol KP10 per kg. Insulin was determined with ELISA (Mercodia) or radioimmunochemically (Linco Res., St Charles, MO, USA). Finally, localization of KP10 in mouse islets was investigated using immunocytochemostry of mouse islet cells using an antibody directed against the N-terminal end of KP10.
Results: KP-13 inhibited insulin secretion in isolated mouse islets and the inhibitory action was most pronounced at glucose concentrations below 11.1 mM. At 5.6 mM glucose the insulin response was 121±15 pg/islet/h and in presence of KP13 the insulin response was reduced to 69±10 pg/islet/h (p=0.01, n=4). The inhibitory action was also seen at 8.3 mM glucose where the insulin response was 205±18 pg/islet/h in control islets and 152±16 pg/islet/h (p=0.04, n=4) in presence of KP13. In vivo, the insulin response to IV glucose was inhibited by the endogenous mouse kisspeptin, KP10. Thus the 1 min increase in plasma insulin after IV glucose was 715±200 pmol/l (n=9) and this was reduced by 60% to 290±94 pmol/l by KP10 (n=9, p=0.008). The presence of KP10 was shown in dispersed primary mouse beta cells using immunocytochemsitry.
Conclusion: Kisspeptins inhibit insulin secretion as evident both by in vivo and in vitro studies, and in vitro the effect was evident at glucose concentrations below 11.1 mM. This argues that signaling of GPR54 through kisspeptins is inhibiting regulating insulin secretion at physiological concentrations of glucose. Since kisspeptins are expressed in beta cells, as is GPR54, our results suggest a novel feed-back mechanism for restraining beta cell secretion. The mechanisms and physiological relevance of this remains now to be explored.
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405

Palmitate-stimulated hormone secretion in relation to GPR40 expression in pancreatic islets of spontaneous obesity and type 2 diabetes in rats
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Background and aims: The role of GPR40 in FFA-induced β-cell signalling is unclear. We examined GPR40 expression in relation to palmitate-induced hormone release in isolated islets from two rat models of spontaneous type 2 diabetes displaying either hyperlipidemia (fa/fa) or hyperglycemia (GK).
Materials and methods: GPR40 expression was analysed by confocal microscopy, Western blot and RT-PCR in islets from young prediabetic Zucker diabetic fatty (ZDF, fa/fa) rats or diabetic Goto-Kakizaki (GK) rats and their healthy controls. Palmitate-stimulated hormone secretion from isolated islets was analysed.
Results: Confocal microscopy of control islets showed expression of GPR40 protein in insulin, glucagon and somatostatin cells. This expression was strongly increased in islets of prediabetic fa/fa rats and had its functional counterpart in increased palmitate-induced release of insulin and glucagon and a pronounced inhibition of somatostatin release. Conversely, GK islets displayed an extremely faint expression of GPR40 as did high-glucose-cultured Wistar control islets. In GK islets this was reflected in an abolished hormone response to palmitate. In accordance, both Western blot and mRNA

expression of GPR40 showed a strong increase in fa/fa islets and a marked decrease in GK islets as compared to control islets and high-glucose cultured Wistar islets.
Conclusion: GPR40 is abundantly expressed in pancreatic islets and influences palmitate-induced secretion of insulin, glucagon and somatostatin. Mild hyperlipidemia increases GPR40 expression and palmitate-induced effects on hormone secretion, whereas hyperglycemia abrogated GPR40 expression and abolished palmitate-induced secretory effects. GPR40 could be a target for therapeutic approach in certain types of obesity and type 2 diabetes.
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406

Evidence for inhibitory autocrine effects of proinsulin C-peptide on pancreatic beta cell function and insulin secretion
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Background and aims: There is evidence to suggest that proinsulin C-peptide may exert various physiological effects in addition to representing a component of proinsulin that is cleaved by proconvertase enzymes during insulin biosynthesis. C-peptide has been shown to affect several cellular processes and bind to cell membranes including insulin-secreting beta cells. However, very little attention has been given to the possible effects of C-peptide on insulin secretion. The present study was undertaken to explore the actions of various forms of proinsulin C-peptide on pancreatic beta cell function and insulin secretion.
Materials and methods: Insulin releasing activity of C-peptide (human, rat-1 and rat-2) was studied in the clonal pancreatic cell line, BRIN-BD11. Findings were substantiated by further studies in pancreatic islets isolated by collagenase digestion from normal Swiss TO mice (n=5). Acute insulin secretion studies were performed at 5.6mM and 16.7mM glucose. A range of C-peptide concentrations were tested with various stimulators of insulin release, and comparative studies performed with known inhibitors of beta cell function. Insulin release was measured by radioimmunoassay, and intracellular calcium ($[Ca^{2+}]_i$) determined by fluormetric assay kit using FLEXstation™.
Results: Acute exposure of clonal beta cells to human C-peptide (10^{-9}-10^{-6}M) resulted in concentration-dependent inhibitory effects on insulin secretion at 5.6mM (P<0.05-P<0.001) and 16.7mM (P<0.01-P<0.001) glucose. Inhibition of glucose-induced insulin secretion with C-peptide ($3x10^{-7}$M) was accompanied by suppression of the insulin-secretory responses to alanine (10mM, P<0.05), elevated Ca^{2+} (7.68mM, P<0.001), arginine (P<0.05), tolbutamide (P<0.001) and GLP-1 (P<0.001) at 5.6mM glucose. Likewise, C-peptide inhibited insulin release induced by KCl (30mM, P<0.05) and IBMX (200μM, P<0.01) at 16.7mM glucose. Additionally, the inhibitory effects of C-peptide were shown to be concentration dependent for alanine (10^{-8}-10^{-6}M, P<0.05-P<0.001) and IBMX (10^{-9}-10^{-6}M, P<0.01-P<0.001) stimulated insulin release at 5.6mM and 16.7mM glucose, respectively. Broadly similar results were found using isolated pancreatic islets as C-peptide ($3x10^{-7}$M) reduced insulin release at 5.6mM (P<0.001) and 16.7mM (P<0.001) glucose. In isolated islets C-peptide also inhibited alanine (10mM), GIP (10^{-8}M), IBMX (200μM) and tolbutamide (200μM) stimulated insulin release at 5.6mM glucose (P<0.01). Acute exposure of clonal beta cells to C-peptide ($3x10^{-7}$M) resulted in no modification of the changes in $[Ca^{2+}]_i$ induced by alanine, whereas inhibition of insulin secretion with diazoxide (300μM), clearly reduced $[Ca^{2+}]_i$ (P<0.001, n=6). Human C-peptide ($3x10^{-7}$M, P<0.01) and established inhibitors of insulin secretion, somatostatin-14 ($3x10^{-7}$M, P<0.001) and diazoxide (300μM, P<0.001) reduced glucose-stimulated insulin release in the acute studies performed at 5.6mM glucose in the presence of alanine. Glucose-induced insulin secretion was inhibited by 25% (somatostatin-14), 43% (C-peptide) and 48% (diazoxide). The various observations made with human C-peptide were reproduced using rat C-peptide I and II.
Conclusion: This study provides evidence that C-peptide is a biologically active endogenous peptide hormone that exerts tonic inhibitory effects on pancreatic beta cell function mediated through intracellular signalling pathways.

407

Silencing mitogen-activated protein 4 kinase 4 (MAP4K4) protects beta cells from TNF-alpha inhibition of glucose-stimulated insulin secretion
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Background and aims: Obesity and type 2 diabetes mellitus are widespread metabolic disorders characterized by an overlapping phenotype of insulin resistance with a relative deficiency in insulin secretion underlying hyperglycemia in type 2 diabetes. Systemic inflammation is also a feature of obesity and type 2 diabetes, raising the hypothesis that elevated cytokine levels may contribute to peripheral insulin resistance as well as decreased beta cell functional mass. In healthy humans, TNF-α infusion induces skeletal muscle insulin resistance. We now explore the impact of TNF-α on glucose-stimulated insulin secretion from primary beta cells and the underlying signalling pathways.
Materials and methods: Human and rat primary beta cells were sorted by FACS and cultured for 24h +/- 20 ng/ml TNF-α to explore the impact on apoptosis (TUNEL assay), proliferation (BrdU incorporation) and short-term insulin secretion (1h 2.8 mM glucose followed by 1h 16.7 mM glucose at the end of the 24h culture period) as well as key signalling protein phosphorylation and expression. siRNA was used to silence MAP4K4 expression: cells were transfected with liposome-siRNA (Lipofectamine 2000 and siRNA (100 nM)) 3 days prior to the experimental period to ensure knockdown of MAP4K4 before TNF-α treatment. Data are mean±SE, for n=4 independent experiments.
Results: Culture for 24h with TNF-α had no effect on either beta cell proliferation (rat) or apoptosis (rat and human). By contrast, TNF-α treatment decreased short-term glucose-stimulated insulin secretion (GSIS) by 42±6% (for human) and 49±8% (for rat) when compared to control (without TNF-α), with no significant effect on basal secretion. In rat primary beta-cells, 1h of glucose stimulation (16.7mM) induces phosphorylation of proteins from the insulin signalling pathway including Akt, AS160, other Akt Substrates, ERK and insulin receptor: 24h TNF-α treatment abolished this effect of glucose on these particular proteins. Strikingly, TNF-α treatment decreased IRS-2 protein levels by 46±7% vs. control, although mRNA expression was unchanged. TNF-α treatment also induced activation of p38, p70S6Kinase and JNK, as well as NF-κB in rat primary beta cells. In order to identify which kinases were responsible for TNF-α inhibition of GSIS, we used either a pharmacological approach or siRNA. Neither NF-κB (Bay 11-7032; 5μM) nor eNOS (LNMMA; 1mM) inhibition protected beta cells from the impact of TNF-α, whereas MAP4K4 knockdown using siRNA preserved GSIS and IRS-2 protein levels as well as the impact of glucose on components of the insulin signalling pathway. MAP4K4 knockdown also prevented activation of JNK and p70S6Kinase by TNF-α, whereas it had no influence on NF-κB or p38 activation.
Conclusion: We show here that 24h exposure to TNF-α inhibits GSIS in primary beta cells. This is associated with an alteration in phosphorylation of key proteins in the insulin signalling pathway after glucose stimulation as well as decreased levels of IRS-2 protein. We identify MAP4K4 as a key upstream mediator of TNF-α action on the beta cell, making it a potential therapeutic target for preservation of beta cell function in type 2 diabetes.
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408

Targeting diabetes through multiple pathways using VPAC2 receptor selective peptide agonists
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Background and aims: Vasoactive intestinal polypeptide (VIP) is one of the most abundant neuropeptides in the pancreas. VIP potently enhances insulin secretion from pancreatic islets but may also stimulate glucagon secretion. VIP may act through either VPAC1 or VPAC2 receptors.
Materials and methods: We have used two different VPAC2R selective peptides, P17 and P104, to determine (1) if VPAC2R specific compounds can dissociate the VIP-mediated enhancements of glucagon and insulin secretion in *in vitro* and (2) to evaluate their efficacy on insulin secretion in normal or diabetic rats.

Results: Both peptides potently enhanced insulin secretion from isolated rat islets; for example, 30 nM P104 enhanced insulin secretion by 3.5-fold versus controls in the presence of 10 mM glucose. The same concentration of GLP-1 resulted in a 4-fold enhancement. In difference to PACAP-27 that activates both VPAC1 and VPAC2 receptors, neither GLP-1 nor P104 enhanced glucagon secretion from rat islets. This observation suggests that a VPAC2R selective compound may selectively enhance insulin secretion without stimulating glucagon release. P17 also protected ß-cells against apoptosis induced either by cytokines (IFNγ + IL1ß) or by H_2O_2. When given acutely in a rat intravenous glucose tolerance test (ivGTT), P104 enhanced the insulin response to an intravenous bolus of glucose by a factor of 4 (measured as the area under the insulin curve from 0 to 10 min after glucose). The half-maximal efficacious dose was 0.1 µg/kg. P104 was also efficacious in an ivGTT in diabetic GK rats, where 3 and 10 µg/kg stimulated insulin secretion by 3 and 4-fold, respectively. In order to assess to which extent VPAC2 receptors desensitize when chronically stimulated, P17 was subcutaneously infused in normal rats at a rate of 2.6 or 8 µg/kg/h for 72 h, after which insulin secretion was studied at two fixed iv glucose infusion rates (5 & 15 mg/kg/min). Both P17 doses enhanced insulin secretion by 4-fold compared to vehicle. The safety profile of P17 was assessed in a parallel study in healthy rats with infusion rates of 8 and 27 µg/kg/h for 72 h; which corresponded to 3- and 10-fold the maximal efficacious dose on insulin secretion. No adverse events were observed, but interestingly, plasma triglycerides and free fatty acids were dose-dependently reduced after the 72h infusion. Both doses reduced food intake by 20% and 50% for 8 and 27 µg/kg/h, respectively. No signs of liver damage or perturbations of plasma GH, corticosterone, prolactin or total cholesterol levels were observed.
Conclusion: VPAC2R-selective peptides enhanced insulin secretion in rat pancreatic islets, without effects on glucagon release. They also potently enhanced insulin secretion in both normal and diabetic rats, improved plasma lipid levels and reduced food intake; a set of properties which is particularly desirable for the treatment of type 2 diabetes.

409

Role of class 1A PI3-kinase in pancreatic beta cell functions and growth
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Background and aims: Recent studies using several knockout models have shown that autocrine insulin (and/or IGF-1) regulates growth and some functions of pancreatic β cells through activation of insulin signaling. Downstream of insulin receptor signaling, Class IA phosphoinositide 3-kinase (PI3K) composed of a catalytic subunit (p110) and a regulatory subunit (p85) plays a pivotal role. Thus, in this, study, we tried to identify the role of Class IA PI3K in β cells.
Materials and methods: To this end, we have generated β cell specific Pik3r1 gene knockout encoding p85α and its splicing variants (βPik3r1KO) mice using Cre-LoxP system, and also ablated the genes of two major regulatory subunits, p85α and p85β, of PI3K by crossing mice lacking Pik3r1 in β cells with Pik3r2 (p85β gene) null mice, for creating pancreatic beta cell-specific double knockout mice (βDKO mice).
Results: βPik3r1KO mice were indistinguishable from control RIP-Cre transgenic mice. βPik3r1KO mice exhibited impaired glucose tolerance and suppressed glucose stimulated insulin secretion (GSIS) *in vivo* with decreased β cell mass. Moreover, βDKO mice showed more prominent glucose intolerance and decreased islet mass than βPik3r1KO mice. βDKO mice exhibited markedly blunted 1st phase insulin secretion in GSIS in vivo and in situ pancreatic perfusion test. Furthermore, apoptotic cells estimated by TUNEL assays were significantly increased in islets of βPik3r1KO and βDKO mice. We found that islets of βPik3r1KO and βDKO mice show impaired glucose-stimulated synchronized Ca influx and subsequent insulin secretion due to a reduction in Connexin 36, a key molecule of gap-junction. We also found that these mice show impaired insulin secretion even when intracellular Ca concentrations were elevated by caged Ca because these islets have reduced levels of SNARE protein complex. These reductions were normalized when the isolated islets were treated with the adenovirus expressing a constitutively active Akt.
Conclusion: These data suggest that Class IA PI3K is required for normal insulin secretion (especially in 1st phase secretion) and age-dependent islet growth, and that decreased Class IA PI3K activity causes postprandial hyperglycemia. Thus, these results may provide a novel therapeutic approach for diabetes by enhancing insulin/PI3K signaling in pancreatic β cells, leading to the normal insulin secretion and the normalization of postprandial hyperglycemia.
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410

Deterioration of islet structure and function precedes alterations of metabolic profile in Zucker diabetic fatty (ZDF) rats
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Background and aims: The ZDF rat has been widely used to demonstrate preclinical efficacy of anti-diabetic drugs. In this study we investigated if the changes in islet function and morphology precede alterations in metabolic profile.
Materials and methods: Measurement of metabolic parameters under basal and glucose challenge (oGTT at 2g/kg) together with quantitative analysis of islet morphology and *in situ* pancreas perfusion were performed longitudinally for 6 weeks starting in 6-week old male ZDF (ZDF) and lean (Lean) rats. Pancreata were stained for insulin, glucagon and nuclei.
Results: Despite near normoglycemia (FBG: 6.5±0.3 vs. 4.1±0.1 mM) and normo triglyceridemia (1.2±0.1 vs. 1.6±0.2 mM), 6w-old ZDF rats were characterized by moderate glucose intolerance ($AUC_{Glu\text{-}2h}$=1138±57 vs 814±21 mM*min) compared to 6w-old Lean ($AUC_{Glu\text{-}2h}$=820±28 mM*min). However, the dynamic insulin secretion profile measured during in situ pancreas perfusion showed a tendency for a reduced phase II compared to Lean (AUC: 84.0±10.7 (n=3) vs 147.6±19.3 ng/ml*min (n=6), n.s.).Total pancreatic insulin content was significantly elevated (p<0.001) and an increase in islet area by ~40% (n.s.) could be observed vs. 6w-old Lean. At 8 weeks of age, ZDF rats were normoglycemic but exhibited hypertriglyceridemia (6.0±0.6 vs 1.2±0.1 mM, p<0.01) and fasting hyperinsulinemia (6.2±0.5 vs. 2.3±0.3 ng/ml, p<0.01) compared to 6w-old ZDF. In the perfused pancreas we observed increased basal insulin secretion (7.2±1.2 vs. 0.1±0.0 ng/m, p<0.01) and a tendency in increased phase I+II secretion (AUC: 116±14 vs. 155±24 ng/mL*min, n.s.) upon glucose stimulation to compensate for evolving insulin resistance (Matsuda: 130±10 vs 390±40, p<0.01). Analysis of islet morphology showed at this age disrupted islet architecture with increased islet area by ≥70% (p<0.001), reduced insulin staining intensity by 26% (p<0.0001) and a 240% (p<0.0001) increase of α-cell infiltration into the islet core compared to 6 w-old ZDF. At the age of 10 and 12 weeks, ZDF rats evolved toward type 2 diabetes showing severe hyperglycemia (FBG: 11.2±1.7, n.s. and 19.0±2.2 mM, p<0.01), hypertriglyceridemia (6.7±0.7, p<0.001 and 5.8±0.6 mM, p<0.001) and both peripheral and hepatic insulin resistance (Matsuda: 165±12, p<0.0001 and 176±16, p<0.0001) compared to 6-w old ZDF. In situ pancreas perfusion showed an insulin secretion profile characterized by a suppressed phase I amplitude and complete flat phase II leading to an overall suppression of insulin secretory capacity. Analysis of islet morphology at 10 and 12 weeks showed an increased islet area by ~90% (p<0.001) and ~114% (p<0.0001), decreased insulin staining intensity by 32% (p<0.0001) and ~48% (p<0.0001) and severe islet destruction measured by the increase in α-cell invasion in the islet core (+380%, p<0.0001 and +448%, p<0.0001).
Conclusion: Our results demonstrate that pre-diabetic 6-week ZDF rats show significant changes of islet structure and function compared to age-matched Lean. During disease progression, further functional and morphological islet changes compensating for insulin resistance precede the development of hyperglycemia and suggest that the ZDF rat model is suitable for preclinical characterization of anti-diabetic drugs modifying islet function and early disease progression.

PS 18 Beta cell transcription factors and transporters

411

Differential microRNA expression in human pancreatic islets from patients with type 2 diabetes

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Background and aims: MicroRNAs (miRNAs or miRs) are a recently identified class of small cellular RNAs, whose function is to pair the mRNAs of protein-coding genes with subsequent transcriptional and post-transcriptional regulation of gene expression. MicroRNAs have emerged as important regulatory factors involved in developmental processes, such as neural progenitor cell growth and differentiation and their altered expression has been observed in a large number of malignancies. In addition, increasing evidence has shown that miRNAs are involved in the development of endocrine pancreas as well as in the regulation of insulin secretion and insulin signaling. In the light that type 2 diabetes is characterized by a defective insulin secretion combined to impaired insulin signaling, we performed a miRNA expression profiling in isolated human pancreatic islets obtained from 5 type 2 diabetic and from 5 age-matched control multi-organ donors.
Materials and methods: To this end, RNA isolation from tissue samples was performed using TRIZOL reagent and quantitative analysis of 150 miRNAs was performed using the specific stem-loop primers for reverse transcription followed by real-time PCR. All values were normalized to endogenous controls U6 and U66. Hierarchical clustering analysis and dendrograms were performed on the Q-PCR expression levels of miRNAs employing Spotfire software.
Results: Among the 150 microRNAs analyzed, 142 were expressed in pancreatic islets and 16/142 resulted differently expressed in type 2 diabetic vs control human islets. An online search of miRNAs targets by miRanda and TargetScan databases revealed that a subset of these differently expressed miRs targeted several genes involved in regulation of beta cell development and function and in insulin signaling. We specifically studied miR-124 which resulted 30-fold hyperexpressed in type 2 vs control diabetic islets, by silencing its expression in MIN6 cells which, interestingly, resulted in increased expression of a number of predicted target genes such as myotrophin (involved in insulin exocytosis), Foxa-2 and NeuroD and in a 5-fold increase in basal insulin secretion (measured at 3.3mM glucose). Indeed, when over-expressed in 293T cells miR-124 was able to repress the translation of constructs in which the myotrophin -3'UTR and Foxa-2 -3'UTR were fused to the renilla-luciferase open reading frame.
Conclusion: In conclusion, we here report a differentially miRNA profiling in type 2 diabetic vs control human pancreatic islets which includes a major hyperexpression of miR-124, whose silencing resulted in increased insulin secretion and expression of genes involved in beta cell function and development, leading to the hypothesis that an altered miRNA expression may contribute to beta cell defects in type 2 diabetes.
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412

Foxa1 and Foxa2 are critical regulators of glucagon gene expression and of genes controlling alpha-cell differentiation

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Background and aims: The Foxa1 and Foxa2 members of the Forkhead family are involved in glucose homeostasis, particularly in the protection against hypoglycaemia in mammals. Foxa proteins are expressed during embryonic development and in adulthood in liver and pancreas. They are essential for the regulation of hepatocyte-specific expression of several target genes and contribute to the specification of the pancreas. Gene ablation studies revealed a critical role for Foxa1 in the regulation of glucagon biosynthesis and in pancreatic islet function and for Foxa2 in the regulation of the terminal differentiation steps and the maturation of α-cells. The aims of the present study are to discriminate the respective role of the *foxa1* and *foxa2* genes on *glucagon* gene transcription by the precise identification of their cognate DNA elements and characterize their target genes to better understand the differentiation of the pancreatic α-cells.
Materials and methods: Specific inactivation of the *foxa1* and *foxa2* genes were performed by transient transfection with siRNA in islet hamster (InR1G9) glucagon-producing cells and by creating InR1G9-derived stable clones overexpressing a dominant negative (DN) form of Foxa2 lacking the transactivation domain.
Results: Both Foxa1 and Foxa2 activate the *glucagon* gene promoter in non islet BHK cells. *In vitro*, Foxa1 specifically interacts with G1, G2 and site A of the *glucagon* gene promoter whereas Foxa2 only with G1 and G2. *In vivo*, both factors also bind to the *glucagon* gene promoter as demonstrated by ChiP assay. SiRNA experiments in InR1G9 cells revealed that inhibition of the expression of Foxa1, Foxa2 or both factors 72h after transfection decreased glucagon mRNA levels respectively by 60%, 40% and 68% and that Foxa2 could not compensate Foxa1 inhibition and *vice versa*. Similarly, both glucagon and its mRNA levels were decreased in stable clones expressing the DNFoxa2. SiRNA directed against Foxa1, Foxa2 or both decreased *glucagon* promoter activity respectively by 63%, 62% and 77% and Foxa binding sites mutagenesis experiments indicated that the effects of Foxa1 and Foxa2 on *glucagon* gene expression are exerted independently through the binding to G2 but not G1 or site A. In addition to the *glucagon* gene, Foxa1 and Foxa2 regulate a number of genes which are important for the α-cell differentiation. Indeed, we found that inhibition of *foxa1* and *foxa2* genes down-regulate the genes coding the G protein-coupled receptor GPR40 affecting glucagon secretion, MafB involved in α-cell differentiation and function and *glucagon* gene expression and HNF4α which is known to be regulated by Foxa2 in pancreatic β-cells. The expression of transcription factors such as Pax6, cMAF and Glut1 was not affected.
Conclusion: These results highlight the critical importance of Foxa1 and Foxa2 in glucagon biosynthesis and secretion, α-cell differentiation and glucose homeostasis.
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413

Functional effects of the common *SLC30A8* Arg325Trp polymorphism on isolated human pancreatic islets

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Background and aims: Genome-wide association studies have shown that Arg325Trp polymorphism in *SLC30A8* (rs13266634) is associated with type 2 diabetes (T2D) susceptibility. *SLC30A8* is mainly expressed in beta-cells and encodes a zinc transporter protein member 8 (ZnT-8) involved in insulin maturation, storage and secretion. The major risk C allele has been found to associate with decreased in-vivo insulin release in a few, but not all studies. To shed further light on this issue, we assessed the relationships between *SLC30A8* genotypes and some properties of isolated human islets.
Materials and methods: DNA from multiorgan donors (n: 82; age: 59.1±16.6 yrs; gender: 38 males and 44 females; body mass index: 24.8±4.2 kg/m2) was genotyped for the Arg325Trp polymorphism; then, pancreatic islets were isolated and insulin secretion in response to 3.3 mmol/l glucose (G), 16.7 mmol/l G, 3.3 mmol/l G plus 20 mmol/l arginine and 3.3 mmol/l G plus 100 μmol/l glibenclamide was determined by the batch incubation technique (45 min per stimulus). Finally, quantitative RT-PCR expression of ZnT-8, insulin and glucagon was assessed.
Results: Overall, 42 CC (51.2%), 36 CT (43.9%) and 4 TT (4.9%) samples were studied. When analyzed together, insulin release (μU/islet/min) was 0.040±0.018 at 3.3 mmol/l G and increased to 0.136±0.090 at 16.7 mmol/l G (stimulation index, SI: 3.42±1.75), 0.112±0.067 at 3.3 mmol/l G plus arginine (SI: 2.83±1.67), and 0.127±0.087 at 3.3 mmol/l G plus glibenclamide (SI: 3.13±1.57). Whatever the experimental condition, the release of insulin was similar in the CC, CT and TT genotype groups. Islet expression (2-ΔCt) of ZnT-8 did not differ significantly between the CC (0.194±0.272, n:17), CT (0.119±0.171, n: 10) and TT (0.361±0,646, n: 4) genotypes. Furthermore, insulin and glucagon gene expressions were similar in the three genotypic groups. Interestingly, *SLC30A8* expression was positively correlated with insulin (r: 0.39, p=0.03) and glucagon (r: 0.80, p<0.001) expressions. Electron microscopy of islet cells was performed in 3 CC and 1 CT cases. In the pres-

ence of the CT genotype, insulin granules ultrastructure appeared clearly different, with larger and less electrondense core; in addition, a lower amount of alpha-cells (< 5%) was observed.
Conclusion: In isolated human islets, the risk C allele of the Arg325Trp polymorphism does not affect ex-vivo insulin secretion and *SLC30A8* expression. However, the Arg325Trp genotype may be associated with changes of insulin granules ultrastructure.
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414

The granular zinc transporter SLC30A8 in beta cells is linked to changes in expression of enzymes involved in proinsulin conversion
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Objective: ZnT8, the product of the SLC30A8 gene, has been implicated in perturbing the processing of proinsulin in non-diabetic relatives of Type 2 diabetic subjects. The common non-synonymous SNP encoding a W>R change at position 325 in the c-terminus confers genetic risk of developing disease. Our aim was to investigate whether the differences in the zinc transport in MIN6 beta cells were affected by overexpression or suppression of ZnT8 and how this might impact prohormone processing and secretory granule biogenesis and secretion.
Methods: MIN6 cells adenovirally expressing either human ZnT8(R325) or ZnT8(W325) variants or subjected to siRNA knockdown of endogenous ZnT8 were analyzed via western blot for protein expression, and ELISA for insulin secretion. Zinc-sensitive BHK cells were used to assess ZnT8 zinc transport ability.
Results: ZnT8 reduces cytoplasmic zinc in zinc-sensitive BHK cells compared to controls, but not as efficiently as the lysosomal ZnT2. Swapping the carboxyl terminus of ZnT2 with that of ZnT8 diminishes the zinc transport ability of ZnT2. siRNA knockdown of endogenous ZnT8 in MIN6 cells resulted in a decreased expression of insulin processing enzymes PC1, PC2 and CPE. Conversely, overexpression of ZnT8 in MIN6 cells resulted an increased expression of PC1, PC2, and CPE. Glucose-stimulated insulin secretion is enhanced in ZnT8-overexpressing MIN6 cells.
Conclusion: Changes in levels of transcription including the diabetes-associated R allele impact upon the levels and presumably activity of pro-protein convertases and provide a possible explanation for differences in circulating proinsulin/insulin ratios in pre-diabetes.
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415

Whole genome profiling approaches to understand the function of Math6 during endocrine cell differentiation
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Background and aims: Basic helix-loop-helix (bHLH) transcription factors often function in cascades to regulate cell fate decisions and differentiation programs in numerous tissues. In the pancreatic endocrine cell lineage, neurogenin3 (neurog3) turns on endocrine differentiation and activates neuroD1, which maintains the differentiation program. Another bHLH factor whose gene is induced by neurog3 is math6. We have recently shown that this factor is expressed in differentiating endocrine cells in the embryonic pancreas and, like neurog3, it is absent from mature islet cells. Math6 has a major non-redundant role in organismal development as demonstrated by early embryonic lethality of math6 knockout embryos. Yet, the precise function of math6 in pancreas formation remains elusive. This work aims to gain insight into the function of math6 in the endocrine transcriptional cascade through the identification of its gene targets.
Materials and methods: We have used math6 gain-of-function (ectopic expression) and loss-of-function (RNA interference) strategies in a neurog3-dependent cellular model of endocrine differentiation using mouse pancreatic duct cells. The effects of overexpressing or silencing math6 on the expression profile of duct cells have been assessed using Affymetrix expression microarrays.
Results: Overexpression of math6 alone has a minor impact on the transcriptome of mouse duct cells (28 genes were significantly different from B-galactosidase-expressing control cells), whereas concomitant expression of math6 and neurog3 results in the inhibition of 11 neurog3 gene targets. In contrast, silencing of math6 during the differentiation process promoted by neurog3 affects the expression of nearly 300 genes. Of these, 46% are also modulated by neurog3 and, remarkably, in many cases in an opposite way. Finally, gene ontology analysis of the identified math6 targets has revealed the potential involvement of this factor in the regulation of important developmental processes such as ubiquitination, chromatin remodelling and the wnt signalling pathway.
Conclusion: This study identifies for the first time a cohort of potential targets of math6 in the endocrine differentiation program. Remarkably, math6 and neurog3 appear to exert opposite effects on specific gene locus, raising the possibility that math6 may act as a regulatory component of neurog3 function. These data may be useful for the development of *in vitro* protocols for the generation of replacement beta cells for the treatment of diabetes.
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416

Impact of c-Maf, one of the large Maf transcription factors, on pancreatic beta cell function
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Background and aims: Beta-cell specific transcription factors, such as PDX-1, BETA2, and MafA, play an important role in regulating insulin gene expression. MafA, a large-Maf factor, positively regulates insulin gene expression. We previously showed small-Maf factors (MafF, MafG and MafK), that lack an N-terminal transactivation domain present in the large-Maf factors, are also expressed in beta cells and thus, can function as a negative regulator of insulin gene expression. These observations suggest that both large- and small-Maf factors are important for the regulation of insulin gene expression through Maf Response Element (MARE) on insulin promoter. c-Maf, one of the large-Maf transcription factors, is reported to be expressed in helper T cell, chondrocyte, and lens, and involved in the differenciation of these cells. Recent studies have reported that not only MafA but also c-Maf was expressed in adult pancreatic beta-cells and could positively regulate insulin expression. However, physiological roles of c-Maf in pancreatic beta-cells is not known. To investigate the effect of c-Maf on insulin gene expression and the underlying mechanisms by which c-Maf expression is regulated in response to glucotoxicity, lipotoxicity, and oxidative stress in beta-cells.
Methods and results: Experiment 1): MIN6 cells, a mouse beta-cell line, were cultured and transfected with mouse c-Maf siRNA by lipofection. Control siRNA was used as negative control. Western blot analysis, real-time RT-PCR, and reporter assay using insulin promoter:luciferase reporter construct were performed to evaluate both cMaf mRNA and protein levels and to examine the effect on insulin promoter activity. Knockdown of c-Maf in MIN6 cells resulted in significant reduction of c-Maf, but not MafA, mRNA and protein levels and concomitant repression of insulin gene. Experiment 2): To investigate the impact of glucotoxicity, lipotoxicity, and oxidative stress on c-Maf expression in beta-cells, MIN6 cells were cultured in different concentrations of glucose, with palmitate or with tert-butyl hydroperoxide (tBHP). c-Maf expression was increased under lipotoxic condition. Experiment 3): To evaluate c-Maf expression in vivo, C57BL/6 mice were fed normal diet (ND) or high-fat diet (HFD) from 5 weeks of age. At 12 weeks of age, serum levels of total cholesterol, triglycerides (TG), free fatty acid (FFA), insulin and blood glucose level were analyzed. Islets were isolated and lysed for western blotting. Pancreatic sections were obtained for immunohistochemistry. Feeding HFD resulted in higher levels of TG, FFA, insulin and blood glucose, and increased beta-cell mass. cMaf expression level was unchanged, while MafA expression in a HFD group was significantly increased.
Conclusion: These data clearly demonstrate that c-Maf has an impact on insulin gene expression in beta-cells and reduction in c-Maf expression may cause beta-cell dysfunction. However, further studies are necessary for elucidation of the physiological roles in vivo.

417

Posttranslational modifications of Pax6 and Pax6(5a) in pancreatic beta cells

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Background and aims: Pax6 is an important transcription factor of the glucagon gene expression in pancreatic alpha cells, while in beta cells the influence of Pax6 on insulin gene expression is discussed controversially. We identified Pax6 as an inhibitor on the rat insulin gene 2 promoter. However the impact of Pax6(5a), an alternative splice product, is still unclear. *In vitro* both Pax6 isoforms are phosphorylated by CaMK2 at S122 and CK2 at S206. Using starved and high glucose conditions we further studied posttranslational modifications by kinases and glycosyl-transferases of both isoforms in pancreatic beta cells.
Materials and methods: Microscopical and reporter gene analyses with overexpressed wild type or mutated (YFP-)Pax6 and (YFP-)Pax6(5a) were done to study their intracellular localization and effects on insulin gene expression in dependence on glucose. Glycosylation of both isoforms was investigated using specific antibodies after immunoprecipitations and Western Blots. Stably transfected BRIN-BD11 cells overexpressing siRNA against Pax6 transactivation domain or exon 5a were generated to verify the inhibitory actions of Pax6 in pancreatic beta cells using northern blots and radioimmunoassays.
Results: Co-Immunprecipitations confirmed a direct interaction between CK2 or CaMK2 and Pax6. CaMK2-phosphorylation site S122 was very sensitive to mutations, while mutation of S206 showed no effects on intracellular translocation and insulin gene expression. Glycosylation of Pax6 was only detectable in cells after glucose deprivation. Both constructs of siRNA diminished Pax6 expression and effects.
Conclusion: Pax6 and Pax6(5a) are posttranslational modified by phosphorylation and glycosylation which may modulate their functional activities.

418

Pax6 is crucial for beta cell functions and represents a component of glucose action on insulin biosynthesis and secretion

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Background and aims: Pax6 is a paired box homeodomain protein expressed in all cells of the endocrine pancreas during development and in the adult stage. We previously demonstrated that Pax6 exerts a major role in glucagon gene transcription and more widely in α-cell differentiation and functions. Pax6 is also involved in β-cell development. Inasmuch as specific knock-out for Pax6 in β-cells of mice leads to a marked decrease in insulin levels and diabetes. In humans, Pax6 heterozygous mutations are also associated with diabetes mellitus.To better understand the role of Pax6 in the differentiation and function of β-cells, we investigated the transcriptional network of Pax6 in HIT insulin-producing cells through a candidate gene approach study.
Materials and methods: We developed two Pax6-deficient models, a partial knock-down with specific siRNA and selected clones expressing a dominant negative form of Pax6 in HIT cells. We also investigated the effect of glucose on Pax6 gene expression in isolated rat islets.
Results: In both models, insulin gene expression was decreased by 50 %, reflecting the important role of Pax6 in insulin gene transcription. In addition to the insulin gene, we report that Pax6 deficiency in β-cells led to a significant decrease of Pdx1, Isl1, c-Maf and Sox4 mRNA levels which are all critical for β-cell differentiation and functions. Furthermore, we also identified several new Pax6 target-genes coding for key proteins involved in glucose metabolism and insulin biosynthesis among them glucokinase (GK), the insulin receptor (IR), the glucose transporter (GluT2) and the fatty-acids receptor GPR40. We further show that Pax6 controls directly or indirectly the promoter activities of these genes in insulin-producing cells.As Pax6 is involved in the control of several genes implicated in insulin biosynthesis and secretion, we examined whether Pax6 might be regulated by glucose in rat islets. We found that high glucose increases Pax6 mRNA levels 2-fold compared to low glucose.
Conclusion: We conclude that Pax6 regulates key genes involved in insulin biosynthesis and secretion and that Pax6 itself is regulated by glucose. These results underline the multiple steps at which glucose affects β-cell function and the critical role of Pax6 in this regulation.
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419

Islet Brain 1 controls insulin secretion by maintaining the contents of annexin A2

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Background and aims: Islet Brain 1 (IB1) plays a key role in the beneficial effects of the long acting agonist of the glucagon-like peptide 1 receptor exendin 4 (ex-4) on pancreatic beta cells. IB1 is rather recognized as an inhibitor of the c-Jun N terminal Kinases (JNK) pathway in pancreatic beta cells and thereby, prevents inhibition of insulin expression and apoptosis induced by stress-activated JNK pathway. Several lines of evidence support an additional role for IB1 in beta cells as a regulator of insulin secretion. First, induction of IB1 expression parallels the stimulatory effects of ex-4 on insulin secretion. Second, impairment of IB1 dimerization diminishes glucose-induced insulin secretion. In the latter case, the loss of secretion is not linked to activation of the JNK pathway and subsequent apoptosis, suggesting that IB1 controls insulin secretion through a JNK-independent mechanism. The goal of this study was to clarify the role of IB1 in the control of insulin secretion.
Materials and methods: The short hairpin RNAs (shRNAs) directed against IB1 or scrambled shRNA were either cloned into plasmids or lentiviral vectors. Insulin secretion elicited by various secretagogues (glucose, KCl, IBMX and forskolin) was monitored by measuring either the release of insulin by the EIA kit (Spi-Bio) or by ELISA (Roche). mRNA and protein levels were assessed by quantitative real-time PCR and western blotting respectively. Proteomic analysis was performed by 2D-gel electrophoresis followed by protein sequencing. Calcium influx was measured by fluorescence microscopy.
Results: Suppressing IB1 expression markedly reduced glucose-induced insulin secretion in INS-1E, MIN6 and isolated rat islets. This effect was specific since the introduction of an IB1-encoding plasmid, resistant to shRNA, restored the capacity of the cells to secrete in response to glucose. Diminution in IB1 contents did not affect calcium influx but still impaired secretion in response to KCl, suggesting a defect in exocytosis. We then assessed whether activation of the JNK pathway could mediate the loss of this beta cell task. Co-treatment of the cells with either the JNK inhibitor peptide or the JNK catalytic activity inhibitor SP600125 did not prevent loss of insulin secretion evoked by silencing of IB1 expression. Proteomic analysis revealed a drastic reduction in the protein content of annexin A2 (Anxa2), a calcium-dependent phospholipid binding protein that regulates exocytosis of neuroendocrine cells. Suppression of Anxa2 in insulin-secreting cells with interference RNA impaired nutrients-induced hormone release, thus mimicking the effects of IB1 silencing.
Conclusion: IB1 is required for the control of nutrients-stimulated insulin secretion. This IB1-dependent function relies on the adequate levels of Anx2A.
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420

Effects of nitric oxide on Cap-dependent and Cap-independent insulin biosynthesis in human islets

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Background and aims: Although insulin biosynthesis has been extensively studied, most of the work has been done in rodent islets. Due to the significant differences between rodent and human islets in islet structure, beta cell content and sensitivity to cytokines and stress, there might also be differences in the regulation of insulin biosynthesis. Based on the idea that polypyrimidine protein 1 (PTBP1) upon binding to the insulin mRNA 5'-UTR could induce cap-independent translation of insulin and that such a mechanism would uphold insulin production during cellular stress, we wanted to investigate both if insulin could be translated in a cap-independent manner and how nitric oxide induced cellular stress would affect the insulin mRNA translation.
Materials and methods: The affinity of PTBP1 to the human insulin mRNA 5'-UTR was measured using cytoplasmic extracts from human islets incubated in either 1.67 or 16.7 mM glucose for 2 hours w/wo 2.5 mM DETA/NO (a nitric oxide donor). These cytoplasmic extracts was then incubated with

biotinylated RNA oligonucleotides corresponding to the insulin mRNA 5'-UTR. The sequence specific proteins were then eluted, using dynabeads, and separated on SDS-PAGE for immunoblotting. Insulin biosynthesis was estimated by measuring the incorporation of L-[3,4,5-3H] leucine into insulin of 20 islets incubated for 2 h in KRBH. For the different experimental groups 1.67 or 16.7 mM glucose was added either in presence or absence of 1 mM DETA/NO and 0.1, 1.0, 10 mM hippuristanol (an eIF4A inhibitor that specifically inhibits cap-dependent translation). Labeled islet insulin was immunoprecipitated using guinea pig anti-insulin serum and protein A-sepharose while total protein was precipitated using trichloroacetic acid.
Results: The PTB pulldown experiments using RNA oligonucleotides corresponding to either the first 29 or the following 30 nucleotides of the 5'-UTR sequence clearly showed that PTB binds specifically to the second part of the insulin mRNA 5'-UTR. The insulin biosynthesis data show that a very high proportion of the insulin mRNA translation (93%) is cap-dependent at a high glucose concentration, but also that part of the insulin biosynthesis takes place through a cap-independent mechanism. Furthermore, DETA/NO treatment significantly decreased the translation of insulin by 83% and total protein biosynthesis by 73%. By expressing insulin biosynthesis as a percentage of total protein biosynthesis we observed that the percentage insulin biosynthesis was lower in DETA/NO treated islets than in control islets. In addition, DETA/NO-induced inhibition of insulin biosynthesis was less pronounced in the presence of hippuristanol. In low glucose, however, DETA/NO did not decrease insulin biosynthesis in relation to total protein.
Conclusion: Our studies shows that PTBP1 binds to the insulin mRNA 5'-UTR in vitro. The main bulk of the insulin synthesis occurs via cap-dependent translation and this process is preferentially inhibited by NO. We also show that cap-independent insulin translation occurs and that this event is to a lesser extent affected by NO. It is tempting to speculate that PTBP1 binding to insulin mRNA maintains a basal rate of insulin biosynthesis at conditions of low glucose or cellular stress.
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PS 19 Beta cell transcriptional regulation

421

Pancreatic duodenum homeobox-1 (PDX1) is phosphorylated in living pancreatic beta cells (MIN6) at serine 269
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Background and aims: Pancreatic duodenum homeobox-1 (PDX1) is a crucial regulator of pancreatic development and mature β-cell function. At present, the molecular mechanisms, including post-translational modification(s), through which PDX1 nuclear uptake and DNA binding activity are regulated are poorly understood. In order to identify physiologically relevant phosphorylation sites for PDX1 we have undertaken phospho-proteomic analysis of the protein over-expressed in clonal β-cells.
Materials and methods: An adenovirus expressing wild type c-*myc*-tagged PDX1 was generated for infection and expression of pancreatic β-cell lines. PDX1 protein was immunoprecipitated from infected β-cells with an anti-c-*myc* antibody and separated on SDS-PAGE. A PDX1-containing band was identified and subjected to mass spectrometry for phosphorylation site mapping. A phospho-specific antibody was accordingly generated and validated.
Results: We revealed by mass spectrometric analysis of PDX1 expressed in INS1 (832/13) β-cells that Ser269 is a novel *in vivo* PDX1 phosphorylation site. Therefore we generated a phosphospecific antibody against phopsho-Ser269 and we used it to confirm further in MIN6 cells and in mouse islets that PDX1 is effectively phosphorylated *in vivo*. Wild type c-*myc*-tagged PDX1-expressing adenovirus was mutagenized to replace Ser269 either with an alanine, to produce a dephosphomimetic S269A mutant, or with a glutamic acid, producing a phosphomimetic S269E mutant. MIN6 cells were infected with either PDX1 wild type or the mutants and immunostained with anti-c-*myc* antibody. Whereas in response to an increase (3-30mM) in glucose concentration both the wild type and the S269A mutant translocated from the nuclear periphery to the nucleoplasm, the phosphomimetic S269E mutant localized preferentially to the nuclear periphery at both glucose concentrations. ^{35}S-Met/Cys pulse-chase analysis revealed that neither S269E nor S269A mutations displayed altered protein stability. Homeodomain Interacting Protein Kinase 2 (HIPK2) has recently been reported to be expressed in pancreatic endocrine cells and to directly phosphorylate the C-terminal domain of PDX1, which contains two potential phosphorylation sites that meet HIPK2 requirements: threonine^{214}-proline (Thr214), and serine^{269}-proline (Ser269). Hence, we postulated that this latter serine residue may be a specific target for HIPK2: indeed, the antiphospho-Ser269 antibody recognized recombinant PDX1 after it was subjected to *in vitro* phosphorylation by HIPK2 but not by several other kinases.
Conclusion: We demonstrate that Serine 269 is phosphorylated in living clonal pancreatic β-cells. We observed that a phosphomimetic S269E mutant of PDX1 failed to respond to glucose with translocation from the nuclear periphery to nucleoplasm. We identified HIPK2 as the most likely responsible kinase for phosphorylation at Ser269. Further investigations will be necessary to establish the physiological significance of this newly reported post translational modification of PDX1.
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422

Conditional overexpression of Pax4 in pancreatic beta cells protects mice from developing streptozotocin-induced diabetes
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Background and aims: We have previously shown that the forced expression of the diabetes-linked transcription factor Pax4 is crucial for islet beta-cell proliferation and survival *in vitro.* We have now generated the first animal model in which Pax4 expression can be specifically induced in beta-cells, allowing us to determine the protective role of Pax4, by challenging animals with streptozotocin, to induce a rapid and sustained hyperglycaemic state.

Material and methods: We generated two transgenic mouse lines harbouring either the wild type mouse Pax4 (mPax4WT) or a Type 2 diabetes-associated variant (mPax4R129W), under control of a doxycycline (DOX) inducible TRE-CMV promoter. Transgenic Pax4 animals were then bred to the Rat Insulin Promoter-Reverse Tetracycline Transactivator (RIP-rtTA) transgenic mouse line to restrict transgene expression to beta-cells. Pax4 expression and localization were determined by QT-PCR and immunohistochemistry respectively. Double transgenic animals were continuously given 1 g/L DOX in the drinking water and challenged once with 200 mg/Kg streptozotocin i.p., an agent that causes massive beta-cell death.
Results: QT-PCR revealed a 14.7 ± 6.5 fold increase in mPax4 mRNA levels in isolated islets from DOX-treated animals, as compared to those of control untreated animals. No expression was detected in other organs. Immunohistochemistry of pancreas sections confirmed Pax4 expression only in beta-cells of mice treated with DOX. Double transgenic animals exhibited normal glucose tolerance and insulin sensitivity, indicating that Pax4 expression did not alter glucose metabolism. Streptozotocin treated control animals developed a severe hyperglycaemia (n=9, 22.2 ± 2.1 mmol/L glucose) whereas double transgenic animals expressing Pax4WT maintained normal blood glucose (n=10, 8.17 ± 0.47 mmol/L glucose $P < 0.001$). Double transgenic animals expressing Pax4R129W developed an intermediate hyperglycaemia (n=6, 15.2 ± 3.5 mmol/L glucose). Immunofluorescence staining of sections of pancreas sampled 1 month after the streptozotocin treatment revealed a massive decrease in the number of beta-cells in control animals whereas Pax4WT overexpressing animals sustained a normal beta-cell mass.
Conclusion: When pancreatic beta-cells are challenged by a pharmacological insult such as streptozotocin, overexpression of Pax4WT confers increased protection against apoptosis to beta-cells, thus preventing the development of a hyperglycaemic state.
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423

Regulation of the wnt signalling pathway by endocrine differentiation bHLH factors
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Background and aims: In the embryonic pancreas, the *atonal*-related bHLH gene neurogenin3 (ngn3) initiates the differentiation program that culminates in the formation of the endocrine cells of the islets of Langerhans. One of the genes activated by ngn3 is the gene for the bHLH factor math6. Math6 is expressed in ngn3+ endocrine progenitors and, like ngn3, is absent from differentiated endocrine cells. However, the specific role of math6 in the endocrine cascade is unknown. Using expression microarrays, we identified several genes related to the wingless (wnt) signalling pathway whose expression is modulated by a siRNA specific for math6 (simath6) in ngn3-expressing duct cells. These data raised the hypothesis that math6 may regulate this pathway during endocrine differentiation. However, while it is well established that activation of the canonical wnt/b-catenin pathway is needed for proper growth and progenitor proliferation in the embryonic pancreas, the involvement of wnt signals in the differentiation of the endocrine cell lineage remains controversial. Here, we have aimed at dissecting the potential link between endocrine differentiation bHLH factors, namely ngn3 and math6, and the wnt signalling pathway.
Material and methods: Overexpression and RNA interference using recombinant adenoviruses in mouse pancreatic duct cells. Gene expression profiles by Affymetrix. Gene expression quantitation by real time PCR. TOP-FLASH luciferase reporter system for measurement of canonical wnt activity.
Results: Microarray analysis reveals that knock-down of math6 activation by ngn3 results in significant changes in the expression of genes coding for several components of the wnt signalling pathway, including ligands, modulators, effectors and targets in duct cells. Likewise, microarray analysis has shown that ectopic expression of ngn3 alone promotes changes in the expression of a number of wnt-related genes in the same cellular context. Differential expression has been confirmed by real time PCR. Some of the identified genes are regulated by both ngn3 and simath6 (*wnt9a, bambi, igfbp4, cfos, tmem46* etc) whereas others are modulated by ngn3 alone (*wnt7b, dkk1*) or by simath6 alone (*TCF7L2*). It is noteworthy that math6 and ngn3 appear to exert opposite effects on the expression of many of the identified common gene targets, suggesting that these factors counter-regulate each other. In this regard, we demonstrate that ngn3 activates while math6 inhibits the activity of the canonical wnt activity as measured with a TCF-dependent luciferase reporter system. To this date, we have confirmed expression of all the identified genes in the embryonic pancreas at the time of endocrine cell differentiation. On-going work in the lab is aimed at understanding the molecular bases for the regulation of the activity of this pathway by ngn3 and math6.
Conclusion: Altogether, this work supports the existence of a link between endocrine bHLH factors and the wnt signalling pathway and points to the need of reassessing whether wnt signals participate in endocrine cell fate decisions during pancreatic organogenesis.
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424

Wnt/Klotho interaction regulates the proliferation of pancreatic beta cells
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Background and aims: Acute stimulation with Wnt-agonists induces beta-cell proliferation and insulin secretion. However, in fibroblasts chronic Wnt-stimulation represses cell proliferation and induces cellular aging. Klotho is a regulator of aging and has recently been identified as an extracellular Wnt-antagonist. The expression and function of klotho in pancreatic beta-cells is not known. Therefore, we investigated the effect of chronic Wnt-stimulation and the role of klotho on the proliferation of insulin-producing cells.
Materials and methods: Transient transfections of insulin-producing Ins-1 cells with a Wnt-reporter gene (TOPFLASH). Isolation, culture, mRNA- and protein extraction of primary murine beta-cells. PCR and Western Blot analysis of Ins-1 and primary beta-cells. Proliferation assays (3H-thymidine uptake). Immunohistochemistry of pancreata of wildtype and NZO (New-Zealand Obese) mice.
Results: We found klotho to act as an antagonist of canonical Wnt-signaling in Ins-1 beta-cells as recombinant klotho inhibited Wnt-mediated transcription (TOPFLASH reporter gene). In primary murine beta-cells we detected klotho expression on the mRNA and protein level. An obese pre-diabetic mouse model (NZO mice) showed an increase in the expression of Wnt4 and a decrease in the expression of klotho in pancreatic islets as compared to wildtype mice (assessed by immunohistochemistry). On the functional level, we found acute (24h) stimulation of Ins-1 cells wíth recombinant Wnt3a protein to induce beta-cell proliferation (129%; $p<0.001$) according to our previous findings. However, chronic Wnt-stimulation over 35 days repressed the proliferation of Ins-1 cells almost completely (to 18% as compared to untreated controls; $p<0.05$). The proliferation was restored by co-stimulation with klotho in addition to Wnt3a to 136% ($p<0.001$).
Conclusion: These data demonstrate the expression of endogenous Wnts and klotho in pancreatic beta-cells and their differential regulation in a pre-diabetic mouse model. Here we show that chronic Wnt-stimulation represses beta-cell proliferation and that klotho acts as a survival factor, suggesting that the Wnt/klotho interaction acts as a long-term regulator of the beta-cell mass.

425

β-catenin as a novel glucose-sensor in INS1E beta cells
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Background and aims: Canonical Wnt signaling is a key feature of growth, differentiation and metabolism. Previous studies have already shown that the Wnt/ β-catenin pathway induces proliferation and insulin secretion in β-cells and can increase glucose-induced insulin secretion. In an earlier study, we have demonstrated that changes in glucose levels can acutely regulate the levels of β-catenin, in different cell lines and also *in vivo*, as β-catenin levels are increased in insulin sensitive tissues (liver, adipose tissue, skeletal muscle) of diabetic rats (unpublished data). Glucose-induced β-catenin increase involves autocrine Wnt activation and N-linked glycosylation. In our current study, we investigate the effect of glucose on the Wnt/ β-catenin pathway in a pancreatic β-cell line to see if these insulin-secreting cells respond to glucose in the same way as the insulin-sensitive tissues.
Materials and methods: As a model of β-cells, we use glucose-responsive and insulin-secreting INS1E cells. To explore the mechanisms, we treat INS1E β-cells with different kind of drugs, such as insulin secretion supressors, Hex-

osamine Biosynthesis Pathway (HBP) inhibitors, Wnt agonist or inhibitors, and also Wnt-conditioned-medium.
Results: Our most significant finding is that glucose itself increases β-catenin levels in INS1E β-cells. More precisely, glucose induces β-catenin translocation to the nucleus and the transcription of β-catenin target genes, such as CyclinD1. The Hexosamine Biosynthesis Pathway (HBP) is also involved as glucosamine can mimic the effect of glucose on β-catenin in INS1E cells. To check a potential autocrine Wnt activation, we looked at phosphorylation of Wnt-co-receptor LRP6 (low density lipoprotein receptor-related protein 6). We observed that in response to glucose, LRP6 phosphorylation is also increased in INS1E β-cells. Finally insulin secretion does not seem to be responsible for β-catenin stabilization as its inhibition in high or low glucose conditions does not affect β-catenin levels.
Conclusion: In this study, we present a new mechanism for pancreatic β-cells to sense glucose levels. Glucose increase stabilizes β-catenin and induces the canonical Wnt/β-catenin pathway via autocrine activation. This mechanism involves the HBP pathway but not insulin secretion. Taken together, these findings suggest that glucose-induced β-catenin increase occurs in insulin-secreting cells as observed in insulin-sensitive tissues such as skeletal muscle and liver. We will now focus on the precise mechanism leading glucose to activate Wnt/β-catenin pathway in β-cells and to regulate energy consumption and metabolism. The current findings establish β-catenin as a promising new sensor of energy availability within cells that regulate glucose levels in the whole body through insulin secretion.
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426

Signalling in islets isolated from individuals with type 2 diabetes mellitus
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Background and aims: Type 2 diabetes mellitus (T2DM) is connected with β-cell failure. Cellular mechanisms responsible for this development have been proposed. The scarcity of human islets in general and islets from T2DM individuals in particular have led to that most studies addressing the T2DM islet pathophysiology have been conducted in animal or cellular models of the disease. We have optimized a gel- and label-free tandem mass spectrometry approach to generate protein expression data sets from approximately 100 islets. The aim was to use this approach to protein profile islets from T2DM and control individuals and analyze the expression data sets for changes in pathway signaling in T2DM islet.
Materials and methods: Islets were isolated from five T2DM and ten age- and weight-matched control individuals. Approximately 30 islets per donor were handpicked directly after isolation. Islets from the T2DM and control donors were pooled into two separate samples. From one T2DM individual approximately 150 islets were obtained which allowed that a separate sample and a matching control sample was prepared. Sample proteins were extracted and digested by trypsin. Generated peptides were separated by nano-liquid chromatography followed by mass determination by Fourier transform ion cyclotron mass spectrometry. Peptides were identified by comparing MS/MS data with the IPI Human database using the Mascot search engine. Identified proteins were mapped on known signaling networks using the databases TRANSPATH and TRANSFAC and analyzed for differential pathway activation.
Results: In samples from pooled T2DM and control islets the number of identified non-identical peptides was 2135 corresponding to 837 proteins. The data sets were analyzed for differential pathway signaling activation. In T2DM islets several pathways controlling β-cell arrest and apoptosis (p53, caspase, stress-associated, Apo2L/TRAIL) were activated. Pathways controlling islet regeneration and proliferation were both activated (E2F, EGF, PLK1, parkin associated) and inactivated (insulin, PRL, PDGF, mTOR, OSM). In addition, pathways involved in immunological responses were activated (Fas, RANKL and TLR3) and inactivated (IFN, LPS, TCR, BCR) in T2DM islets. Expression data sets were also generated from one T2DM and one control individual. Analysis of differential pathway activation of islets from this T2DM individual, who had accentuated functional impairment, revealed that several pathways of proliferation and immunological response were altered. In contrast, pathways of apoptosis and cell arrest were essentially the same.
Conclusion: Information about how signaling of specific pathway are altered in islets from T2DM individuals, once verified and validated, may be used to compare with and validate altered signaling in animal and cellular models of the disease. The differential signaling observed in T2DM islet may open for new ways of developing strategies aiming at maintaining or restoring islet function and survival in individuals with T2DM.
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427

The protein tyrosine phosphatase, PTP-BL, interacts with the Wnt signalling pathway to regulate cell proliferation in INS-1 cells
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Introduction: The multifunctional protein tyrosine phosphatase, PTP-BL, has been shown recently to regulate pancreatic β-cell proliferation by interacting with components of the Wnt signalling pathway, resulting in altered β-catenin levels. B-catenin exists in various cellular pools and can also be present in active and inactive forms but the effects of altered expression of PTP-BL on the various pools of β-catenin and its activation state have not been characterised. Therefore, changes in the localisation and activation of β-catenin have been studied in the present work, after treatment of β-cells with activators of Wnt signalling and following induction of PTP-BL expression.
Methods: The rat β cell line INS-1 was used to generate clones stably expressing either wild type (WT) PTP-BL or a tyrosine phosphatase deficient mutant (PTP-BL-CS) under the control of an inducible promoter. INS-1 cells were grown to 80-90% confluence and treated with either purified Wnt3a, a low molecualr weight synthetic Wnt agonist (2-Amino-4-(3,4-(methylenedioxy)benzylamino)-6-(3-methoxyphenyl)pirimidine) and/or the Wnt co-ligand, R-spondin-1, to activate Wnt signalling. In some experiments, Exendin-4 was also used as this has been reported to regulate β-catenin responsive genes in β-cells. Whole cell protein extracts were analysed by Western blotting with anti-total β-catenin and anti-active β-catenin antibodies and with antibodies against Cyclin-D1. Subcellular fractionation of the cells was also performed to reveal the distribution of PTP-BL, total β-catenin and active β-catenin.
Results: Conditional expression of PTP-BL and PTP-BL-CS in INS-1 cells was confirmed by Western blotting. Induction of WT PTP-BL decreased the amount of active β-catenin in unstimulated INS-1 cells and it also antagonised the rise caused by Wnt 3a, Wnt3a+ R-spondin 1 or Exendin-4. These effects were not seen with the phosphatase deficient mutant, PTP-BL-CS. Surprisingly, although INS-1 cell growth was attenuated by induction of PTP-BL (uninduced: 0.30±0.02 A_{490} units at 72h; induced: 0.14±0.01 units; ($p<0.001$)) the increase in cyclin D1 caused by Wnt3a or Exendin-4 was not reduced following induction of PTB-BL. Thus, the attenuation in proliferation was apparently not mediated via Cyclin-D1. In unstimulated INS-1 cells, active β-catenin was found mainly in the nuclear and cytoskeletal fractions. Active β-catenin was also seen in the nucleus and cytoskeleton of the cells over-expressing PTP-BL and, additionally, it accumulated in the membrane fraction under these conditions.
Conclusion: The protein tyrosine phosphatase, PTP-BL, regulates the Wnt signalling pathway by modulating active β-catenin levels in pancreatic β cells but the attendant attenuation of cell proliferation appears to be independent of changes in cyclin-D1 expression.
Supported by: Diabetes UK

428

Evaluation of histology pattern recognition and color deconvolution image analysis of beta cell and islet area
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Background and aims: The etiology of diabetes varies and there are a number of rodent models which mimic type 1 (T1D) and type 2 (T2D) forms of the disease. Evidence indicates that reduced functional beta-cell mass underlies insulin insufficiency in both conditions; thus, new therapies are increasingly being targeted towards the restoration or preservation of beta cell mass. In order for rodent models to be useful for the evaluation of new therapies, islet morphology and insulin positive area (beta cell area) need to be well characterized using histological approaches. Manual morphometric approaches can be tedious and time-intensive; therefore, most labs are now calculating beta cell area using immunohistochemistry (IHC) with an insulin antibody and image analysis using color separation to estimate insulin stained area. The

objective of the current study is to analyze results for beta cell & islet area using manual morphometry, color separation, and histology pattern recognition image analysis.
Materials and methods: Analysis was performed on an established T2D mouse model, Tallyho (TH), and age, gender-matched control mice. Representative pancreatic sections from six TH and six wild-type animals were compared. Two parameters were analyzed, insulin positive area and total islet area. Pancreas sections were processed for histology using hematoxylin and eosin (H&E) or an insulin antibody, detected by DAB. Whole slide images were captured using an Aperio Scanscope and were analyzed with ImageScope tools and algorithms. Histology pattern recognition was accomplished using a genetic algorithm which uses multiple features, such as color, shape, cell morphology, and staining texture, to separate tissue into "classes".
Results: We report strong concordance between results obtained with histology pattern recognition and manual morphometry for both beta cell area and islet area. There was no significant difference in beta cell area calculated using the two methods. While color separation showed similar trending, the values for beta cell area were much higher than those obtained with either manual morphometry or histology pattern recognition (2-6% vs. 0-2% for other methodologies). Presumably, this increase is due to presence of background IHC staining which is inadequately removed by thresholding; therefore, it is included as positive area in the data.
Conclusion: These results suggest that automated histology pattern recognition is a quicker alternative to manual morphometry and is more reliable than color separation alone for analyzing islet morphology in animal models.

429

Human beta cell sorting for characterisation of beta cell specific transcripts and functions

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Background and aims: For some years, researchers have been able to isolate rat and mouse beta cells for studying in isolation based on endogenous fluorescence of the beta cells relative to the non-beta cells, but this has not been possible in human islet cells until recently. We have used a method for sorting beta and non-beta human islet cells based on labeling with a zinc-binding fluorescent dye (Newport Green), which labels the insulin granules of the beta cells allowing separation of the beta and non-beta populations. This study aimed to first characterise the beta and non-beta cell fractions obtained by this method and then to identify beta cell-specific transcripts that may be associated with beta cell dysfunction or proliferation.
Materials and methods: Human islets were dissociated with trypsin and DNAse I and sequentially labelled with an antibody marker for ductal cells, Newport Green to identify the beta cells and 7-aminoactinomycin D to label dead cells. The sorting protocol discards ductal and dead cells and sorts the remaining population on the basis of size and Newport Green fluorescence to give two populations of endocrine beta and non-beta cells. RNA was extracted immediately after sorting and reverse transcribed to cDNA for quantitative PCR. For immunofluorescence, cells were fixed to slides by Cytospin followed by 2 % paraformaldehyde fixation. For insulin secretion measurements, beta cells were seeded on 804G extracellular matrix, and insulin secretion and content measured during 1hr incubation with glucose or KCl with an enzyme-linked immunoassay.
Results: This method successfully sorts human beta cells with a purity of consistently 90 % or greater. However, the non-beta cell fraction was more variable between different islet donors with varying amounts of glucagon transcript, as measured by Q-PCR. We also observed by immunofluorescence varying proportions of glucagon-positive to glucagon and insulin double negative cells in the non-beta fraction. Total RNA yields per cell were significantly higher in the beta cell fraction (4.58 pg/cell for non-beta versus 8.77 pg/cell for the beta cells, $n > 4$, $p < 0.01$) possibly indicating higher protein synthetic activity in this fraction. The beta cell fraction retained an insulin secretory response to high glucose and to KCl after sorting and monolayer culture, confirming their functional identity and demonstrating that they are still capable of insulin secretion in the absence of other endocrine cells. Gene profiling of the beta cell fractions from multiple donors suggested possible genes that could correlate with donor body mass index (BMI), which may be associated either with beta cell dysfunction or proliferation.
Conclusion: This method provides a means of acquiring purified beta and non-beta cells from human islets in larger quantities than can be obtained with other current methods such as laser capture microdissection. This will enable functional analysis of beta cells in the absence of other endocrine cells and allow comparisons to be made between diabetic and non-diabetic beta cells. Identification of beta cell enriched transcripts in these cells may provide novel targets for diabetes therapy.
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430

Orexin-A stimulates insulin secretion and proinsulin gene expression

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Background and aims: Orexin-A (OXA) regulates food intake and energy homeostasis. We recently demonstrated that OXA inhibits glucagon secretion and gene expression via forkhead-1 (foxo1)-dependent pathway. Discrepant observations were reported regarding the effects of OXA on insulin secretion in vivo. We therefore aimed to characterize the role of OXA in regulating pancreatic B-cell function.
Methods: Orexin receptor 1 (OX1R) expression in the endocrine pancreas, isolated pancreatic islets and INS-1 cells was identified by Western blots and confirmed immunohistochemically. Intracellular signalling encompassed the measurement of cyclic AMP, calcium, proinsulin gene expression and insulin secretion by ELISA, RIA, Fura-2, Western blots and real-time PCR. In situ pancreas perfusion studies were performed on anaesthetized male rats.
Results: OXR1 was expressed on pancreatic B-cells. OXA stimulated insulin secretion from in situ perfused rat pancreas and INS-1 cells. The changes were accompanied by an increase of intracellular cyclic AMP and calcium concentrations. OXA raised proinsulin mRNA-levels with a maximum after 24 hours of exposure.
Conclusion: Our study provides the first evidence for OXA as a stimulus of insulin secretion and proinsulin gene expression. In addition we identify cAMP/AKT/PDK-1 and calcium as intracellular target molecules for OXA action in pancreatic B-cells.

PS 20 Ion channels in beta cells

431

Gliquidone features different from glibenclamide in the insulin secretion pattern and closing the K_{ATP} channels profiles in HIT-T15 cells

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Background and aims: The aim of this study is to determine whether gliquidone has any different effect on the insulin-secretory profiles of HIT-T15 cells in response to glucose stimuli and the closing ATP-sensitive potassium (K_{ATP}) channels profiles in this βcells membrane from the other two sulphonylureas i.e. gliclazide and glibenclamide.
Materials and methods: HIT-T15 cells (β-cells line) were cultured for 8h in RPMI-1640 with three different glucose concentrations (5, 10, 15mmol/l), the cells were re-cultured with gliquidone ,gliclazide and glibenclamide in physiological concentrations ,and then, insulin levels in the medium at 0,10,20,30,45,60,120 and 180 minutes were measured by radiommunoassay(RIA). The action of the drugs with the therapeutic concentrations was studied by whole-cell current recording of K_{ATP} channels in single HIT-T15 cells.
Results: In this study , all three sulpholureas strongly stimulated the insulin release by β-cells in the presence of glucose in different patterns. Gliquidone and gliclazide induced a similarly rapid biphasic insulin secretion, reaching the first peak at 10 minutes and the second lower peak at 30 minutes, followed by gradual decline to near the basal values at the third hours. In contrast, glibenclamide induced a strong but monophasic insulin release, reaching its peak at 45 minutes followed by somewhat decline of the hormone release but did not return to basal values in 3 hours later. Gliquidone blocked the whole-cell βcells K_{ATP} current rapidly, the mean time of the 50% K_{ATP} channels closing is much shorter than that of glibenclamide (2.5min Vs 6.8 min. P<0.001),but is similar to gliclazide (2.5min Vs 2.4 min. P>0.05)
Conclusion: Gliquidone stimulates insulin release of HIT-T15 cells in biphasic secretion which similar to gliclazide, but different from glibenclamide. gliquidone closes the K_{ATP} channels of βcells more quickly than glibenclamide. This unique feature in insulin secretion of gliquidone may have important implications in its clinical use.
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432

Excessive expression and activity of Kv2.1 channels in islet beta cells of type 2 diabetic GK rats

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Background and aims: Voltage-dependent delayed rectifier K^+ (Kv) channels are involved in repolarization of excitable cells. In pancreatic β-cells, activation of Kv channels might repolarize cells and attenuate glucose-stimulated action potentials to suppress insulin secretion. Among Kv channel families, Kv2.1 is reportedly expressed in islet β-cells as the major component of Kv currents in rodents. This study aimed to determine the pathophysiological role of Kv2.1 channels in the impaired glucose-induced insulin release in islet β-cells of type 2 diabetic rats.
Materials and methods: Expression of Kv2.1 channels in the pancreas of male normal Wistar and type 2 diabetic Goto-Kakizaki (GK) rats was measured by immunohistochemistry. Islets were isolated from these rats by collagenase digestion, and insulin release was determined by ELISA. In rat single β-cells, whole cell Kv channel currents were measured in the presence of tolbutamide by patch-clamp technique, and cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured by fura-2 microfluorometry.
Results: Immunohistochemical analysis revealed that Kv2.1 channels were expressed in islet β-cells, but not exocrine part, of the pancreas. The Kv2.1 immunofluorescence intensity in the islets of diabetic GK rats was stronger than that in normal Wistar rats. In isolated islets of Wistar and GK rats, tetraethylammonium, a non-selective blocker of Kv channels, increased glucose (8.3 mM)-induced insulin release. Moreover, Kv2.1 channel blockers, stromatoxin-1 (ScTx) and guangxitoxin-1E (GxTx), significantly increased glucose-induced insulin release, without altering basal insulin release at 2.8 mM glucose. Kv channel current density in β-cells was larger in GK rats than Wistar rats. Kv2.1 channel blockers markedly attenuated Kv channel currents in β-cells, and the channel currents that remained in the presence of GxTx were identical between Wistar and GK rats. Blockade of Kv2.1 channels by ScTx and GxTx potentiated glucose (8.3 mM)-induced $[Ca^{2+}]_i$ increases in β-cells of normal and diabetic rats, whereas basal $[Ca^{2+}]_i$ levels and KCl (25 mM)-induced $[Ca^{2+}]_i$ increases at 2.8 mM glucose were not affected by these channel blockers.
Conclusion: Kv2.1 channels may physiologically limit glucose-induced Ca^{2+} influx and thereby attenuate insulin secretion in β-cells. Excessive expression and/or activity of Kv2.1 channel may be causally related to impaired insulin secretion in diabetic GK rats. Blockade of this channel can promote glucose-induced insulin release in diabetic as well as normal rats, providing a potential therapeutic tool to treat type 2 diabetes.

433

Essential role of NAADP-evoked calcium release in glucose-mediated depolarization, [Ca2+]i spiking and insulin secretion in mouse pancreatic beta cell

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Background and aims: Current mechanisms of stimulus-secretion coupling in pancreatic β-cells emphasize a role for glucose-enhanced plasma membrane ion fluxes as a consequence of the closure of KATP (Kir6.2/SUR1) channels. However, glucose may still evoke Ca2+ signals and insulin exocytosis in β-cells from Kir6.2 or SUR1 knockout mice by KATP-independent pathways. We have investigated the role of the NAADP-targeted acidic Ca2+ stores in glucose-mediated Ca2+ oscillations and insulin secretion in primary mouse β-cell.
Materials and methods: We have used Ca2+ imaging, electrophysiology and insulin secretion measurement.
Results: We found that the release of Ca2+ from acidic Ca2+ stores plays a critical role in triggering and shaping glucose-evoked Ca2+ signals since these signals are abolished by the vacuolar proton pump inhibitor, bafilomycin. Using new membrane-permeant probes, we further show that the intracellular messenger NAADP mobilizes Ca2+ from acidic stores, but not the endoplasmic reticulum, depolarizes the plasma membrane by activating a cation current. Inhibition of NAADP-evoked Ca2+ release, either by Ned-19, a selective NAADP antagonist, or self-desensitization, abolishes not only glucose-induced depolarization, Ca2+ signals and insulin secretion but also tolbutamide-induced insulin secretion. We have recently discovered that the two-pore channel (TPC2) located on acidic stores is the target receptor of NAADP (Nature, in Press). We show that glucose-induced [Ca2+]i signalling is dramatically altered in TPC2 knockout mice
Conclusion: We propose that NAADP-mediated Ca2+ mobilization from acidic stores is a key step in triggering stimulus-secretion coupling in mouse pancreatic β-cells. In turn this activates a calcium-dependent cation current which, when KATP channels close, can depolarize the membrane potential allowing the activation of voltage-gated Ca2+ channels, Ca2+ influx and exocytosis of insulin granules.
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434

Analysis of membrane potential oscillation mechanism in the novel pancreatic beta cell simulation model

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Background and aims: Several types of pancreatic beta cell models have been published. However, the application of these models is still very limited because of very simplified ionic channel models. We aim at developing an electrophysiological pancreatic beta cell model to analyze the role of ion channels and transporters in the oscillation mechanisms of membrane potential oscillation underlying in the regulation of insulin secretion.
Materials and methods: Mathematical models of twelve ion channels (INa, ICaL, IKr, Ito, IKATP, IKCa, IKb, ICRAN, ITRPM4) and transporters (INa/K,

IPMCa, INa/Ca) were developed according to electrophysiological studies, and were implemented into a novel beta cell computer model based on the framework of previous cell models. Then we examine contributions of ion channels and transporters to oscillatory membrane potential response using this new model.
Results: The model was quiescent at glucose([G]) below 6 mM with a most negative resting potential of about -70 mV at 0 [G]. Brief bursts of action potential with an overshoot = ~ -10 mV and a plateau level = ~ -40 mV were initiated at [G] higher than 6.5 mM accompanied with an increase in intracellular Ca2+ ([Ca2+]). The burst duration was prolonged with increasing [G] as observed in experimental studies. The burst of action potentials was generated by alternating activation of L-type Ca2+ channel and the delayed rectifier K+ channels. The open probability of the ATP-sensitive K+ channel is gradually increased because of increased consumption of ATP during the burst, and the increased outward current finally terminated the burst. The mechanisms underlying the cyclic burst activity is explained along the sequential events as described below. During the inter-burst period, the glucose-dependent ATP production exceeds the ATP consumption and decreases the [ADP] / [ATP] ratio. Thereby, the open probability of ATP-sensitive K+ current is decreased and the membrane potential is depolarized with time. Finally at around -55 mV, the bust of action potential is triggered. During the burst of repetitive Ca2+ spikes, [Ca2+] accumulate, which then leads to acceleration in the ATP consumption and an increase in the [ADP] / [ATP] ratio. As a result, outward current through the ATP-sensitive K+ channel is increased and the burst is terminated. With increasing [G], the ATP production rate is increased in a dose-dependent manner, and the burst duration is prolonged because of the delayed increase in the [ADP] / [ATP] ratio. At [G] higher 12 mM, the increase in [ADP] / [ATP] ratio becomes much depressed. Under this condition, the role of the ATP-sensitive K+ channel is replaced by the increase in the Na+/K+ pump current. Namely, the accumulation of Ca2+ is converted to a Na+ accumulation by the Na+/Ca2+ exchange. Thereby, the outward Na+/K+ pump current is enhanced to finally terminate the burst activity after more than ~90 sec burst. Switching the terminatory role from the ATP-sensitive K+ channel to the Na+/K+ pump current is achieved smoothly through the ultra slow inactivation of the Ca2+ channel.
Conclusion: We concluded that the new B cell model is relevant for analyzing mechanisms underlying the [G]-dependent activation of the cell.

435

Oscillations of Zn^{2+} beneath the plasma membrane in glucose-stimulated beta cells

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Background and aims: Glucose is the main physiological stimulus of insulin secretion from pancreatic β-cells. Glucose increases the cytoplasmic Ca^{2+} and cAMP concentrations in β-cells, and cAMP is an important amplifier of Ca^{2+}-triggered pulsatile insulin release. Insulin is stored in secretory vesicles as a crystalline zinc complex and Zn^{2+} is co-secreted with insulin. Zn^{2+} has been proposed to exert a negative feedback on insulin secretion as a potent and reversible activator of hyperpolarizing K^{+}_{ATP} channels. Zn^{2+} has also been found to interfere with cAMP turnover in β-cells. We therefore studied how glucose affects Zn^{2+} in insulin-secreting β-cells.
Materials and methods: MIN6 β-cells were transfected with a fluorescent translocation biosensor reporting phosphatidylinositol-3,4,5-trisphosphate in the plasma membrane ($[PIP_3]_{pm}$) in response to autocrine insulin receptor activation. This assay was used as readout for insulin secretion from single cells. The cytoplasmic cAMP concentration beneath the plasma membrane ($[cAMP]_{pm}$) was detected by another translocation biosensor based on fluorescence-labeled protein kinase A subunits. Measurements of intracellular Zn^{2+} and Ca^{2+} concentrations were performed in cells loaded with the fluorescent indicators FluoZin-3 and Indo-1, respectively. Conventional epifluorescence microscopy was used to detect the global changes of Zn^{2+} and Ca^{2+} in the cell cytoplasm ($[Zn^{2+}]_i$, $[Ca^{2+}]_i$). Local changes of these cations in the sub-membrane space ($[Zn^{2+}]_{pm}$, $[Ca^{2+}]_{pm}$) as well as changes in $[PIP_3]_{pm}$ and $[cAMP]_{pm}$ were recorded with evanescent wave microscopy.
Results: MIN6 cells reacted to glucose stimulation with pronounced $[Ca^{2+}]_i$, $[Ca^{2+}]_{pm}$ and $[cAMP]_{pm}$ as well as $[PIP_3]_{pm}$ oscillations reflecting pulsatile insulin release. Glucose stimulation did not affect $[Zn^{2+}]_i$ but induced oscillations of $[Zn^{2+}]_{pm}$, which were heterogeneous with irregular amplitude and frequency. Hyperpolarization with 250 μmol/l diazoxide or inhibition of voltage-dependent Ca^{2+} channels with 100 μmol/l methoxyverapamil prevented the glucose-induced $[Ca^{2+}]_i$ and $[Ca^{2+}]_{pm}$ signaling without affecting the $[Zn^{2+}]_{pm}$ oscillations. Also chelation of extracellular Zn^{2+} with 2 mmol/l Ca-EDTA failed to affect the glucose-induced $[Zn^{2+}]_{pm}$ and $[cAMP]_{pm}$ oscillations. However, the membrane permeable Zn^{2+} chelator TPEN (50 μmol/l) abolished the Zn^{2+} signal and much amplified the $[cAMP]_{pm}$ and $[PIP_3]_{pm}$ responses to glucose stimulation. Application of 30 μM Zn^{2+} to glucose-stimulated MIN6 cells resulted in an increase of $[Zn^{2+}]_{pm}$ and markedly inhibited $[cAMP]_{pm}$ and $[PIP_3]_{pm}$ responses as well as suppressed insulin release detected by ELISA.
Conclusion: These data provide support for intracellular Zn^{2+} as inhibitory messenger affecting cAMP and insulin secretion from β-cells.

436

Rapid changes in surface expression of L-type Cav1.2 channels in stimulated INS-1 cells

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Background and aims: Voltage gated calcium channels (VGCCs) in the plasma membrane (PM) play an essential role in linking glucose metabolism to insulin secretion. Under resting condition, the number of VGCCs in the PM (surface expression) maintains a dynamic equilibrium that is controlled by trafficking to and from the cell interior. eIF3e (eukaryotic translation initiation factor 3, subunit E) is widely expressed in eukaryotic cells and is involved in both initiation of protein translation and degradation. Interestingly, eIF3e has been suggested to be involved in the regulation of activity-dependent internalization of L-type Cav1.2 channels in neurons. However, it remains unclear whether eIF3e plays a similar role in pancreatic beta-cells.
Materials and methods: Cav1.2 clusters were observed by 3D confocal imaging in INS-1 cells. Cluster internalization was quantified using the ImageJ and Matlab programs. To assess the rate of Cav1.2 transport to plasma membrane, the Fluorescence Recovery After Photobleaching (FRAP) technology was used.
Results: Depolarization causes internalization of Cav1.2. Cav1.2 is expressed in clusters in Cav1.2-EGFP transfected INS-1 cells. The ratio of the cluster number in the PM over clusters residing in the cytosol was calculated in time-lapse series taken from the middle section of the cells, which decreased from 4.1±1.09 to 1.36±0.18. Double labeling experiments with Cav1.2 and the PM indicator FM-64, verified that the number of Cav1.2 clusters in the PM decreased by 50% upon glucose stimulation. To allow a careful quantification of Cav1.2 cluster internalization, we calculated the geometrical centre of the cluster scatter and measured the distance from that point to each cluster, which decreased from 6.2±0.11 to 5.2±0.16 μm after 30 min stimulation with glucose ($P<0.001$).
Cav1.2 cluster internalization can be detected also in native cells. To validate the physiological significance of our initial results, we measured Cav1.2 expression by immunostaining in INS-1 cells before and after 15-30 min stimulation with glucose. The ratio of the number of clusters in the PM over clusters in the cytosol amounted 3.32±0.48 which decreased to 0.83±0.14 after glucose stimulation ($P<0.01$). Similarly, Cav1.2 redistribution from the PM to intracellular membranes upon glucose stimulation could be detected by Cav1.2 immunoblotting in fractionated cells.
eIF3e silencing reduces surface expression of Cav1.2 and inhibit their internalization upon stimulation. After knockdown of eIF3e by RNAi, the number of Cav1.2 cluster in the PM was reduced by 40% in resting condition ($p<0.05$). Interestingly, stimulation with HK or glucose now failed to internalize Cav1.2 to the cell interior.
eIF3e silencing decreases the rate of Cav1.2 supply to the PM. A FRAP approach was used to investigate the dynamics of Cav1.2 clusters in the PM. The average recovery intensity was fitted by an exponential curve and the time constant (τ) indicates how slow the Cav1.2 moves to PM. eIF3e silencing increased τ for Cav1.2 transport to the PM from 0.79 s to 1.51 s under non-stimulatory conditions. Under stimulation with HK, eIF3e silencing increased τ from 0.21 s to 0.63 s.
Conclusion: Depolarization causes internalization of Cav1.2 clusters in INS-1 cells. eIF3e enhances Cav1.2 surface expression under resting condition, and
eIF3e is also required for activity-dependent internalization of Cav1.2 clusters.
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437

Ca^{2+}/Calcineurin/NFAT signalling pathway regulates glucose-induced IRS-2 expression in rat pancreatic beta cells

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Background and aims: Insulin receptor substrate 2 (IRS-2) plays an essential role in pancreatic beta-cells by promoting cell growth and survival and so represents an interesting target for therapeutic means in type 2 diabetes. We have previously shown that glucose induces a rapid increase in IRS-2 expression in rat pancreatic islet beta-cells, mediated at the transcriptional level and requiring presence of Ca^{2+}. However how Ca^{2+} regulates IRS-2 gene expression remains unknown.
Materials and methods: Rat pancreatic islet beta-cells or INS-1E cell line were exposed to various inhibitors and/or infected with adenovirus expressing CAIN or VIVIT. IRS-2 expression was analyzed by RT-PCR and Western blot. NFAT activation and translocation were analyzed by Western blot and confocal immunofluorescent microscopy.
Results: Using nifedipine, a selective inhibitor of L-type Ca^{2+} channels, we showed that elevated intracellular concentration of Ca^{2+} is required for glucose-induced IRS-2 gene expression in isolated rat islet beta-cells. Moreover, in rat islets beta-cells exposed to 30 mM KCl, which leads to an $[Ca^{2+}]_i$ increase via depolarization, specific increased in IRS-2 mRNA and protein levels was observed at stimulatory glucose concentrations (≥5 mM). Inhibition of Ca^{2+}/calmodulin-dependent phosphatase 2B (calcineurin) by FK506 or Cyclosporin A inhibitors markedly blocked the glucose-induced IRS-2 expression at both mRNA and protein levels in both INS-1E cells and rat islet beta-cells. Moreover, infection of islets beta-cells with the adenovirus expressing CAIN (Adv-CAIN; a selective peptide inhibitor of calcineurin), totally suppresses glucose-induced IRS-2 gene expression compare to cells infected with an adenovirus control expressing luciferase. As expected, decrease in IRS-2 protein level correlates with mRNA decrease in presence of these inhibitors. The transcription factor nuclear of activated T cells (NFAT) is well known to be activated by the Ca^{2+}/calcineurin pathway which induces its dephosphorylation and then translocation to the nucleus. NFAT protein phosphorylation state and localization are modified by glucose in INS-1E cells. Four putative NFAT binding sites within the proximal region of the rat IRS-2 promoter (-1250 to +1 bp) were identified in the IRS-2 promoter sequence. To investigate the potential role of NFAT in IRS-2 expression, we infected INS-1E cells with an adenovirus to express VIVIT (Adv-VIVIT; a selective peptide inhibitor of calcineurin-mediated NFAT activation). We found that selective NFAT inhibition abolished glucose-induced IRS-2 expression at the mRNA and protein levels compare to control cells. Furthermore, direct binding of NFAT on IRS-2 promoter was demonstrated by a chromatin immunoprecipitation assay.
Conclusion: Taken together, these results clearly suggest that Ca^{2+}/calcineurin/NFAT pathway in islets beta-cells is implicated in IRS-2 gene expression in response to glucose. Considering the pivotal role that IRS-2 plays in pancreatic beta-cells, a better understanding of the IRS-2 transcriptional regulation could eventually provide novel therapeutic means in type 2 diabetes by improving beta-cells survival and proliferation.
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438

BLX-1002, a novel thiazolidinedione with no PPAR affinity, selectively enhances insulin secretion in diabetic animal islets stimulated with high glucose, GLP-1 or tolbutamide

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BLX-1002 is a novel amino acid conjugated, small thiazolidinedione with no apparent affinity to peroxisome proliferator-activated receptors (PPAR) that was shown to reduce glycemia in type 2 diabetes without adipogenic effects. We have investigated the actions of the drug on insulin secretion in pancreatic islet cells from diabetic and non-diabetic animal models. In the obese, leptin-deficient ob/ob mice, BLX-1002 enhanced insulin secretion stimulated by high (20 mM), but not low or intermediate (3 or 8 mM) glucose concentrations during a 20 min-incubation, while its major metabolite BLX-1015 had no effect. BLX-1002 also potentiated pioglitazone-, but not fenofibrate-induced insulin secretion. BLX-1002, but not BLX-1015, restored glucose-sensitive insulin secretion in pancreatic islets from type 2 diabetic, leptin receptor-deficient db/db mice. Under perifusion conditions, BLX-1002 significantly potentiated insulin secretion induced by GLP-1 or the sulfonylurea tolbutamide in pancreatic islets from type 2 diabetic Goto-Kakizaki (GK) rats, effects that were not observed in islets from non-diabetic Wistar rats. In contrast, BLX-1015 was without effect. Studies in single islet cells from ob/ob mice revealed that BLX-1002 augmented [Ca2+]i at high glucose, which was abolished either by the L-type Ca2+ channel blocker nifedipine or by pre-treatment of the cells with the Ca2+-ATPase inhibitor thapsigargin. The BLX-1002-induced rise in [Ca2+]i was significantly suppressed by the selective phosphatidylinositol (PI) 3-kinase (PI3K) inhibitor LY294002. However, BLX-1002 interfered neither with voltage-gated Ca2+ channel nor with KATP channel activities as demonstrated using patch-clamp technique. In addition, cellular NAD(P)H stimulated by glucose was not affected by the drug. The stimulatory effect of BLX-1002 on insulin secretion at high glucose was completely abolished by treatment of the cells with the PI3K inhibitors wortmannin or LY294002. Furthermore, stimulation of the b-cells with BLX-1002 induced activation of AMPK at high glucose. Our study suggests that BLX-1002 selectively potentiates insulin secretion induced by high glucose, GLP-1 or tolbutamide in b-cells from obese or diabetic animal models, but not from healthy animals. The effect of BLX-1002 on glucose-induced insulin secretion is dependent on PI3K activity and is associated with an increased [Ca2+]i and AMPK activation. The glucose-sensitive stimulatory impact of BLX-1002 on b-cell function may translate into substantial clinical benefits of the drug in the management of type 2 diabetes, by avoidance of hypoglycemia.
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PS 21 Beta cell - exocytosis

439

Roles of the GDP-dependent Rab27a effector coronin 3 in membrane traffic in the pancreatic beta cell

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Background and aims: Membrane trafficking is crucial for the regulation of the size of the readily releasable pool of the secretory granules and for the recovery of the granule membranes. Although many proteins have been found to participate in membrane trafficking, knowledge about its regulatory mechanisms is still limited. Rab27a is involved in the control of membrane traffic in the beta cell typically through its GTP-dependent effectors. We have recently identified the actin-bundling protein coronin 3 as a novel Rab27a effector which paradoxically bound GDP-Rab27a in the pancreatic beta-cell line, MIN6. In the present study, we investigated GDP-dependent roles of Rab27a via its interaction with coronin 3 in the pancreatic beta cell.
Materials and methods: A pull-down assay was performed to quantify GTP- and GDP-bound Rab27a. The slp homology domain of the GTP-dependent Rab27a effector and the GDP-dependent Rab27a binding site of coronin 3 were used as specific affinity ligands. For immunostaining and live imaging, MIN6 cells were transfected with plasmid DNAs or siRNA using Lipofectamine 2000. After 24 h of transfection, the cells were analyzed with a confocal laser microscopy system. For the observation of endocytosis, cells were incubated in culture media containing the endocytosis marker FM4-64 or anti-phogrin antibody which reacts with its extracellular domain. The rate of cells with cytoplasmic pattern was evaluated.
Results: Glucose stimulation promptly shifted Rab27a from the GTP- to GDP-bound form and redistributed coronin 3 to the cell periphery. The redistribution was inhibited in Rab27a-silenced MIN6 cells. Coronin-3-silencing inhibited FM4-64 uptake and phogrin recycling. Disruption of the coronin-3-GDP-Rab27a interaction with the dominant-negative coronin 3 reproduced these changes, which were restored by co-expression of the GDP-Rab27a mutant.
Conclusion: The interaction of GDP-Rab27a with coronin 3 regulates endocytosis of the insulin granule membranes. We propose a model where Rab27a is a key protein in stimulus-endocytosis coupling in the pancreatic beta cell, and plays a pivotal role in membrane recycling of the insulin granules.
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440

Sorting nexin 19 regulates the number of insulin-containing secretory vesicle and insulin content by affecting IA-2 expression

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Background and aims: IA-2 is a member of the transmembrane protein tyrosine phosphatase (PTP) family and a major autoantigen in type 1 diabetes. Autoatibodies to it are positive in about 70 % of newly diagnosed patients. They also are a predictive marker of the disease because of appearance years before the onset of the clinical disease. This molecule is 979 amino acids in length consisting of an intracellular, transmembrane and luminal domain. IA-2 is an intrinsic transmembrane component of dense core secretory vesicles. Studies of knockout in mice and knocked down in a mouse beta-cell line MIN6 showed that IA-2 is a regulator of insulin secretion. In addition, overexpression of IA-2 in MIN6 cells increases insulin content and insulin containing secretory vesicle numbers in part through stabilizing half-life of the vesicles. However, it is not fully understood how IA-2 regulates insulin content or insulin-containing secretory vesicle numbers. To address this question, we examine the involvement of sorting nexin 19 (SNX19), an IA-2 interacting protein identified by yeast two-hybrid system. SNX19 is a member of the SNX family which has a phospholipid binding motif, phox domain, and is proposed to regulate intracellular trafficking.
Materials and methods: Endogenous interaction of SNX19 and IA-2 was confirmed by immunoprecipitation analysis and Western blot analysis using anti-SNX19 antibody and/or anti-IA-2 antibody. Binding of SNX19 to phospholipids were determined by protein/lipid overlay assay. Permanent SNX19-knocked-down cell lines were established by transfection of SNX19 siRNA expression vector and selection by limit dilution with hygromycin. Insulin content was measured by insulin ELISA kit. Numbers and size of insulin-containing secretory vesicles in SNX19-knocked-down or control cells were measured by electron microscopy.
Results: SNX19 was endogenously expressed in MIN6 cells and pancreatic islets isolated from *C57BL/6 mice* and *Wister rats*. Immunoprecipitation analysis revealed endogenous interaction of SNX19 and IA-2 in MIN6 cells. Protein/lipid overlay assay indicated that SNX19 could mainly bind phosphatidylinositol-3-, or -4-, or -5- phosphate, and phosphatidylinositol-(3,5)-bipospate. In SNX19-knocked-down MIN6 cells, SNX19 expression was decreased by one-fifth with a parallel decrease of insulin content as compared to that in control siRNA-transfected MIN6 cells. Interestingly IA-2 expression also was decreased in SNX19-knocked-down MIN6 cells. Electron microscopy revealed that the number of insulin-containing secretory vesicle were about 75% decreased and the average size of the vesicles was about 40% smaller in SNX19-knocked-down MIN6 cells as compared to that in control siRNA-transfected MIN6 cells, respectively.
Conclusion: SNX19 regulates the number of insulin-containing secretory vesicle and insulin content in pancreatic beta-cells via modulating IA-2 expression.

441

Protein expression and distribution of syntaxin isoforms in INS-1 cells, mouse, rat and human pancreas

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Background and aims: In previous studies we showed the differential expression of SNARE proteins at different glucose concentrations in the insulin secretory cell line INS1. At high glucose concentration (20mM), the expression of several key proteins for insulin secretion, syntaxin1, VAMP2, synaptotagmin I and connexin36 was reduced. In contrast, the expression of t-SNARE proteins, SNAP25 and SNAP23 augmented suggesting a specific effect of high glucose concentration on the expression of proteins involved in the exocytic molecular machinery. Plasma membrane syntaxin (sytx) isoforms (1, 2, 3 and 4) have been related to insulin secretion, but their particular differential implication is still obscure and poorly studied. The aim of this study was to define both the distribution of syntaxin isoforms in mouse, rat and human pancreas and in the cell line INS-1, and the expression pattern of syntaxin isoforms (1 to 4) at different glucose concentrations in the cell line INS1 and rat isolated islets.
Materials and methods: The distribution of syntaxin isoforms was analyzed by immunofluorescence and confocal microscopy using isoform specific antibodies. The level of protein expression at different glucose concentrations was determined by western blot. Cell line INS1 was cultured for 1, 3 and 7 days at 3,3; 11,1; 20 and 33,3 mM glucose in RPMI medium 10%FBS. Sprague-Dawley rats of 150-175 g were used for studies on pancreas and isolated islets. Rat isolated islets were cultured for 6 days at the same glucose concentration used in the experiments with INS1cell line. Human pancreas were obtained from brain-dead donors and processed for immunofluorescence and confocal microscopy.
Results: A) Syntaxin isoform distribution: Sytx1 was found in the beta-cell plasma membrane in all models used while sytx 2, 3 and 4 showed a cytosolic distribution in the endocrine tissue. Sytx3 was also present in the plasma membrane in cells from the rat exocrine tissue. Sytx4 was mainly found in the alpha-cells from mouse and rat pancreas. None of the studied sytx isoforms showed immunofluorescence signal in a human diabetic pancreas, with the exception of sytx2.
B) Protein expression at different glucose concentrations: 1- INS-1: expression of sytx2 was decreased (44±15%, $p \leq 0.05$) after 7 days at high glucose concentration; sytx3 was increased at 3,3, 20, and 33,3 mM compared with 11,1 mM glucose, and sytx4 was increased (114±37%, $p \leq 0.05$) after 7 days at high glucose concentration. 2- Rat isolated islets: expression of sytxs 2 and 3 showed a tendency to increase at 3,3, 20, and 33,3 mM compared with 11,1mM glucose; sytx4 showed a tendency to decrease at high glucose concentration. Western blot results were confirmed by immunofluorescence and confocal microscopy.
Conclusion: The isoforms of syntaxin (1, 2, 3 and 4) studied in mouse, rat and human pancreas showed differential distribution patterns and protein expression at different glucose concentrations. The differential distribution

and expression of syntaxin isoforms in islet pancreatic cells indicates the presence of different pathways for intracellular membrane trafficking. However, further studies are required to establish the functional significance of these differences.
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442

Both rapid exocytosis and granule mobilization in glucagon secreting cells are reduced by antibodies against synaptosomal protein of 25 kDa (SNAP-25) and Syntaxin 1
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Introduction and aims: Glucagon is important for regulation of glucose metabolism by enhancing synthesis and mobilization of glucose in the liver. Glucagon resides within granules in the alpha-cells (A-cells) of the pancreas that upon A-cell activation fuses with the plasma membrane whereby glucagon is released into the bloodstream. A crucial mechanism behind the fine tuned release of glucagon is a functional exocytotic machinery constituting the SNARE-complex including the two target-SNARE proteins on the plasma membrane, syntaxin 1A and SNAP-25. We have previously shown that the SNARE-proteins are key players in the exocytotic process in beta-cells: Our aim is to investigate whether this is also true for A-cell exocytosis.
Material and methods: Primary A-cells extracted from mouse pancreas were identified by their small size and Ca^{2+} current inactivation properties using the standard whole-cell configuration of the patch-clamp technique. Exocytosis was measured as an increase in membrane capacitance following membrane depolarisations.
Results: The exocytotic granules are differently positioned within the cell where the granules most approximate to the plasma membrane are called the readily releasable pool (RRP), also constituting an immediately releasable pool (IRP), which are primed to the plasma membrane so that glucagon release may occur. The larger portion of the granules is contained in a non-releasable reserve pool that needs to be mobilized and primed prior to release, and is responsible for the refilling of the RRP. Single A-cells were stimulated by a train of ten 500-ms depolarisations from -70 mV to 0 mV. The total increase in membrane capacitance evoked by a train was 691 ± 67 fF (n=17) under control conditions. This response was reduced to 304 ± 112 fF (n=8; p<0.01) when anti-SNAP-25 was included in the pipette solution. Close inspection of the responses revealed that exocytosis during the first two depolarisations, correlating to RRP, was significantly decreased from 322 ± 32fF to 160 ± 41 fF (p<0.01). The release of mobilized granules as defined by the exocytotic response evoked by pulses 2-10, was also significantly decreased from 371 ± 54 fF to 145 ± 74 fF (p<0.05). It was controlled using IgG that antibodies do not affect exocytosis through steric hindrance. A similar setup showed a reduction in total membrane capacitance from 1615 ± 123 fF (n =18) in control to 798 ± 176 fF following immunoneutralization of Syntaxin 1 (n=18; p<0.001). Likewise, the release of the RRP was significantly reduced by 40% (p<0.001) and the mobilized granules were reduced by 47% (p<0.001). In addition, pulse length- measurements were performed to create an image of the rapid exocytosis occurring during the fast exocytosis and to get an estimate of IRP. In the presence of the Syntaxin 1 antibody (n= 13), there was a 50% (p<0.001) reduction of the exocytosis evoked by a 50-ms depolarisation as compared to the control (n=17), and a 45% (p<0.05) reduction in the plateau reached at 250 ms corresponding to a decrease in IRP.
Conclusion: Investigating the effect of SNARE-proteins on the processes involved in the exocytotic machinery of glucagon secreting A-cells revealed that SNAP-25 and Syntaxin 1 are important for both rapid exocytosis and refilling of IRP/RRP by granule mobilization. Thus, we suggest that SNAP-25 and Syntaxin 1 are necessary for glucagon exocytosis in A-cells.

443

Evidence for PKA-independent priming of insulin secretory granules: insight from mathematical modeling
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Background and aims: Incretins, such as GLP-1 and glucagon, raise the concentration of cAMP in beta-cells. cAMP acts through both Epac-dependent and PKA-dependent pathways and is believed to be an important player in the maturation of secretory granules leading to calcium triggered exocytosis and insulin release. The aim of this study was to investigate the interplay between cAMP and calcium in the final steps preceding membrane fusion by estimating the size of the immediately releasable pool, calcium sensitivity of exocytosis, maximal rate of exocytosis and the rate of priming from capacitance data from single beta-cells under various conditions using a biophysical model.
Materials and methods: We developed a mathematical model of calcium induced exocytosis of insulin secretory granules, and applied it to experimentally obtained whole cell patch clamp capacitance data from single beta-cells. The model includes the immediately releasable pool, priming of granules, measured calcium currents and exocytosis. The simulations were done by solving numerically the ordinary differential equations of the model, and parameter estimation was done using Levenberg-Marquardt nonlinear regression, as implemented in the software Octave.
Results: The measured calcium currents were used as input to the mathematical model, and the predicted capacitance increases were fitted to the experimental data. To obtain insight in the role of cAMP for priming and fusion of secretory granules, we analyzed data from groups of cells stimulated with various concentrations of cAMP (0-500 μM) in the patch pipette, as well as from cells where PKA was inhibited by including Rp-cAMPS (0.5 μM) in addition to cAMP. The outcome of the model demonstrated that the differences between groups could be explained by varying just three parameters: Addition of 100 μM cAMP increased the priming rate 12-fold with respect to the control group (from 1.26 (+/- estimated standard deviation=0.05) fF/s to 15.2 (+/-1.9) fF/s). This rate was not changed by further addition of Rp-cAMPS (14.7 (+/-0.6) fF/s). The increased priming rate is thus PKA-independent, likely mediated by Epac2/cAMP-GEFII. Since the size of the immediately releasable pool is assumed to be proportional to the priming rate, we also estimated a 12-fold PKA-independent increase in pool size. The EC50 value of exocytosis with respect to calcium was slightly lowered by addition of 100 μM cAMP (to 12.6 (+/-0.8) from 14.7(+/-0.7) pA) but increased to 30.1(+/-0.7) by further addition of Rp-cAMPS. 100 μM cAMP + Rp-cAMPS did not change the maximal exocytosis rate (2.5 (+/-0.1) pr. sec in both cases), but was only 0.8(+/-0.1) pr. sec with 100 μM cAMP alone. In spite of the lower exocytosis rate, the group with 100 μM cAMP showed the largest total capacitance increase. Inspection revealed that this was due to a PKA-dependent increase in calcium currents in this group.
Conclusion: Our results differ from estimates using simpler analytical methods on the same data set and demonstrates that it is important to include calcium currents directly in the analysis of capacitance data. This is best done by mathematical modeling due to the nonlinear dependence of the exocytosis rate on the calcium concentration. The model strengthens the suggestion that priming of insulin secretory granules is largely mediated by Epac2/cAMP-GEFII. The PKA-dependent modified fusion properties might reflect the shift to a highly calcium sensitive state of the granules.
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444

Membrane depolarization: a fail-safe parameter to assess the triggering pathway of insulin secretion?
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Background and aims: A classical experimental manoeuver to elicit insulin exocytosis is to subject pancreatic B-cells to depolarization by increasing the external K^+ concentration or by current injection. Typically, the extent of depolarization is made equal or even larger than the peak values of the action potentials resulting from K_{ATP} channel closure by nutrient and non-nutrient secretagogues. We tested whether this procedure is appropriate to gain insight into the physiological regulation of insulin secretion.
Materials and methods: NMRI mouse islets and beta cells were used to measure insulin secretion by perifusion and ELISA, membrane potential by patch clamping (perforated patch mode).
Results: When the blocker of voltage-dependent K^+ channels, TEA (10 mM), was added to a maximally effective concentration of the K_{ATP} channel blocker glipizide (2.7 μM) in the absence of glucose, an increase in action potential amplitude by 11.7 ± 4.1 mV was noted. Addition of KIC (10 mM) to glipizide led to a transient repolarization of the B-cell, thereafter an elevated plateau depolarization was observed. Again, the additional presence of TEA led to a significant increase in action potential amplitude (8.1 ± 1.4 mV). When

secretion of perifused islets was measured using the same experimental protocol, the presence of TEA did not increase secretion rather the secretory effect of glipizide in absence of glucose was reduced by 46.8%. When the fuel secretagogue KIC (10 mM) was added to the perifusion in both cases (presence or absence of TEA) a fast increase in secretion was elicited. When the data were normalized to the last value prior to addition of KIC, the increase in the absence of TEA was not significantly different and from that in the presence of TEA. When the blocker of K_{ATP} channels, tolbutamide (50 μM) was added to high KCl (15 mM), the moderate membrane depolarization (20.7 mV) established by KCl was further enhanced and action potentials ocurred. The difference in plateau depolarization was 5.5 ± 1.8 mV. Here, the enhanced depolarization had a corresponding effect on secretion in that the amount of insulin secreted during 30 min was increased 2.5fold.
Conclusion: The extent of depolarization of the B-cell membrane per se does not define the strength of a secretory stimulus. Rather, the underlying mechanisms have to be taken into account. Action potential amplitude is largely determined by membrane resistance and an increased amplitude does not necessarily transduce into a proportionate increase of secretion. Depolarization methods circumventing K_{ATP} channel closure may represent unphysiologically strong stimuli when depolarization levels equal to action potential peaks are attained.

445

Involvement of protein kinase A and Epac in initiation and maintenance of glucose-stimulated pulsatile release of insulin
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Background and aims: Glucose stimulation of β-cells results in pulsatile release of insulin. This process is controlled by synchronized oscillations of the Ca^{2+} and cAMP concentrations beneath the plasma membrane ($[Ca^{2+}]_{pm}$ and $[cAMP]_{pm}$). The effects and mediators of glucose-induced cAMP signals in β-cells are poorly characterized, and the aim of the present study was to clarify the contribution of protein kinase A (PKA) and the cAMP-regulated guanine nucleotide exchange factor Epac to pulsatile insulin secretion from glucose-stimulated MIN6 β-cells.
Materials and methods: Evanescent wave fluorescence imaging was used to record $[cAMP]_{pm}$ or $[Ca^{2+}]_{pm}$ in parallel with phosphatidylinositol-3,4,5-trisphosphate (PIP_3). This membrane lipid is formed by autocrine insulin receptor activation and was used as readout for insulin secretion from single MIN6 β-cells.
Results: Elevation of the glucose concentration from 3 to 11 mM induced oscillations of $[Ca^{2+}]_{pm}$ and $[cAMP]_{pm}$. The second messenger signals were tightly associated with PIP_3 oscillations reflecting pulsatile release of insulin. After inhibition of PKA with 100 μM Rp-8-CPT-cAMPS or 2 μM KT5720 the PIP_3 response to a subsequent glucose challenge was reduced by 30±7% (n=37 cells, P<0.05) and 59±10% (n=19, P<0.01), respectively, without corresponding changes in $[Ca^{2+}]_{pm}$. In contrast, already manifested PIP_3 oscillations in glucose-stimulated cells were little affected by PKA inhibition. cAMP was nevertheless required for the glucose-induced PIP_3 oscillations, and inhibition of adenylyl cyclases with 100 μM 2',5'-dideoxyadenosine or 400 μM SQ22,536 (SQ) reduced the time-average PIP_3 response by 66±5% (n=54, P<0.001) and 42±3% (n=101, P<0.001), respectively. This inhibition was partially reversed by 1 μM of the Epac-selective nucleotide analogue 8-pCPT-2'-O-Me-cAMP-AM restoring the PIP_3 response in SQ-treated cells to 80±5% (n=78, P<0.001 vs SQ) of that obtained with 11 mM glucose alone.
Conclusion: cAMP contributes significantly to pulsatile insulin release from glucose-stimulated β-cells. While PKA is required for appropriate initiation of the secretory response, the cAMP-dependence of maintained pulsatility is mediated by Epac.

446

Secretagogue-induced elevation of the plasma membrane PtdIns4*P* concentration in individual insulin-secreting cells
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Background and aims: The phospholipid phosphatidylinositol 4-phosphate (PtdIns4*P*) is a critical regulator of a variety of cellular processes, including membrane and vesicle trafficking. Generation of PtdIns4*P* by phosphatidylinositol 4-kinases has been implicated in the priming of secretory granules in pancreatic β-cells. However, it is unknown whether stimulators of insulin secretion regulate plasma membrane PtdIns4*P*. The aim of this study was to clarify how glucose and the phospholipase C-activating agonist carbachol affect plasma membrane PtdIns4*P* concentration, $[PtdIns4P]_{PM}$, by real-time monitoring of the lipid in individual insulin-secreting cells.
Materials and methods: The PtdIns4*P*-binding pleckstrin homology domain from the oxysterol binding protein fused to cyan fluorescent protein (OSBP-CFP) was used as a biosensor for PtdIns4*P* in individual MIN6 β-cells. The subcellular distribution and plasma membrane localization of the biosensor were monitored with confocal and evanescent wave microscopy, respectively. Response amplitudes were expressed as the ratio of OSBP-CFP over membrane bound yellow fluorescent protein signal.
Results: Confocal imaging of MIN6 β-cells expressing OSBP-CFP showed distinct localization of the biosensor at intracellular membranes, whereas the plasma membrane fluorescence was only slightly brighter than the diffuse fluorescence from unbound biosensor in the cytoplasm. However, background reduction by release of free cytoplasmic OSBP-CFP after membrane permeabilization with digitonin revealed a clear plasma membrane localization of the PtdIns4*P* biosensor (n=13). This membrane localization was specific, since a PtdIns4*P* binding-deficient biosensor mutant was localized exclusively in the cytoplasm (n=9). Evanescent wave microscopy recording of plasma membrane OSBP-CFP fluorescence showed that elevation of the glucose concentration from 3 to11 mM induced a prompt increase of $[PtdIns4P]_{PM}$ (22.2 ± 0.1% increase of the fluorescence ratio; n=56), which was transient, sustained or oscillatory in 39%, 35% and 26% of the cells, respectively. The glucose-induced elevation of $[PtdIns4P]_{PM}$ was prevented by inhibition of type III PI4-kinases with LY-294002, by removal of extracellular Ca^{2+} (n=24), hyperpolarization with 250 μM diazoxide (n=21) or blockage of L-type voltage-gated Ca^{2+} channels with 50 μM methoxyverapamil (n=41). Elevation of the cytoplasmic Ca^{2+} concentration by depolarization with 30 mM K^+ (n=9) or 1 mM tolbutamide (n=7) triggered a similar rise in $[PtdIns4P]_{PM}$ as glucose. Activation of phospholipase C with 100 μM of the muscarinic receptor agonist carbachol induced sustained elevation of $[PtdIns4P]_{PM}$ (12.5 ± 0.7% increase of the fluorescence ratio; n=52). In contrast to the glucose-induced elevation of $[PtdIns4P]_{PM}$, the carbachol response persisted even after removal of extracellular Ca^{2+} and depletion of intracellular Ca^{2+} stores.
Conclusion: Glucose and carbachol stimulation of insulin-secreting cells is associated with elevation of the plasma membrane PtdIns4*P* concentration. Whereas the glucose effect is mediated by Ca^{2+}, carbachol operates via a Ca^{2+}-independent mechanism. The demonstration of stimulus-induced changes of the plasma membrane PtdIns4*P* pool reinforces the importance of this lipid in signaling underlying insulin secretion.

447

Glucose models cAMP-linked signalling pathways in pancreatic beta cells
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Background and aims: Glucose regulates gene expression in pancreatic β-cells and prolonged exposure to elevated levels induces considerable changes in acute effects of the hexose. In this study, we are investigating the long-term effects of glucose on cell signalling pathways namely GPCR- and cAMP ; these effects are determined using the physiologically relevant hormone glucagon-like peptide 1 (GLP-1) as well as forskolin (FSK), a general activator of adenylyl cyclases (ADCYs) and IBMX, a general inhibitor of phosphodiesterases.
Materials and methods: INS-1E cells or pancreatic islets were cultured for 24 to 72h at 5.5, 11 or 20 mM glucose (G5.5, G11, G20). Intracellular calcium concentration ($[Ca^{2+}]_i$) was determined in single cells using microspectrofluorimetry and Indo-1 as fluorescent probe, cAMP and insulin were measured by commercial assays. Microarray analysis was performed on Affymetrix arrays (REA 230 2.0) and analyzed by ARRAY-Assist or INGENUITY. Exocytosis was determined by capacitance measurements.
Results: GLP-1- or FSK-induced $[Ca^{2+}]_i$ increases were abolished or largely reduced by culture of INS-1E cells for 72h at G11 or G20 as compared to G5.5. Similarly, increases in cAMP levels by FSK/IBMX were reduced at G20

and amplification of glucose effects on insulin secretion by GLP-1 or FSK were lost. Microarray analysis revealed profound changes at G11 and at G20 within several canonical pathways and ranked pyruvate metabolism, glycolysis, cAMP- and GPCR-mediated signalling as the most significantly affected ($p<0.005$, Fischer's exact test). The latter two showed a loss in GLP-1 receptor already at G11 and a major reduction in the calcium-sensitive adenylyl cyclase 8 (ADCY8) at G11 and at G20. Other ADCYs including ADCY10 or cAMP targets were not altered. Expression profiles were validated by quantitative PCR as well as immunoblots and similar changes were observed in rat or human islets. Interestingly, transient re-expression of ADCY8, but not of GLP-1 receptor, restored GLP-1-induced calcium signalling in cells cultured at G20. In line with these observations, knock-down of ADCY8 in cells cultured at G5.5 abolished GLP-1-induced changes in $[Ca^{2+}]_i$ and reduced by two-thirds the glucose response. In contrast, thapsigargin- or KCl-induced changes in $[Ca^{2+}]_i$ remained unchanged. Moreover, this knock-down abolished amplification of calcium-induced exocytosis by FSK/IBMX.
Conclusion: The expression of key proteins involved in GPCR- and cAMP-linked signalling, such as ADCY8 and GLP1R, is controlled by glucose. These findings strongly suggest a central role for ADCY8 in glucotoxicity.
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PS 22 Beta cell metabolism

448

Protein GAS6 and its receptor Axl are expressed and operate in pancreatic beta cells
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Background and aims: Growth arrest-specific protein 6 (GAS6) belongs to the family of plasma vitamin K-dependent proteins. It shows homology (~40%) to the plasma anticoagulant protein S, another gamma-carboxylated protein. GAS6 exhibits growth factor-like effects, as it interacts with a receptor tyrosine kinase: Axl. GAS6 and the Axl have further been implicated in inflammation and immune responses, hemostasis, and cancer. Here, we studied whether this system occurs in pancreatic islets.
Materials and methods: TaqMan qRT-PCR was used to quantify mRNA levels. GAS6 and Axl were detected by immunoblotting, immunocytochemistry and ELISA in pancreatic beta-cells. Insulin secretion was determined by RIA.
Results: Quantitative RT-PCR analysis demonstrated a low level of Axl mRNA expression in INS-1 832/13 clonal beta-cells. In contrast, expression of GAS6 mRNA was abundant in INS-1 832/13 cells. Immunoblotting of protein extracted from INS-1 832/13 cells with an antibody to GAS6 showed an immunoreactive band of the expected molecular weight (75 kD). Furthermore, immunoblotting with an antibody to Axl detected expression of full length Axl receptor. Immunocytochemical analysis revealed that GAS6 and Axl are expressed in mouse and rat islets. Secretion of GAS6 from INS-1 832/13 cells was analysed in conditioned RPMI-1640 medium containing 11.1 mM glucose in which INS-1 832/13 cells had been cultured for 24 h, using a highly sensitive ELISA. The level of GAS6 protein was 100 pg/ml in the medium (corrected for concentration of medium); this was several-fold higher than that in medium alone, ensuring that it is not a constituent of serum. Next, we observed that glucose affects Axl receptor expression in INS-1 832/13 cells: Axl mRNA was upregulated two-fold upon 48 h culture of INS-1 832/13 cells in 16.7 mM glucose compared to cells cultured with 2.8 mM glucose. Axl mRNA levels were reduced by 92-98% using three different siRNAs to Axl. Knockdown of Axl resulted in a significant decrease in glucose-stimulated insulin secretion, whereas basal insulin secretion did not change. GAS6 induces Axl-mediated PI3-kinase/Akt signalling in INS-1 832/13 beta-cells. Immunoblotting with an antibody to Axl[pY779] and Akt/PKB[pS473] detected phosphorylation of Axl and Akt/PKB induced by 400 ng/ml GAS6. Axl-phosphorylation was blocked by preincubation GAS6 with soluble Axl-receptor or GAS6 antibody. Akt-phosphorylation was inhibited by 100 nM wortmannin (inhibitor of PI3-kinase).
Conclusion: By using different methods we identified protein GAS6 and its receptor Axl in clonal beta-cells and pancreatic islets. For the first time we showed that GAS6/Axl system operates in pancreatic beta-cells: 1) Axl knockdown resulted in impairment in glucose-stimulated insulin secretion in beta-cells; 2) GAS6 activates Axl/PI3-kinase/Akt signalling in INS-1 832/13 beta-cells. Studying the GAS6/Axl system opens a new avenue for research in pancreatic islet cell biology.
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449

Role of mitochondrial fission and fusion in beta cell stimulus secretion coupling
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Background and aims: In pancreatic beta cells glucose-induced insulin secretion is mediated by the glucose sensor enzyme glucokinase (GK) and accompanied by mitochondrial membrane potential (MMP) hyperpolarization. Previous studies show, that mitochondrial fission followed by selective fusion segregates dysfunctional mitochondria and permits their removal by autophagy. Thus, this machinery is vital to maintain mitochondrial morphol-

ogy and ATP generation. The aim of the study was to determine fusion and fission events in beta cells with respect to the glucose metabolism and MMP.
Materials and methods: Insulin-producing RINm5F-R-GK, expressing GK in response to the synthetic inducer RSL1 and primary rat beta cells were incubated at different glucose concentrations. The MMP was measured using the potentiometric dye TMRE in combination with MitoTracker Green. The change of MMP induced by 10 mmol/l glucose was analyzed over 60 min by fluorescence microscopy. The slope of the TMRE/MitoTracker Green ratio change of individual cells and mitochondria was calculated. Fission and fusion events were determined by the use of the green to red photoconvertible fluorescent protein Dendra2 fused to cytochrome-c-oxidase (Dendra2-Mito). Photoconversion of a single mitochondrium was performed with a confocal microscope equipped with a second scan unit. Mitochondrial dynamics were analyzed with Imaris software.
Results: RINm5F-R-GK cells were pre-cultured for 48 h at 10 mmol/l glucose without or in the presence of 500 nmol/l of the inducer RSL 1. Thereafter the increase in the MMP after 90 min starvation in response to 10 mmol/l glucose was significantly faster in cells with GK expression. Interestingly, RINm5F-R-GK cells pre-cultured at 30 mmol/l glucose for 48 h showed also a faster increase in MMP in comparison to cells pre-cultured at 10 mmol/l. This increase was further amplified by GK expression during pre-culture at 30 mmol/l glucose. Likewise, in primary rat beta cells pre-culture for 48 h at 30 mmol/l glucose resulted in a significantly faster increase in the MMP in comparison to cells pre-cultured at 5 mmol/l. Furthermore pre-culture at 30 mmol/l glucose resulted in a higher cellular MMP homogeneity. Visualized mitochondrial morphology by Dendra2-Mito in RINm5F-R-GK cells was comparable to that observed with MitoTracker Green and thus, feasible to describe mitochondrial dynamics. Mitochondrial fusion and fission was not affected by GK expression, but occurs more frequently in cells pre-cultured at 30 mmol/l glucose.
Conclusion: GK expression temporally activates MMP hyperpolarization after glucose stimulation, but is not involved in the regulation of the mitochondrial morphology and maintenance of the functional network. Mitochondrial adaptation to high glucose exposure acts in beta cells of healthy rats. Analysis of mitochondrial fusion and fission with Dendra2-Mito will be a useful tool to analyze mitochondrial dysfunction in type 2 diabetes.

450

Isocitrate dehydrogenase 1 knockdown in INS 832/13 cells by siRNA increases glucose-induced insulin secretion
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In pancreatic β-cells, metabolic coupling factors produced by glucose metabolism regulate insulin secretion. Recent studies have suggested that NADPH could act as a metabolic coupling factor since NADPH concentration correlates with glucose-induced insulin secretion (GIIS). In β-cell, very low amount of glucose is metabolised via the pentose phosphate pathway, such that cytosolic NADPH level is thought to be controlled primarily by the activity of the cytosolic isoform of malic enzyme (ME1) and of isocitrate dehydrogenase (IDH1). In a previous study, we have shown that ME1 expression is important for appropriate insulin secretion in response to glucose. To investigate the role of IDH1 in GIIS, we transfected INS 832/13 cells with siRNA directed against IDH1. IDH1 expression at mRNA level was reduced by 70 % using siRNA, correlating with more than 35% decrease in IDHc activity. Surprisingly, siRNA knockdown (KD) of IDH1 expression led to a 58% increase in insulin secretion at intermediate (5 mM) glucose and of 46% at high (10 mM) glucose. This effect on GIIS was mediated via the K_{ATP}-independent/amplification pathway. Basal and KCl-induced secretion were not affected by IDH1 KD. IDH1 KD cells showed an elevation of glucose incorporation into fatty acids. Taking together, the results suggest that metabolic flux through IDH1 may not be critical for GIIS as previously thought, and that a decrease in the metabolic flux through IDH1 is compensated by an elevation of pyruvate/citrate cycling possibly linked to enhance production of metabolic coupling factors such as NADPH and malonyl-CoA. Current experiments aim at assessing the metabolic consequence of IDH1 KD.
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451

Q268X mutation of HNF4α/MODY1 gene affects the mitochondrial function in cultured living beta cells; evaluation by a new original system
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Background and aims: Mitochondrial pH is known to reflect its functional viability. However, there has been no useful system to measure quantitative and serial mitochondrial pH to date. Recently, a pH of organum has been successfully calculated by using fluorescence GFP, but still it is not a direct quantitative calculation. Here, we developed a new system to calculate mitochondrial pH of living cell with fluorescence. Since mitochondrial function of the β−cell is a key step for insulin secretion, we aimed to measure a mitochondrial pH of the β-cell carrying the mutation of the hepatocyte nuclear factor (HNF)-4α/ MODY1 gene characterized by impaired insulin secretion, using this unique system.
Materials and methods: A pH calculation probe consists of two probes, a pH dependent fluorescence Venus of which pKa ranged 7.2-8.0, and a pH independent fluorescence mutational GFP. Mitochondrial pH calculated probe made by cytochrome-C subunit IV gene was fused with the pH measurement probe, then transfected stably in cultured β−cells, INS-1 and MIN6 cells, by lipofectum method. Insulin stimulating substance: glucose, tolbutamide, arginine, and potassium are applied to these cells, and calculate mitochondrial pH. Since Q268X mutation of the HNF4α gene is a causative mutation in human diabetes of autosomal dominant inheritance, we have introduced this mutational gene fused with JRED. These mutational genes were transfected to the mitochondrial pH measurement β−cells. Mitochondrial pH was calculated serially and quantitatively under the stimulation of insulin secretion using these cells.
Results: In the wild cultured β−cells, the mitochondrial pH decreased quickly by glucose and potassium, and leisurely decreased by arginine and tolbutamide. A basal level of the mitochondrial pH of β−cells increased by aeration in a cultured medium. Interestingly, in the β−cells with Q268X mutation of HNF4α gene, a response to glucose was clearly disappeared, but a response to arginine was still shown.
Conclusion: Mitochondrial pH is decreased by insulinotropic stimulation in cultured β−cells. We have observed that Q268X mutation of the HNF4α affected mitochondrial function in the living β−cells. This new system worked well and is thought to be a useful tool for evaluation of the mitochondrial function in the living cells.

452

Mitochondrial matrix alkalinisation serves as an essential signal for nutrient-induced second phase insulin secretion
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Background and aims: The mitochondrial matrix pH is more alkaline than the close to neutral cytosolic pH. We recently demonstrated that in the pancreatic beta-cell matrix pH under resting conditions is very low but increased substantially during nutrient stimulation. Furthermore we found matrix alkalinisation to be essential for nutrient-dependent mitochondrial ATP synthesis in alpha toxin permeabilised INS-1E cells. Here we have tested whether mitochondrial matrix alkalinisation serves as a mitochondrial activation signal necessary for metabolism-secretion coupling.
Materials and methods: INS-1E cells or intact rat islets were infected with Adenoviruses expressing reporter proteins to measure mitochondrial pH (mitoAlpHi), ATP (cytosolic Luciferase), mitochondrial calcium (mitochondrial Aequorin) or cytosolic calcium (cytosolic YC3.6). The different parameters were measured using either single cell fluorescence imaging or by following luminescence changes. Nigericin was utilized to clamp nutrient-dependent matrix alkalinisation. This ionophore can be used to equilibrate proton gradients across biological membranes with only little impact on the much larger potassium gradients.
Results: Nigericin (500nM) attenuated glucose-induced mitochondrial matrix alkalinisation, whereas cytosolic pH remained constant close to 7.1. Preventing mitochondrial alkalinisation and the associated increase in ΔpH lowered glucose-stimulated insulin secretion by more than 70%, whereas basal release was unchanged. In rat islet perifusion experiments second phase

insulin secretion was almost abolished by nigericin. Insulin secretion per se was not affected as insulin exocytosis to PKC activation (independent of mitochondrial function) was normal in the presence of the ionophore. Nigercin also strongly reduced the glucose-mediated net increase of cytosolic ATP and the amplitude and frequency of cytosolic calcium transients consistent with the observed pronounced secretion defect. In contrast, the cytosolic calcium response to tolbutamide was unaffected by nigericin, demonstrating that nigericin did not clamp the plasma membrane potential. We find however that tolbutamide may in part act as a secretagogue via mitochondria as cytosolic ATP levels were increased by 12% in response to tolbutamide at basal glucose concentration. Nigericin prevented this increase and consequently lowered insulin secretion to tolbutamide despite a normal cytosolic calcium response.

Conclusion: Attenuation of mitochondrial alkalinisation not only inhibits mitochondrial ATP synthesis preventing closure of KATP channels, but may also affect other energy-dependent aspects of insulin exocytosis. Our results demonstrate that matrix alkalinisation is an essential signal in metabolism-secretion coupling.

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453

Hyperthyroidism decreases islet pyruvate dehydrogenase kinase 1 (PDHK1) expression and attenuates islet starvation adaptations

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Background and aims: Glucose metabolism in the pancreatic β-cell controls glucose-stimulated insulin secretion (GSIS), the routes by which carbons enter and exit the TCA cycle being critical. Two major regulatory fates are reactions catalyzed by pyruvate carboxylase (PC) and pyruvate dehydrogenase (PDH), respectively. PC controls the major anaplerotic pathway in β-cells, and its inhibition results in impaired GSIS. Inhibitory phosphorylation of PDC by the PDH kinases (PDHKs) conserves pyruvate for PC-mediated entry into the TCA cycle during starvation. Here, we aimed to determine whether reports of increased glucose oxidation by pancreatic islets in the hyperthyroid state reflected altered islet PDK expression.

Materials and methods: Insulin secretion, PDHK expression and GSIS were examined in rat islets after 72 h of *in vivo* treatment with tri-iodothyronine.

Results: Islet PDHK1 protein expression was reduced by 61% ($P<0.001$), and PDHK2 protein expression was decreased by 27% ($P<0.001$), as assessed by Western Blot. At basal glucose (2 mM), insulin secretion by islets was unaffected by hyperthyroidism. The GSIS response to hyperthyroidism differed depending on dietary status. Insulin secretion was decreased (by up to 58%) in the fed state, but the insulin secretory response to starvation (associated with enhanced expression of PDHK4) was attenuated.

Conclusion: We suggest that augmented PDH activity in response to hyperthyroidism in the fed state lowers GSIS via suppression of PC flux and pyruvate cycling; however, through maintenance of low PDHK1 and PDHK2 expression, keeping PDH in a relatively dephosphorylated and active state, hyperthyroidism opposes the response of GSIS of pancreatic β-cells to fasting.

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454

Differences in metabolic regulation between glucose responsive INS-1 832/13 and its glucose non-responsive INS-1 832/2 sister clonal rat beta cell line decides their beta cell functionality

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Background and aims: The biochemical mechanisms underlying glucose-stimulated insulin secretion from pancreatic beta-cells are not completely understood. The main coupling signal is a rise in ATP:ADP ratio, mainly accounted for by the mitochondrion. This closes ATP-dependent K+ channels, depolarizating the plasma membrane. Voltage-gated Ca2+ channels opens and allow Ca2+ ions to flow into the cell triggering insulin vesicles to merge with the plasma membrane. In order to investigate the putative metabolic disturbances in β-cells that affects glucose-stimulated insulin secretion we investigated two subclones of INS-1 exhibiting different properties: 832/13 cells are glucose-responsive while the 832/2 cells are glucose-nonresponsive. The insulin content of the cells is the same and ATP-independent closing of K+ channels evokes insulin release in both cell types. This leads us to hypothesize that the difference in insulin response could have a metabolic origin.

Materials and methods: Insulin secretion for one hour in static incubation was measured by RIA. Differences in glucose metabolism were assessed by Extracellular flux analyzer XF24 (Seahorse Bioscience, Billerica, MA). Lactate in the cells incubated at 2.8 and 16.7 mM glucose was measured using a colorimetric lactate assay kit (Biovision, Mountain view, CA, USA). ATP production was measured by using an ATP monitoring reagent based on firefly luciferase (BioThema AB, Handen, Sweden)

Results: The INS-1 832/13 were robust secretors of insulin (>8-fold) when stimulated by an elevation in glucose from 2.8 mM (low glucose) to 16.7 mM (high glucose) whereas the same settings failed to release insulin in INS-1 832/2. The Oxygen Consumption Rate (OCR) is basically a measurement of the respiratory rate in the mitochondrion. INS-1 832/13 cells showed a considerable increase in OCR when stimulated with high glucose; this effect was abundant in INS-1 832/2. They also displayed a poorer response to the metabolic modulators oligomycin, FCCP and Rotenone. The ATP production rate of the INS-1 832/13 was more than twice that of the INS-1 832/2 in a 14 mM glutamate/succinate buffer. INS-1 832/2 cells secreted 140 nmol/mg prot/h of lactate at high glucose while lactate secretion from the INS-1 832/13 was not detected. Extracellular acidification rates (ECAR) can be attributed to lactate and/or CO2 production. The ECAR of 832/13 cells basically paralleled the OCR, suggesting that it is mainly accounted for by CO2 production. In the 832/2 cells, the ECAR was 3.6 fold higher, and increased in response to oligomycin and FCCP. This suggests that increased glycolytic rate compensates for the inhibition of ATP production caused by these drugs.

Conclusion: We conclude that tight coupling between the glucose stimulus, via glycolysis, and the rate of oxygen consumption determines ATP production. This aspect of mitochondrial metabolism is crucial for the ability of the β-cell to respond to high glucose with insulin secretion. The INS-1 832/2 cells which express LDH have regained their ability to speed up glycolysis to reflect energy demands and substrate levels. This gives them an advantage as it makes them less vulnerable but occurs at the expense of β-cell functionality, such as their ability to secrete insulin in response to glucose.

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455

Complement factors are expressed in pancreatic islets and affect glucose induced insulin secretion

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Background and aims: Complement factors form an important part of the humoral innate immune system and have been implicated in the pathogenesis of autoimmune diseases, including type 1-diabetes. There is however accumulating evidence that the complement system may also be an important player in the development of type 2-diabetes. First, the complement system modulating peptide adrenomedullin is increased in patients with type 2-diabetes. This peptide, together with its binding partner the complement inhibitor factor H, has been reported to affect insulin secretion. Furthermore, patients with type 2-diabetes exhibit increased neutrophil levels of complement factor 5a. C5a is produced as a result of complement activation and exerts its effect by binding to its receptor C5aR. In this study we have addressed the expression of the complement system in human pancreatic islets and the effects of adrenomedullin, Factor H and C5a on islet function.

Materials and methods: Expression in human islets: Total RNA was isolated from pancreatic islets islets from 17 non-diabetic donors (6 female, 11 male aged 26-73, BMI 17.7-29 kg/m2) using the AllPrep DNA/RNA Mini Kit (Qiagen) and analyzed using Gene 1.0 ST whole transcript based assays (Affymetrix).

Expression in rat islets and INS1 832/13 cells: Isolated rat pancreatic islets and cDNA from liver were used to study the complement gene expression by Q-RT-PCR technique with specific primers. Secretion assay: The role of C5a, factor H and adrenomedullin on insulin secretion was identified by insulin secretion assay in the presence of 2.8mM and 16.7mM glucose in Ins1 823/13 cells.

Results: The microarray data confirmed the presence of all the complement genes in human pancreatic islets. Complement genes C2, C3, C5aR, factor H and factor I were selected for validation in rat. The rat-derived clonal Ins1 832/13 cells expressed low levels of the transcripts for the selected complement genes. Rat pancreatic islets exhibited higher transcript levels. Of the selected transcripts expression was highest for factor H and complement component 2 (C2) relative HPRT. The mRNA relative HPRT expression values of C2, factor H, C3, C5aR, and factor I were 0.047, 0.043, 0.018, 0.014, 0.0026 respectively, with 0.00336, 0.00127, 0.00045, 0.00107, 0.00018 respective standard error means. Liver is considered as a rich source for the complement factors and we therefore compared expression of complement in islet with liver. Interestingly, it was observed that the expression of C5a mRNA was higher in islets than in liver. Next, we evaluated effects of factor H, adrenomedullin and C5a on glucose evoked insulin secretion in Ins1 832/13 cells. Adrenomedullin and factor H both stimulated glucose-induced insulin secretion by 75% and 52%, respectively with P=0.003 and 0.003, respectively. Their simultaneous presence did not stimulate secretion any further. Interestingly, C5a also had a stimulatory action in the absence of the complement factor glucose stimulated insulin secretion 2.6-fold, whereas in the presence of C5a a 5.6-fold stimulation was observed.
Conclusion: Most, if not all, complement factors are expressed in human and rat pancreas. Adrenomedullin, factor H and C5a all appear to stimulate insulin secretion. Signalling via the C5a receptor (C5aR) is a particularly interesting signalling pathway that may be involved in type 2-diabetes.
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PS 23 Stem cells and beta cell development

456

Non beta cell progenitors of beta cells in pregnant mice
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Objective: Pregnancy is a physiological condition associated with a near doubling in maternal beta cell mass, in a relatively short period of time. In this state, replication of pre-existing beta cells is often presented as the main mechanism responsible for this expansion. The aim of this study was to critically examine whether a non-beta cell source might be also involved in the increase in beta cell mass.
Research design and methods: We used a double transgenic mouse (RIP-CreER^{TAM}/ZAP) of Dor and Melton. In this lineage tracing system, the cre-recombinase is expressed only in beta cells and is activated only upon tamoxifen exposure, leading to a conditional deletion of the translational stop sequence, flanked by two *loxP* sites, upstream of the human placental alkaline phosphatase (HPAP) region in the Z/AP transgene. As a result, the beta cells are irreversibly and heritably labelled with the HPAP reporter, in response to a pulse of tamoxifen before pregnancy.
Results: Following the tamoxifen-pulse, first we conclude that the lineage tracing system was highly specific for β-cells. Secondly, we compared the proportion of the β-cells as HPAP positive during a subsequent chase period in pregnant and non-pregnant females. We observed a dilution in this labelling index in pregnant animal pancreata (0.33±0.06), compared to non-pregnant controls (0.44±0.05). This difference was statistically significant (P =0.021, paired two-tailed t-test). We counted the numbers of HPAP-positive or HPAP-negative insulin-positive cells in the duct epithelium and in several islets in the same sections, to give the labelling index for islets and ductal cells. The labelling indices of insulin-positive ductal cells (0.25, n=44) and islets (0.31, n=5459) from pregnant animals were not significantly dissimilar (2x2 contingency table, Fisher's exact test, P= 0.455). Consequently, we concluded that β-cells associated with the pancreatic ducts were not a preferential source of new β-cells. Finally, there was no transdifferention of β-cells to other cell types in a two and half month period following labelling, including the period of pregnancy.
Conclusion: For the first time in a normal physiological condition we demonstrate that pregnancy is associated not only with β-cell duplication, but importantly also with the activation of a non- β-cell progenitor population.
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457

Modulation of islet cell mass by fetal low-calorie diet and glucocorticoid exposure
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Background and aims: Fetal adverse environment, such as insufficient maternal nutrition, placental insufficiency and stress, alters organ development and leads to poor fetal growth, also called intrauterine growth retardation (IUGR). IUGR is associated with an increased risk of perinatal mortality and morbidity as well as late-onset metabolic diseases, such as obesity, diabetes and hypertension in adulthood. In the rodent model, IUGR is induced by fetal caloric restriction, by isocaloric protein restriction, by restricted placental blood supply or by exposure to high levels of glucocorticoids. Such experimental IUGR models show a decreased beta cell mass and glucose intolerance with aging. To date, the mechanisms responsible for the loss of beta cells are still poorly understood. The aims of the present study were: (1) to examine the effects of intrauterine caloric restriction and dexamethasone exposure on pancreatic islet gene expression in rats; and (2) to characterize changes in gene expression during postnatal catch-up growth.
Materials and methods: To induce IUGR, pregnant rats were subjected to a 70% caloric restriction (CR) or exposed to dexamethasone (Dexa) (100 µg/kg/d for 7 days). Islet cells were then obtained in the offspring at postnatal days P7 and P21 and gene expression profiles compared to cells harvested

from pancreases of rats with a normal birth weight. Using real-time PCR, we quantified the relative changes in gene expression and confirmed the microarray results for a group of selected genes.
Results: At P7, islet cell area and insulin content of the pancreas were significantly reduced in the CR group in comparison to controls ($p<0.05$) and mRNAs of insulin and several beta cell markers (Pdx1, Kir6.2, Glut2 and glucokinase) were down regulated by 50% to 60%. In the Dexa-exposed group the islet cell area was also reduced compared to controls, while the difference for insulin mRNA did not reach significance. At P21 the islet cell area normalized in both groups. The expression of the cell cycle gene, cyclin D1, was significantly decreased at P7 (by 20%), but increased markedly by P21 (230%) in the CR group and paralleled the increase of beta cell markers such as insulin, Pdx1 and Glut2. In the Dexa-exposed group cyclin D1 did not change significantly at P7 or P21. There was no difference in the expression of cyclin D2 in both groups compared to controls. We also tested P16Ink4a to assess premature aging after IUGR, no expression was found at P7 or P21. At P21 the gene profile was different from P7, showing mostly a decrease in transcriptional regulators such as hepatocyte nuclear factor 6 (HNF6) and Krüppel-like factor 5 (KLF5).
Conclusion: In conclusion, we show that postnatal recovery of the islet cell mass after fetal stress exposure is linked to a complex regulation of different groups of genes, including transcription factors involved in prenatal pancreas development and cell cycle genes known to regulate postnatal beta cell expansion.
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458

Liraglutide induces human beta cell proliferation, counteracts low density lipoprotein anti-proliferative effects and protects from IL-1beta induced apoptosis
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Background and aims: Glucagon-like peptide 1 (GLP-1) analogs induce β-cell proliferation and have anti-apoptotic effects in rodent islets. However, the proliferative capacity of human β-cell and its modulation by GLP-1 analogs remain to be fully investigated. The aim of this study was to determine whether the GLP-1 analog liraglutide affects β-cell proliferation and to investigate whether liraglutide is able to counteract the anti-proliferative effect of low density lipoprotein (LDL) and the pro-apoptotic effect of interleukine-1β (IL-1β).
Materials and methods: Human islets from cadaveric organ donors were dispersed into single cells and exposed to liraglutide for 2 days and co-stained for Ki-67 or BrdU incorporation and insulin, allowing the determination of proliferative β-cells. The anti-apoptotic effects of liraglutide against 2 ng/ml IL-1β were tested in human islets cultured for 4 days and analyzed for cell death by the TUNEL assay. Mouse islets were cultured for 4 days in the presence of liraglutide and 3.1 mM LDL, isolated from healthy human plasma. Proliferation was assessed by analyzing BrdU incorporation.
Results: After 2 days incubation, liraglutide increased human β-cell proliferation (unequivocally identified by insulin staining), in a dose dependent manner up to 3 fold. Moreover, 4 days incubation with liraglutide protected human islet cells from IL-1β induced apoptosis. LDL decreased mouse islet cell proliferation and this anti-proliferative effect of LDL was counteracted by the presence of liraglutide.
Conclusion: These results indicate that human β-cell can proliferate in vitro in response to liraglutide. Furthermore, liraglutide protects human islet cells from IL-1β induced apoptosis and counteracts the anti-proliferative effect of LDL in mouse islets, suggesting additional mechanisms for the anti-diabetogenic effects of liraglutide.

459

The downregulation of Raf-1 Kinase Inhibitor Protein (RKIP) allows the formation of new beta cell cluster
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Backgrounds and aims: RKIP (raf-1 kinase inhibitor protein) is a docking protein that regulates different signaling pathways involved in cell proliferation and apoptosis. Previous studies, performed in our laboratory, have demonstrated a downregulated expression of RKIP in a model of beta cell regeneration. To understand the role of RKIP in the plasticity of the beta cell, and a possible role in the inhibition in the replication, we have studied the pancreas in the whole knock out (KO) mouse of RKIP.
Material and methods: Morphometric studies have been performed in samples of pancreas fixed in formalin of 8-weeks old male mice, determining pancreas mass, beta cell mass and area. Intraperitoneal glucose tolerance test was performed in 8-weeks old male mice.
Results: The KO mouse shows a bigger pancreas than the wild type (1% of the body weight in KO and a 0.8% in WT; $p<0.05$). There are no differences between WT and KO animals in the total beta cell mass and pancreatic islet distribution (number of islets/mm^2) but there are significant differences in the distribution of different beta cell area, KO animals present a higher percentage of insulin-positive cluster cells smaller than 1.000 um^2 (KO: 71% ± 4.5, WT: 38.4% ± 5.3; $p<0.001$) and a lower percentage of bigger islets. These smaller clusters represent an 11.28% (± 2.8) of the total mass of beta cell in the KO pancreas compare to the WT who has a 5.16 % ± 0.75. For otherwise, in response to the i.p. glucose tolerance test, KO mice have not elevated the glucose, showing lower glycemia levels at 15 and 30 minutes ($p<0.05$) than WT mice.
Conclusion: With these results, we suggest that RKIP is involved in beta cell plasticity and its absence increases the formation of new insulin-positive clusters. Therefore RKIP should be considered as a new gene which controls formation of beta cell cluster processes in the pancreas and its modulation with some pharmacologic agents could be considered as a new target for the treatment of diabetes.
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460

Novel transgenic mice for inducible gene overexpression in pancreatic precursors or mature beta cells
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Background and aims: The use of genetically modified mice for conditional gene inactivation or gene overexpression in specific cell populations has helped identifying the precise role of genes in pancreatic beta cells. Previously, we have shown that the absence of glucocorticoid receptor in pancreatic precursor cells led to a doubled beta-cell mass while its absence in mature beta cells had no consequence on beta-cell mass. These results showed that genes may have crucial functions at precise developmental windows and that genetic modifications have to be controlled not only in specific cell populations but also during specific time frames. Thus, the aim of the present project was to generate new transgenic mice using the inducible Tetracycline system to overexpress genes of interest either in pancreatic precursor cells or in mature beta cells and during precise time frames.
Materials and methods: We have created two lines of mice : one with the expression of the tetracycline Transactivator (tTA) under the control of the mouse insulin 1 gene promoter (Ins-tTA, expression in mature beta cells), the second with the tTA under the control of Pdx-1 promoter (Pdx-tTA, expression in pancreatic precursor cells). Because minimal promoters may lead to ectopic expression, we used Bacterial Artificial Chromosomes containing large portions (>150 Kb) of the gene environment to drive the tTA expression. Mice from each line were crossed with mice that express the LacZ reporter gene under the control of the tetracycline response element. We then analyzed the sites of expression of the transgenes for each mouse line by determining where the reporter gene is expressed. Since this system can be turned off by the use of Doxycycline (Dox), we have tested if gene expression could be controlled during various developmental windows.

Results: Our results show that in the Pdx-tTA mice, the transgene is expressed as early as embryonic (E) day 11.5 in the two pancreatic buds and at E13.5 and E15.5 in all pancreatic precursor cells. In the adult pancreas, the transgene is present in mature beta, delta and PP cells and in some acinar cells. Transgene expression was also found in the duodenum and stomach. In the Ins-tTA mice, the transgene is expressed from E13.5 but is restricted to beta cells. No expression in other organs than the pancreas was found. Next, we showed in both mouse lines that a 4 week-Dox treatment to adult mice was able to turn off the expression of the reporter gene. Moreover, when Dox was given from conception until postnatal age, the turned off system could be re-induced upon removal of Dox.
Conclusion: In conclusion, we have generated transgenic mice in which the transgene is correctly targeted to precursor cells, mature beta and some acinar cells for Pdx-tTA mice and only to beta cells for Ins-tTA mice. The transgene overexpression can be controlled in time, thus providing new useful tools to overexpress genes in pancreatic cells during defined development periods.
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461

First generation adenovirus vectors affect differentiation of human mesenchymal stem cells
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Background and aims: Type 1 diabetes results from a specific destruction of β-cell by the immune system. The restoration of the damaged β-cell population represents an attractive challenge. In that prospect, the use of adult mesenchymal stem cells (MSC) would represent an attractive therapeutic option since they are easy to isolate and their plasticity allows them to differentiate into the endoderm, mesoderm and ectoderm lineage. Despite these attractive properties, the number of studies documenting efficient endocrine differentiation remains rather limited compared to embryonic stem cell based strategies for instance. Our previous data showed that the use of lentivirus vectors to force expression of Pdx-1 and Ngn3 does not lead to robust expression of any endocrine hormones. However, many studies have reported some evidences of endocrine differentiation by Pdx-1 overexpression used an adenoviral vector. In this study we want to investigate the putative effect of viral carrier on BM-MSC differentiation capacities.
Materials and methods: Functionality of the Pdx-1 lentivirus has been assessed by luciferase assay after co-expression in HepG2 cells of a reporter construct where the luciferase gene is driven by the human insulin gene promoter. Lentiviral and adenoviral transduction efficiency in BM-MSC have been evaluated by FACS and endocrine differentiation progression determined by RT-PCR/qPCR analysis.
Results: After a first transduction with a lentivirus carrying Pdx-1, modified BM-MSC cells were transduced with an irrelevant and replication-deficient first-generation human adenovirus type 5 vector endowed with the fiber of serotype 50. The cells were maintained in high glucose containing media for 4 additional days. Whereas Pdx-1 modified BM-MSC cells did not show any robust sign of differentiation, the subsequent transduction with the adenovirus leads to expression of insulin, glucagon and somatostatin. qPCR and FACS analysis demonstrated that the adenoviral transduction had little or no effect on the CMV promoter that drives expression Pdx-1 in the lentiviral vector, demonstrating that the effect observed was independent on the level of expression of Pdx-1. Moreover, we confirmed by qPCR that the combined treatment LV-Pdx-1 / irrelevant adenovirus trigger BM-MSC to the endocrine pathway since amylase level, used here as control for exocrine pancreas, was not affected. Although the molecular mechanism is still unclear it appears that an adenoviral gene product is involved in this process since no modifications have been observed after Helper-Dependent (HD) adenovirus transduction. Finally, making use of human pancreatic-islet derived MSC, which poorly differentiate into adipocytes, we demonstrate that infection of these cells with a E1-deleted adenovirus vector carrying an irrelevant transgene resulted in robust adipogenic differentiation.
Conclusion: These data demonstrate that subsequent transduction of lentivirally modified BM-MSC with an irrelevant first-generation human adenovirus type 5 fibers 50 (HAdV5-fib50) leads to expression of insulin, glucagon, and somatostatin. Thus, first-generation E1-deleted adenoviruses are not inert gene transfer vectors and can have a broad effect on differentiation capacity of stem cells in vitro. Epigenetic modifications associated with adenoviral transduction could explain the effect of Pdx-1 on its target promoters.
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462

Regulation of beta cell regeneration by the autonomous nervous system in pancreatectomized rat
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Background and aim: The autonomous nervous system (ANS) regulates the activity of endocrine and exocrine pancreatic cells. Numerous in vitro and in vivo studies have shown that acetylcholine, the main neurotransmitter of the parasympathetic nervous system, enhances glucose-stimulated insulin secretion from pancreatic beta cells while activation of sympathetic nerves inhibits insulin release from the islets. However the role of ANS in the growth and proliferation of pancreatic cells is not clearly identified.
Material and methods: We investigated the role of ANS in a model of compensatory beta cell growth after 90% pancreatectomy in adult rat. In order to determine the impact of parasympathetic system on the regulation of beta cell regeneration, we performed sub-diaphragmatic vagotomy on pancreatectomized adult Wistar rats. The beta cell mass was morphometrically measured 7 days after surgery. The processes of cell replication, cell neogenesis and apoptosis were assessed in the time period preceding the partial recovery of beta cell mass after pancreatectomy i.e. 8h, 24h and 48h after surgery.
Results: The follow up of the beta cell mass 7 days after pancreatectomy showed that beta cell regeneration was reduced by 40% in the vagotomized/pancreatectomized group compared to the pancreatectomized group. We showed that during the first 48h following the pancreatectomy, the tremendous increase in ductal cell proliferation normally occurring in this model is significantly reduced in rats with sub-diaphragmatic vagotomy. PDX-1 expression in ductal cells, a feature of beta cell neogenesis, was also reduced in pancreatecomized/vagotomised rats 48h after surgery when compared to pancreatectomized rat. Moreover, vaogotomy associated with pancreatectomy significantly increased the rate of acinar cell apoptosis in the pancreas. Finally, beta cell replication was decreased as a consequence of vagotomy.
Conclusion: Taken together, our data show that vagotomy significantly alters beta cell regeneration in pancreatectomized rats suggesting that neurotransmitters released by parasympathetic nervous system are important for the compensatory growth of beta cells in adult rats.
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463

Upregulation of cell differentiation-related genes in 90%-pancreatectomized rats treated with gastrin
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Background and aim: We have recently found that gastrin treatment improved glucose tolerance and increased beta cell regeneration in pancreatectomized rats through stimulation of beta cell proliferation and neogenesis. Here, we aimed to study the gene expression profile of beta cell neogenesis-related genes induced by gastrin in pancreatectomized rats.
Material and Methods: Sprague-Dawley rats underwent 90% partial pancreatectomy (90%-Px), a well established model of beta cell regeneration, and were treated with [15 leu] gastrin-17 (Px+Gast, n=18) or with vehicle (Px+V; n=17). A sham operated group was added as control (Sham, n=6). Pancreatic remnants were harvested on days 1, 3 (early Px) and 5, 7 (late Px) after pancreatectomy and processed for total RNA extraction. Gene expression was determined by quantitative real time PCR. **Results:** 90%-Px rats developed mild hyperglycemia after surgery with similar blood glucose concentrations between Px+V and Px+Gast groups (Blood glucose on day 7 after Px: Px+V=149 ± 11 mg/dl; Px+Gast=163±37 mg/dl). Gastrin treatment increased *ngn3* and *neurod1* expression on day 1 and *pdx-1, nkx6.1* and *tcf1* on day 3 after Px ($p < 0.05$ compared to sham animals). In late Px, *pdx-1*, *nkx6.1* and *pax6* gene expression levels were dramatically decreased in vehicle treated rats ($p < 0.05$ compared to sham animals). The lower expression of these transcription factors, which are crucial for maintaining the mature/functional beta cell phenotype, was consistent with the downregulation of the insulin gene expression in Px+V rats on day 7 ($p < 0.05$) compared to sham animals. In contrast, pancreatic remnants of Px+Gast rats showed sustained gene expression of all these transcription factors. Accordingly, *insulin2* and *glucagon* gene expression levels were also similar in Px+Gast and normal pancreas of sham animals, and significantly higher than in Px+V group ($p<0.05$).

Summary/Conclusion: Gastrin treatment stimulated the expression of the crucial transcription factors *ngn3*, *neuroD*, *pdx-1* and *nkx6.1* required for beta cell differentiation during the early phase of regeneration after 90%-Px. Moreover, gastrin administration resulted in the maintenance of the mature/functional beta cell phenotype preventing *pdx-1*, *nkx6.1* and *insulin2* gene expression downregulation found in late Px+V rats.
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464

Combined loss of the cell cycle inhibitors p21cip1 and p27kip1 reveals that both are dispensable for maintaining adult beta cell mass and function

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Background and aims: β-cell replication is partially responsible for developing and maintaining beta cell mass and function. The "pRb pathway" is the final regulation step of the G1/S checkpoint in the β-cell cycle. In addition to cyclins and cyclin-dependent kinases, upstream control at the G1/S checkpoint also includes cell cycle inhibitors, the CIP/KIPs and the INK4s. On the CIP/KIP side, p27kip1 play a role in maintaining cell cycle arrest, which becomes apparent only when p27kip1-null mice are placed on a high-fat diet, or on insulin-resistant backgrounds. p21cip1 is also a cell cycle inhibitor, but its loss in mice results in no obvious β-cell phenotype. Since p21 and p27 are functional homologues, we have investigated if it is possible that loss of one is compensated by the other. Three hypotheses are feasible: 1) since p21 and p27 are required for assembly and nuclear import of cdks and D-cyclins, their loss will lead to dramatic beta cell failure, reductions in β-cell proliferation and mass, and diabetes; 2) since p21 and p27 are functional and structural homologues, and are important cell cycle inhibitors, their combined loss will lead to robust β-cell replication, increases in beta cell mass, and marked hypoglycemia; or, 3) since the G1/S checkpoint has multiple layers of redundancy and compensation, combined loss of p21 and p27 will have no important effect.
Matherial and methods: To address these hypothesis, we generated mice that are doubly deficient for p21 and p27.
Results: Mice doubly deficient for p21 and p27 prepared by two different breeding strategies and studied at 2 and 6 months of age, display no striking abnormalities in β-cell mass, islet number, fasting or postprandial glucose or insulin concentrations, intraperitoneal glucose tolerance or any other evidence of metabolic or β-cell dysfunction. Preliminary examination of beta cell proliferation also discloses no abnormalities.
Conclusion: Together, these findings suggest that combined p21 and p27 loss do not have striking effects on β-cell mass or function, and support hypothesis #3 above. Further, they suggest that other cell cycle inhibitors may compensate for this loss. This possibility is currently under investigation.
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465

Selective removal of undifferentiated embryonic stem cells during differentiation towards insulin-producing cells

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Background and aims: Embryonic stem cells (ESCs) are attractive candidates for generation of surrogate insulin-producing cells for cell replacement therapy in type 1 diabetes. However, the major limitation to their approval for clinical use is the risk of uncontrolled proliferation and teratoma formation after transplantation. This form of cellular misbehaviour constitutes a health risk and may contribute to functional failure of the graft. Therefore, a selection process must be developed for removal of residual ESCs from differentiation cultures and for tumour prevention. To tackle this problem we combined an efficient protocol for differentiation of insulin-producing cells with negative selection of undifferentiated cells. Our aim was to establish an embryonic stem cell line expressing a suicide gene HSV-1 thymidine kinase (HSVtk) that renders cells sensitive to the prodrug Ganciclovir (GCV). HSVtk cloned under the promoter of the human *Pou5f1* gene (Oct4), the master regulator of stem cell pluripotency, allows specific elimination of embryonic cells and leaves differentiated populations insensitive to GCV treatment.
Materials and methods: Mouse ES-D3 cells were transfected with an expression vector carrying the HSVtk gene and eGFP under the control of the Oct4 promoter. The clonal cell line eHSVtk was subjected to a new 4-stage differentiation protocol into insulin-producing cells, comprising 7 or 14 days of 1 μM GCV incubation. GCV-treated and untreated cells were analyzed by RT-PCR, flow cytometry, Western blot and immunocytochemistry.
Results: ES cells were successfully transfected with the pOct4-HSVtk-eGFP construct. The clonal cell line eHSVtk remained similar to wild type cells in terms of morphology, ESC marker expression (SSEA-1, alkaline phosphatase, Oct4, Nanog) as well as the potential to follow the differentiation protocol into insulin-producing cells. 19 days of differentiation, including 7 days of 1 μM GCV incubation, showed a very quick loss of eGFP fluorescence and only negligible populations of SSEA-1-positive cells. In contrast, untreated cultures were composed of up to 40 % of eGFP- and SSEA-1-positive cells. Selective ablation of ESCs after GCV treatment was also documented by reduction in expression of many important stem cell markers: alkaline phosphatase, Oct4, Nanog, ERas, Sox2, Rex1, Dax1, Klf4 and Nac1, both on the mRNA and protein level. After an additional 7 days of 1 μM GCV incubation, the percentage of eGFP- and SSEA-1-positive cells further decreased from 1.8 % to less than 0.3 %. Also, a further significant drop in the expression of other embryonic markers was observed. Immunocytochemical staining of Oct4, Nanog, and ERas revealed barely detectable levels, unlike that of untreated cells with a typical embryonic pattern of staining. Finally, cells supplemented with GCV showed higher insulin content than those cultured without GCV.
Conclusion: The results indicate that the suicide gene HSVtk driven by the stem cell-specific Oct4 promoter can be used to specifically target undifferentiated stem cells with teratogenic potential and effectively remove them from the differentiation culture. This technique, together with a new protocol for differentiation of ES cells into insulin-producing cells, provides the basis for a future application of differentiated stem cells in diabetes treatment.

PS 24 Islet and pancreas transplantation

466

Improved metabolic control and quality of life in 10 patients with type 1 diabetes following islet after kidney transplantation: results of the GRAGIL 1 trial

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Background and aims: Islet transplantation with Edmonton protocol has proven its efficiency in non-uremic brittle diabetic patients. We report here the results of islet grafts with this same immunosuppression protocol in type 1 diabetic patients with previous kidney transplantation within the GRAGIL group.
Patients and methods: Sixteen intra-portal islets infusions were performed in 10 type 1 diabetic patients with functional kidney transplant. Target islet equivalent number (IEQ) was 10'000/Kg of body weight administered in two infusions if necessary. Immunosuppression was switched on the day of transplantation to regimen associating sirolimus-tacrolimus-daclizumab. Metabolic results were assessed by HbA1c, fasting glycaemia and C-peptide levels. Insulin needs and hypoglycaemic events were quantified. Quality of life was evaluated by SF36 and DQOL scores.
Results: Patients were 43 years-old (median) with median diabetes duration of 36 years. They had received a kidney graft 7 years before. At 12 months post transplantation, 9/10 patients had a functional islet graft (C-peptide level> 0.5 ng/ml). HbA1c significantly decreased from 7.1%[6.4-7.8] to 6.5%[6.4-6.7] at 6 months and to 6.6%[5.8-6.8] at 12 months ($p<0.05$). Four patients were insulin independent at 6 months but only one at 12 months. However, insulin needs decreased, with a significant decrease of hypoglycaemic events, from 30IU/day to 8IU/day ($p<0.01$) and 9/10 patients needed less than 30% of their previous insulin dose. Global quality of life scores did not reach significant improvement but physical activity and health perception scores were significantly better at 12 months post transplantation. Adverse events included one minor peritoneal effusion, mouth ulceration (n=1) and diarrhea (n=2) with a need for switching sirolimus to mycophenolate mofetil in one patient.
Conclusion: Islet after kidney transplantation can restore good glycaemic control with a reduced occurrence of hypoglycaemia thanks to very low dose of insulin. Impact of this control on kidney transplant function needs further evaluation.

467

Islet cell aggregates are superior to islets for transplantation in microcapsules

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Background and aims: Small islet-cell aggregates were studied to determine if their survival and function were superior to intact islets within microcapsules during low oxygen culture and transplantation.
Materials and methods: Islet-cell aggregates were generated by dispersing rat islets into single cells and allowing them to reaggregate in culture. Islets and islet-cell aggregates were encapsulated in barium alginate capsules and cultured in low (0.5% or 2%) or normal (20%) oxygen, or transplanted. Following culture, tissue was assessed with measurements of oxygen consumption rate (OCR), nuclei counts, insulin to DNA content ratio, glucose-stimulated insulin secretion (GSIS), and gene expression. Syngeneic transplantation experiments were carried out to assess tissue survival in the transplantation setting without an immune response, and xenogeneic transplants were performed to assess whether islet-cell aggregates were superior in their ability to reverse diabetes in streptozocin diabetic mice.
Results: After low oxygen culture islet-cell aggregates were able to survive and function better than intact islets in terms of OCR, nuclei counts, insulin to DNA ratio, and GSIS. Culture in low oxygen (2%) resulted in maintenance of pre-culture levels of viable tissue (determined by OCR) in aggregates, but for whole islets only 61% of pre-culture viable tissue remained, $p < 0.01$. In terms of nuclei counts there was a substantial loss of tissue in the islets cultured at 0.5% oxygen when compared to pre-culture values (59%; $p < 0.05$) but only modest loss of tissue in the aggregate capsules (87% of pre-culture values, $p < 0.05$). The ratio of insulin to DNA content (an index of beta cell viability) decreased markedly in islets cultured in 0.5% oxygen for 2 days, in contrast to no decline in aggregates. After 2 days of culture in 2% oxygen there was no difference in insulin output between low and high glucose with islets, whereas islet cell aggregates showed differential increased secretion to high glucose. Pro-inflammatory genes MCP-1 and tissue factor were expressed at significantly lower levels in islet cell aggregates than in whole islets ($36 \pm 9\%$ and $59\% \pm 11\%$, $p < 0.01$ and 0.05, respectively) after overnight culture in 2% oxygen. Two weeks after encapsulated islets and aggregates were transplanted into syngeneic (Lewis rat) recipients, large areas of central necrosis were apparent in many of the islets and were especially prominent in the larger islets. In contrast, encapsulated aggregates remained healthy in appearance. Encapsulated aggregates cured a greater proportion of diabetic xenogeneic transplant recipients (83%) compared to encapsulated islets (30%) ($p < 0.03$ Fisher exact test, and the overall average blood glucose concentration was substantially lower with encapsulated aggregates. IPGTTs performed on the cured transplant recipients showed aggregates were as responsive as islets to a glucose load in terms of insulin secretion and similar to the normoglycemic controls.
Conclusion: The use of islet-cell aggregates with a smaller diameter than an intact islet reduces oxygen transport limitations to encapsulated tissue. These aggregates were superior to intact islets in terms of survival and function in low oxygen culture and under transplantation conditions.
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468

Effect of hypoxia-inducible timely VEGF gene overexpression in islet transplantation

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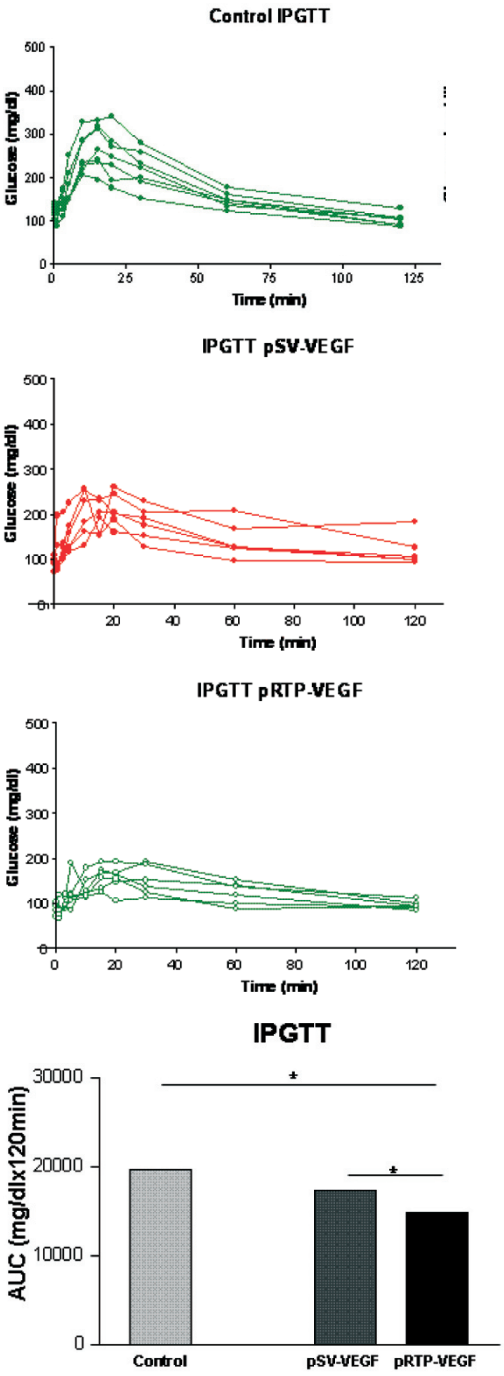
Background and aims: Hypoxic damage is one of the major causes of early islet graft failure. Vascular endothelial growth factor (VEGF) is known to induce a pleiotropic response in favor of angiogenesis and has been investigated as a therapeutic gene for revascularization of islets. In particular, timely and localized overexpression of VEGF in hypoxic cells is desirable, since unregulated VEGF expression in normal cells may induce overgrowth of endothelial cells. In this study, we determined the effect of hypoxia-inducible VEGF gene transfer on the function and revascularization of transplanted islets using a syngeneic mouse model.
Materials and methods: Mouse islets were transfected with hypoxia-inducible gene expression vector, pRTP801-hVEGF or control vector, pSV40-hVEGF using a non-viral carrier, Effectene. After transfection, the islets were incubated at the desired concentration of oxygen (normoxia, 20% oxygen; hypoxia, 1% oxygen) for 20 hrs, and then hVEGF expression was assessed by ELISA. One hundred mouse islets transfected with pRTP801-hVEGF or pSV40-hVEGF were transplanted under the left renal capsule of syngeneic STZ-induced diabetic recipients. At 7-weeks posttransplant, IPGTT and left nephrectomy were performed.
Results: The hVEGF level was significantly induced in the islets transfected with pRTP801-hVEGF and left under hypoxia condition. However, this effect was not observed in the islets transfected with pSV40-hVEGF, suggesting that the RTP801 promoter increased VEGF expression specifically in islets under hypoxia. When 100 mouse islets were transplanted into renal subcapsular space of STZ-induced diabetic syngeneic mouse, AUC in IPGTT at 7-weeks after transplantation was significantly lower in recipients of pRTP801-

hVEGF transfected islets than in recipients of pSV40-hVEGF transfected islets (Fig.).
Conclusion: Theses results indicate that the delivery of pRTP801-VEGF using Effectene induces VEGF level specifically under hypoxia and further enhances islet engraftment and preserves islet function in transplants compared with the pSV40-VEGF delivery. Our results suggest that the construction of plasmids with cytoprotective target genes and the RTP801 promotor combined with nonviral delivery with Effectene may be a useful strategy for ex vivo gene therapy in clinical islet transplantation.



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469

Influence of donor age on islet isolation and transplantation outcome
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Background: It has been suggested that the age of human organ donors might influence islet isolation and transplantation outcome in a negative way due to a decrease of in vivo function in islets isolated from older donors.
Methods: We retrospectively analyzed 332 islet isolations performed in our facilities and divided them into two groups depending on donor age (n=187 and n=145 for below and above 50 years, respectively). Pancreata were procured and processed according to established protocols. Isolation outcome was determined by islet yield, success rate (>250'000 IEQ) and transplantation rate. Beta cell function was assessed in vitro by stimulation indices in static incubation assays. Transplanted patients were divided into two groups depending on donor age of islet preparations (n=49 and n= 31 patients that received just islets from 50 year-old donors, respectively). In vivo function was assessed by the newly developed secretory units of islets in transplantation (SUIT) index and the C-peptide/glucose ratio 1 month after transplantation.
Results: There was no difference in islet yields between the two groups (249'200 ± 11'400 and 245,900 ± 9'800 IEQ for 50 year-old donors, respectively). Success rates were 45% for both groups, respectively. Overall, 85 (45%) islet preparations were transplanted from <50 year-old donors and 56 (39%) islet preparations were transplanted from >50 year-old donors. Stimulation indices were similar for both groups. SUIT indices and C-peptide/glucose ratios one month after transplantation were significantly higher in patients that received islets only from the younger donor population (41 ± 4 vs. 26 ± 4, p=0.008 and 1.38 ± 0.12 vs. 0.87 ± 0.1, p=0.003, respectively).
Conclusion: Our study shows that, in our donor population, donor age does not influence islet isolation outcome, in contrast to islet graft function.

470

Transendoscopic islet transplantation to gastric submucosa (eGSM-ITx) of pigs with streptozotocine induced diabetes - preliminary results
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Islets and pancreas transplantation have become standard treatments of patients with diabetic complications.However pancreas transplantation is associated with high incidence of complications and the long-term results of islet transplantation are still unsatisfactory.Loss of pancreatic islets grafts is not only caused by immunological reactions but also due to the site of grafting and IBMIR.Gastric submucosal space might be an alternative site for transplantation.
The aim of this study was to assess the possibility of endoscopic islets transplantation into the gastric submucosa-its efficacy and complications.
Materials and methods: 20Landrace pigs weighing 19-24kg were obtained for the study.7 animals were controls(C-group)and 13 were Transplantation group(TX group).In both groups diabetes was induced by streptozotocine (stz)infusion at 200 mg/kg.At 7 days post stz infusion pigs of both groups underwent endoscopy-in group-C to assess the feasibility of gastroscopic examination under general anesthesia in pigs with diabetes and to study the influence of basiliximab infusion on pigs,in Tx-group to perform eGSM-ITx. Immunosuppression consisted of tacrolimus 0,2 mg/kg and sirolimus 6mg/m^2.At 7 days post transplantation, control gastroscopy was performed to assess the gastric mucosa and to take biopsies for histopathology.10 to30 days after eGSM-ITx Magnetic Resonance(MRI)examination was performed and pigs were euthanized.Stomach and pancreas were obtained at autopsy for histopathology.C-peptide level was assessed prior to diabetes induction, at 7 days post induction and 3,10,30days post eGSM-ITx.Glycemia was assessed twice daily during the experiment.For 10 days after diabetes induction(up to three days after eGSM-ITx)in both groups,insulin was given to reach glyc-

emia between 150-200mg/dl,after that period insulin was given only when glycemia exceeded 600 mg/dl.
Results: There were no differences in insulin requirement and glycemia up to the day of eGSM-ITx between the groups.Tx-group animals received a mean of 6000±3170 IEQ/kg.Tx-group animals had a significantly lower insulin requirement and significantly lower mean glycemia since the first day post transplantation.This trend was observed till the end of study at 1 month. Beginning at day 4 post eGSM-ITx pigs from the ITx-group did not receive insulin-in 7 cases(63 %)mean glycemia remained below 250 mg/dl;in 4 cases it did not exceed 450 mg/dl.C-group animals all required insulin once daily to keep glycemia below 600 mg/dl. There were no signs of perforation,ulceration or bleeding after the eGSM-ITx on gastroscopy and histopathological examination.In MRI scans unspecific thickening of gastric wall was observed at sites of islet deposition.
Conclusion: Transendoscopic islets transplantation into gastric submucosa is feasible and a safe procedure in an experimental animal setting. Its potential for clinical application in human subjects needs further studies.
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471

Type 1 diabetic patients after successful pancreas kidney transplantation exhibit normal fasting and postprandial liver glycogen content despite systemic insulin secretion
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Background and aims: Insulin replacement in Type 1 diabetes mellitus (T1DM) is usually performed via a systemic (subcutaneous) instead of the physiologic portal route. So far it is unclear whether this systemic route of insulin delivery might contribute to metabolic defects in these patients. This hypothesis is supported by the finding that T1DM with poor glycemic control exhibit reduced hepatic glycogen stores. Successful pancreas kidney transplantation (PKT) with systemic venous drainage is an ideal model of optimized systemic insulin therapy. Therefore, the aim of the present study was to investigate the effects of PKT on fasting and postprandial liver glycogen content.
Materials and methods: Using ^{13}C-nuclear magnetic resonance spectroscopy (^{13}C-NMR), liver glycogen concentrations were assessed in 6 T1DM patients after successful PKT (26±2kg/m², 49±3yrs, 2f/4m, fasting glucose 86±4mg/dl, HbA1c 5.2±0.2%) with systemic venous drainage and in 4 matching nondiabetic controls (CON) (25±2 kg/m², 52±3yrs, 2f/2m, 86±3 mg/dl, 5.5±0.2%) at fasting and after two standardized mixed meals. Fasting insulin sensitivity was assessed using the QUICKI-Index.
Results: Liver glycogen concentrations at fasting (PKT:185±15, CON:191±15mM), after breakfast (PKT:207±10, CON:205±14mM) and after lunch (PKT:233±17 vs. CON:233±11mM) were comparable in PKT and CON. Mean and fasting concentrations of glucose, insulin, C-peptide and glucagon and QUICKI were similar in PKT vs. CON.
Conclusion:Despite systemic insulin secretion, T1DM after successful PKT exhibit unchanged fasting and postprandial liver glycogen stores in comparison with age-and BMI-matched non-diabetic controls. Thus, this study indicates that systemic insulin substitution does not cause alterations of hepatic glycogen storage.
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472

Immunosuppressive drugs inhibit rat islet cell proliferation *in vitro*
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Background and aims: Beta-cell replication is thought to play a significant role in maintaining pancreatic beta-cell mass. Whether immunosuppressive drugs affect replication of beta-cells in transplanted islets is not fully understood. The aim of this study was to determine the effects of immunosuppressive drugs, used in islet transplant recipients, on islet cell replication, *in vitro*.
Materials and methods: Rat pancreatic islet cells were incubated with BrdU (10μM) in the presence or absence of different concentrations of cyclosporine A (CsA), tacrolimus, sirolimus or mycophenolate mofetil (MMF). Cell replication was determined by BrdU incorporation after 1 to 7 days of culture. Data are expressed as % of positive BrdU cells and as mean ± SEM for 3 or more independent experiments.
Results: Sirolimus (10 ng/ml) blocked almost completely islet cell proliferation from 24h (0.17±0.17 vs. 2.00±0.51; sirolimus vs. control, $p<0.001$) until 7 days of treatment (0.14±0.10 vs. 23.80±3.63; sirolimus vs. control, $p<0.01$). Additionally, the inhibitory effect of sirolimus was also observed at low concentrations from 0.1 ng/ml (2.75±0.97 vs. 13.43±0.76; sirolimus vs. control, $p<0.001$). After 2 days of treatment, MMF also inhibited cell proliferation in a dose-dependent manner (control: 12.08±1.53; MMF 1μg/ml: 5.74±0.80; MMF 5μg/ml: 1.83±0.30; MMF 25μg/ml: 1.33±0.35%). Treatment with CsA (200 ng/ml) and tacrolimus (10 ng/ml) during 5 days significantly reduced islet cell proliferation (2.52±0.94; 3.68±1.06; 21.13±2.47 for CsA, tacrolimus and control, respectively ($p<0.01$)). None of the immunosuppressive drugs induced a significant increase of apoptosis. Furthermore, effect of immunosuppressive drugs on cell proliferation was reversible.
Conclusion: Our results indicate that immunosuppressive drugs, at therapeutic target concentrations, inhibit pancreatic islet cell proliferation *in vitro*. It is therefore suggested that progressive graft islet dysfunction may result, in part, from an impairment of beta cell replication induced by immunosuppressive drugs.
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473

Simultaneous pancreas kidney transplant (SPK) improves cardiovascular risk factors in type 1 diabetes mellitus
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Background and aims: SPK is the treatment of choice in patients with type 1 diabetes and stage 4 and 5 chronic kidney disease (CKD). Long term haemodynamic and biochemical outcomes following SPK in a UK population are not well defined. The aim is to evaluate metabolic and biochemical variables in patients with type 1 diabetes following SPK in a tertiary renal centre.
Materials and methods: A retrospective analyses of successful SPK transplant patients over the last 12 years. Successful SPK was defined as insulin and dialysis independence at most recent clinic visit. Clinical and biochemical measures were obtained from patient records prior to SPK (V1) and at the most recent clinic visit (V2).
Results: 47 patients (32 M, 15F) were included with a follow up of 4.2 (1.2-11) yrs (median & range). The age at the time of SPK was 41±7 yrs (mean±SD) and the duration of diabetes was 26.5±8.5 yrs. V1 to V2, systolic blood pressure fell from 139±18 to 127±16mmHg and diastolic blood pressure from 78±12 to 72±10mmHg, HbA1c (n=22) improved from 8.3±1.6 % to 5.6±0.6% and serum creatinine fell from 695±285 to 113± 25μmol/l ($p<0.01$ for all variables). There was a reduction in the number of anti-hypertensive and lipid lowering agents at V2 ($p<0.05$).
Conclusion: Successful SPK not only removes need for insulin and dialysis but also improves cardiovascular risk factors.

PS 25 Immunoprevention in type 1 diabetes

474

GAD_{65} treatment induces high GADA but no changes in epitopes or adverse signs/symptoms in type 1 diabetic children

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Background and aims: GAD_{65} treatment (Diamyd) has been shown to preserve residual insulin secretion in children and adolescents with recent onset Type 1 diabetes (T1D), and to cause an increase of GAD auto-antibodies (GADA). High GADA may be associated with risks for neurological adverse events and we have therefore performed a clinical follow-up of patients 4 years after GAD_{65}-treatment, as well as studied GADA and its binding in more detail.
Methods: 70 T1D patients (10-18 years old) with diagnosis within the previous 18 months, were included in a double-blind, randomised, controlled trial receiving a primary injection on day 1 of 20 µg recombinant human GAD_{65}-alum (Diamyd) (n=35) or placebo (n=35) and a booster dose after 4 weeks. Blood samples were collected before the first injection and after 1, 3, 9, 15, 21, and 30 months. The serum levels and the epitope binding pattern of GADA were analysed. A 48 months follow-up is on-going.
Results: There were no clinical adverse events after 30 months, and none is seen so far in the 48 month follow-up. Patients receiving GAD_{65}-alum or placebo had similar levels of GADA at baseline, but GADA were higher in GAD_{65}-alum treated patients after 3 months ($p=0.001$), and remained so still after 30 months ($p<0.05$). There were no differences in the binding pattern of GADA to a number of selected epitopes of GAD. High GADA, seen in both groups, were not related to any signs of neurological or other clinical adverse events. To further clarify the effect of treatment in the induced GADA, analyses of enzymatic inhibition, anti-idiotypic antibodies and subclasses of GADA are ongoing.
Conclusion: The effect of GAD_{65}- treatment on the humoral response was antigen specific and induced a long lasting specific B cell memory, without inducing epitope spreading or adverse clinical events, seen so far after 48 months follow-up.
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475

Effect of combined oral protease and flavonol treatment in persons at risk for type 1 diabetes

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Background and aims: Past regimens for preventing diabetes have failed in individuals with family history of type 1 diabetes (T1D). We examined in a pilot study, whether combined treatment with proteases and flavonols decreases the incidence of diabetes and affects immune and inflammatory markers.
Materials and methods: This randomised, prospective, multicenter, placebo-controlled, double-blind study included 21 islet antibody positive first-degree relatives of patients with T1D, with one participant dropping out before start of medication. Eleven subjects (age: 12.0±8.0 years) received daily oral treatment with 48mg trypsin, 90mg bromelain and 100mg rutoside, nine subjects (10.2±3.7 years) received placebo for at least 24 months. Serum cytokines and chemokines were measured at 0, 1, 3, 6 and 12 months and diabetes incidence was monitored for 8 years.
Results: During a total of 8 years follow up, 6 of 9 (67%) subjects on placebo and 4 of 11 (36%) on proteases/flavonol developed diabetes (Hazard ratio 2.1 [0.6-7.9]). Serum concentrations of cytokines (IFN-g, IL-10, IL-13, MIF) and chemokines (IL-8, MIP-1ß [CCL4], MCP-1 [CCL2]) were unchanged during 12 months in both groups. Immune mediators measured at study entry were not predictive of later development of T1D. No side effects were reported upon proteases/ flavonol treatment.
Conclusion: Proteases/flavonol treatment was found to be safe and well tolerated but did not significantly decrease diabetes incidence in this small group of subjects at increased risk for T1D. Circulating cytokines were neither related to treatment nor to later manifestation of T1D. Large clinical trials are needed to recognise smaller size effects of the protease/flavonol mixture on the progression towards T1D.
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476

Evaluation of different regimens of a chimeric/humanized aglycosylated anti-CD3 monoclonal antibody (MAb), otelixizumab, in subjects with type 1 diabetes mellitus

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Background and aims: Otelixizumab is an Fc-disabled IgG1 MAb directed against the mature T cell antigen CD3ε. Otelixizumab is being developed for the treatment of autoimmune disorders, including type 1 diabetes mellitus (T1DM). Phase 1b/2 open-label dose-escalation studies were conducted to identify a regimen that minimized pro-inflammatory cytokine (PIC) release, immunogenicity, and perturbation of Epstein Barr Virus (EBV) immunity (PEI), yet preserved clinical activity and pharmacokinetic (PK) and pharmacodynamic (PD) parameters. A prior Phase 2 study and studies in the NOD mouse model guided dose optimization.
Materials and methods: Over 100 T1DM adult subjects were enrolled in 3-, 4-, and 8-day dosing studies. Eleven different regimens with total doses ranging from 0.3 mg to 6.85 mg otelixizumab were assessed. Serum concentrations of cytokines, otelixizumab, and anti-otelixizumab antibodies were measured along with standard safety parameters. PD parameters were monitored using flow cytometry; EBV viral load was measured by a validated, quantitative PCR method.
Results: A cumulative dose of 3.1 mg otelixizumab administered over 8 days was identified for use in a Phase 3 clinical trial in new onset T1DM adult subjects (DEFEND-1). This regimen utilizes increasing doses on the first 3 days, followed by 5 subsequent doses that are higher but without significant PIC release (suggesting that at least partial conditioning is achieved in the first 3 days). This regimen resulted in no lymphocyte depletion or other cytopenias, no electrolyte abnormalities, no clinically significant changes in liver function tests, no rash or unexpected AEs, no SAEs, and no significant immunogenicity or PEI. CD3/TCR modulation during the last 5 days of dosing ranged from between 25% to 40% prior to dosing and 70% to 80% just after dosing. This was accompanied by 30% to 40% saturation prior to dosing and 90% saturation just after dosing. The pattern of CD3/TCR modulation mirrored results seen with efficacious dose regimens in the NOD mice. In the peripheral blood of T1DM subjects, increases in CD4+CD25+FOXP3+ T regulatory cells were observed. Lastly, preliminary results show that new onset T1DM subjects, beta cell function appeared to be comparable to that previously published with higher doses of otelixizumab (Belgian Diabetes Registry Study). Higher cumulative doses resulted in similar but more pronounced PK/PD changes and modest dose-dependent increases in immunogenicity and PIC release.
Conclusion: An optimized 8-day otelixizumab regimen was identified that resulted in targeted PK/PD changes, had no significant safety issues, and was relatively well tolerated. These data provided the support for choosing this dose regimen for evaluation in an ongoing Phase 3 clinical trial (DEFEND-1).

477

C-Peptide response and HLA genotypes in subjects with recent onset type 1 diabetes following immunotherapy with diapep277

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Background and aims: In immunotherapy trials of type 1 diabetes (T1D) response to treatment has not been analysed according to different HLA genotypes (high, moderate or low risk genotypes) due to the low number of enrolled subjects.
Materials and methods: This analysis was carried out in 120 consecutive T1D subjects enrolled in double blind trials with DiaPep277. Age of patients ranged from 4 to 59 years (mean age 19.9 yrs. ± 10.8). Fasting C-peptide, glucagon-stimulated peak C-peptide (Max Cpep) and area under the curve

(AUC Cpep) were evaluated at study initiation (Baseline) and after 12 months therapy. The variances of fasting C-peptide, Max Cpep and AUC Cpep were analyzed using the general linear model repeated-measures procedure. This provided the analysis of groups of related variables representing different measurements of the same attribute (within-subjects factor). The model includes between-subjects factors that divides the population into groups according to their HLA risk groups genotype and treatment (DiaPep277 treatment vs. placebo).
Results: Fasting C-peptide, Max Cpep and AUC Cpep values in T1D subjects with low risk HLA genotypes were significantly higher than those in subjects with high/moderate risk HLA genotypes from baseline through 12 month follow up (p=0.001, p=0.02, p=0.04, respectively). These findings were independent of age at onset. Nonetheless changes in fasting C-peptide, Max Cpep and AUC Cpep over the observational period did not differ significantly between subjects with low or high /moderate risk HLA risk genotypes (for all comparisons). No interactions were observed between HLA genotypes and type of treatment (Diapep277 vs. placebo) on Cpep Max Cpep and AUC Cpep changes from baseline through 12 month follow up.
Conclusion: T1D subjects with low risk HLA genotypes maintain higher C-peptide secretion irrespective of type of therapy (Diapep277 vs. placebo), however a different outcome might result from an analysis separating pediatric vs. adult patients.

478

Treatment with alum-formulated GAD65 in type 1 diabetic children results in early induction of Th2 responses
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Background and aims: Animal studies have shown that treatment with GAD65 can prevent destruction of the β-cells, by induction of immunological tolerance and shifting the normal Th1/Th2 balance toward a Th2 response. In a phase II study, injection of alum-formulated GAD65 (GAD-alum) to children with T1D resulted in the preservation of β-cell function. Here we studied the immunomodulatory effect of the treatment on the early GAD65-induced cytokine and chemokine response.
Materials and methods: Peripheral blood mononuclear cells (PBMC) from patients (n=70) included in the study, collected at day 0 and after 1, 3 and 9 months, were stimulated *in vitro* with antigens (e.g. GAD65, IA-2 peptide, insulin peptide, PHA) for 72 hours. Secretion of cytokines and chemokines was analyzed in cell supernatants and serum samples using Luminex. Expression of transcription factor FOXP3 was analyzed with real-time RT-PCR.
Results: Treatment with GAD-alum expanded a GAD65-specific T-cell population in the periphery that secreted the Th2 associated cytokines IL-5 ($p<0.05$) and IL-13 ($p<0.01$), and expressed FOXP3 ($p<0.05$) one month after injection. The recall response was characterized by a broader range of cytokines and chemokines in samples collected 3 and 9 months later. Remarkably, IL-5 and IL-13 were the only GAD65-induced cytokines that increased over time in the treated group ($p<0.001$). Similar secretion of all cytokines and chemokines was seen in the two groups after stimulation with placebo, IA-2 peptide, insulin peptide and PHA. No difference was detected in serum samples between the two groups.
Conclusion: Immunotherapy with GAD-alum in humans with T1D induces an early Th2-type specific immune response.
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479

GAD-alum treatment induces GAD-specific FOXP3$^+$ cells in type 1 diabetic children
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Background and aims: We have recently shown that alum-formulated GAD_{65} contributes to preservation of residual insulin secretion in newly diagnosed type 1 diabetic children. In parallel, the treatment induced an antigen-specific T cell population with a recall response including a broad range of cytokines. Here we study the immunomodulatory effect on T cells with focus on CD4CD25^{hi} cells after GAD-alum treatment.
Materials and methods: Seventy children (age 10-18) recently diagnosed with T1D (<18 months), fasting C-peptide >0.1 nmol/L and presence of GADA were randomly assigned 20μg GAD-alum (n=35) or placebo (n=35) at two occasions, one month apart. Twelve healthy children (age 11-15) were included as reference. Peripheral blood mononuclear cells (PBMC) were stained with antibodies (CD4, CD25, CTLA-4, Neuropilin, FOXP3) for flow cytometry. At 21 months PBMC were cultured over-night with GAD_{65} and medium alone and thereafter treated as previous samples. As the receptor expression was not normally distributed, two groups were compared with Mann-Whitney U-Test and three or more groups with Kruskal-Wallis test for unpaired observations.
Results: Three months after the initial injection, GAD-alum treated children had lower percentages of CD4CD25^{hi} cells co-expressing Neuropilin and CTLA-4 ($p=0.01$) than the placebo group. Further, median intensity of CD-4CD25^{hi} cells was lower in samples from children receiving GAD-alum compared to placebo ($p<0.05$). Interestingly, at 21 months increased percentage ($p=0.04$) and intensity ($p=0.006$) of GAD_{65}-induced CD4CD25^{hi} FOXP3+ cells were detected in samples from GAD-alum treated children.
Conclusion: Treatment with GAD-alum seems to decrease the inflammatory process in parallel to an induction of GAD_{65}-specific CD4CD25^{hi} cells expressing FOXP3.
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480

Characterisation of patients with new onset diabetes and prospective follow-up from birth to diabetes onset
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Background and aims: Type 1 diabetes (T1D) is preceded by islet autoimmunity. Aim of the study is to characterize patients who have developed T1D at onset and compare the autoimmunity status at onset to the prediabetic period.
Materials and Methods: 55 children who developed T1D between age 1 and age 16 years were investigated. Unique in this cohort was that all children were prospectively followed from birth with repeated blood sample collection. Antibodies to insulin (IAA), GAD, and IA2, were measured in all available samples until diabetes onset.
Results: At diabetes onset, 56% of cases were positive for 3 islet autoantibodies (ab), 33% of cases were positive for 2 islet abs, 11% were positive for 1 islet ab, and no cases were autoantibody negative. At diabetes onset, IAA was present in 91% of the cases, GADA in 73%, and IA-2A in 71% of the cases. 9 of the 18 children with 2 antibodies at onset had all three antibodies during the prediabetic period. 1 of the 6 children with 1 antibody at onset had 2 antibodies and none had 3 antibodies in the prediabetic period. Two offspring lost IAA before onset, 5 offspring lost GADA, and 3 offspring lost IA2A before onset, resectively. The maximum titer of IAA was found 2.7 ± 3.2 yrs prior to T1D onset (mean peak titer 69 U, mean onset titer 55 U), the maximum titer of GADA 2.4 ± 2.3 yrs (mean peak titer 191 U, mean onset titer 137 U), and the maximum titer of IA2A 2.0 ± 2.7 yrs (mean peak titer 170 U, mean onset titer 82 U) prior to T1D onset, respectively. Of the 55 cases, 22 had developed rapid progressive diabetes defined by progression from first autoantibody positivity to T1D within 2 yrs. In those cases T1D onset age ranged from 0.7 to 9.9 yrs, indicating that they were younger than slow progressors (mean onset age 3.1 ± 2.0 yrs vs. 8.9 ± 3.4 yrs, $p<0.001$).
Conclusions: Maximum expression of autoantibodies usually occurs prior to diabetes onset. A subgroup of rapid progressors differs by younger onset age and higher IAA titers from slow progressors.
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481

Prevention of autoimmune diabetes mellitus by modulatory antibodies in the LEW.1AR1-*iddm* rat: protective effects of CD4 modulation and diabetes aggravation by CTLA-4 agonism
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Background and aims: The LEW.1AR1-*iddm* rat is an animal model of autoimmune diabetes mellitus with a strong genetic component and an incidence of 60 %. Modulation of CD4+ T cells by monoclonal antibodies (mabs) is a promising approach to induce immune tolerance. It was the aim of this study to test whether (1) the modulating anti CD4+ antibody (RIB5/2) or (2) the agonistic CTLA-4 antibody (H4) could prevent autoim-

mune diabetes and affect the cellular immune state when applied during the prediabetic period.
Materials and methods: Normoglycaemic LEW.1AR1-*iddm* rats were treated on day 40, 42, 44, 46 and 48 after birth with the mabs (5 x 5 mg/kg b.w.) RIB5/2 (n = 17) or H4 (n = 16). Blood glucose was measured biweekly and blood samples were taken on day 50, 60, 75 and 120 for flow cytometry (FACS) analysis. Animals were killed on day 120 (normoglycaemia) or after diabetes manifestation (blood glucose > 10 mmol/l). Blood samples were analyzed for immune status via FACS for the T-cell markers CD4, CD8, CTLA-4 and CD25. FACS data were expressed as % of monuclear cell population. Pancreas samples were examined for beta cell destruction and immune cell infiltration.
Results: Application of RIB5/2 mab resulted in a 40 % reduction of T helper cells within 48 h and a 91 % reduction of CD4+ fluorescence intensity on T helper cells indicating modulatory receptor internalization. RIB5/2 treatment significantly ($p<0.01$) reduced diabetes incidence from 60 % to 11 %. Diabetes manifestation was delayed from d 60 to d 68 ($p<0.05$). RIB5/2 antibody treatment significantly reduced the portion of CD4+ T-cells in blood on day 50 (16 % vs. 48 %, $p<0.001$), day 60 (normoglycaemic 29 % vs. 46 %, $p<0.001$; diabetic 30 % vs. 48 %, $p<0.05$) and day 75 (36 % vs. 43 %, $p<0.05$) (RIB5/2 treated cohort vs. untreated control). Blood CD8+ T-cells were increased at day 50 in RIB5/2 treated animals who eventually developed diabetes (39 % vs. 30 %, $p<0.05$). Interestingly, regulatory CD4+/CD25+ cells were significantly increased in diabetic RIB5/2 treated animals on day 50 (6.2 % vs. 1.6 %, $p<0.05$). H4 treatment slightly reduced the portion of blood CD8+ T-cells at day 50 but did not affect the portion of CD4+ T cells. The time point of diabetes manifestation was not different to that of the control cohort. At day 50 regulatory CD4+/CD25+ T-cells were significantly reduced in H4-treated animals. The portion of CTLA-4+ T-cells was significantly increased after H4 treatment at day 50 but significantly decreased after day 60 in rats developing diabetes. Treatment with the RIB5/2 or H4 mab did not affect the degree of islet infiltration in diabetic animals in comparison to untreated controls.
Conclusion: The modulatory downregulation of CD4 by the RIB5/2 mab during the critical period of islet infiltration proved to be a promising strategy to induce persisting tolerance against autoimmune diabetes. The increase of regulatory T cells could be at least in part a possible mechanism to control autoimmunity. Stimulation of the CTLA-4 feedback pathway showed aggravating effects upon beta cell autoimmunity. Thus, modulation of costimulatory pathways appears problematic as prevential strategy of tolerance induction in type 1 diabetes.

482

Specific immunomodulatory effect of GAD_{65} in type 1 diabetics
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Background and aims: In animals prevention of autoimmunity with GAD_{65} induced immunological tolerance and shifting toward Th2 responses. We intended to clarify the effect of GAD_{65}-alum in the immune responses of type 1 diabetic (T1D) patients.
Materials and methods: Samples from T1D children receiving 20µg rh-GAD_{65}-alum (n=35) or placebo (n=35) and a booster after 4 weeks were collected at baseline and at 1, 3, 9, 15, 21 and 30 months. Isolated PBMC were stained for flow cytometry with anti-CD4, CD25, CTLA-4, Neuropilin and FOXP3. At 21 months cells were cultured over-night with GAD_{65} before staining. After 72 hours stimulation, cytokines IL-5, -6, -10, -12, -13, -17, TNF- α, IFN- γ were analyzed by Luminex, FOXP3 by RT-PCR and serum GADA, IA-2A, tetanus toxoid and IgE by RIA.
Results: After one month GAD_{65} induced IL-5,-13 and FOXP3 ($p<0.05$) in GAD-alum treated. At three and nine months, IL-17, TNF-α and IFN-γ ($p<0.05$) were additionally induced, and GADA titers increased ($p<0.001$). At nine months also IL-10 was higher in GAD-alum group. Reduction of C D4+$CD25^{high}$Neuropilin+CTLA-4+ cells ($p=0.01$) and lower CD4+$CD25^{high}$ MFI ($p<0.05$) detected at three months was at nine months only observed in children with shorter disease duration. At 21 months GAD_{65} induced CD4+$CD25^{high}$FOXP3+ cells ($p<0.05$) while CD4+CD25+ ($p<0.005$) were reduced. No differences in IA-2A, tetanus antibody or IgE were observed
Conclusion: GAD-alum induced long-lasting specific T and B cell memory, and a specific T cell population characterized by early Th2 and regulatory immune responses that increased over time, in parallel to reduction of the inflammatory process.
Supported by: Swedish Child Diabetes Foundation, Medical Research Council of Southeast Sweden and Diamyd Medical AB.

PS 26 Markers of autoimmunity in type 1 diabetes

483

Distinct phenotype and function of natural killer cells in the pancreas of non obese diabetic mice
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Background and aims: Natural Killer (NK) cells are part of the early innate immune response and play an important role in fighting certain viruses, tumours and in rejection of bone marrow transplants. A role for NK cells in autoimmune disease has been suggested, but the literature is conflicting. The objective of this study was to investigate the presence, phenotype and function of NK cells in the pancreas of non obese diabetic (NOD) mice.
Materials and methods: NK cells from NOD mice pancreases were analysed by immunohistochemistry and flow cytometry. Proliferation was assessed by BrdU-incorporation. Functional responses were recorded using antibody-mediated stimulations and measurements of intracellular cytokines. Adoptive transfers were performed to investigate NK cell homing to the pancreas.
Results: NK cells infiltrated the pancreas of NOD mice at young age, had a distinct phenotype and proliferated more compared to spleen and pancreatic lymph node NK cells. NK cells could home to the pancreas in a T and B cell-independent fashion and adoptive transfer experiments showed that the spleen contained NK cells or precursors that could traffic to the pancreas, where they displayed the typical phenotype. Despite expression of activation markers, most pancreatic NK cells showed diminished IFN-γ secretion and degranulation compared to spleen NK cells after stimulation in vitro, reflecting exhaustion. However, some pancreatic NK cells produced IFN-γ spontaneously, suggesting that the pancreas contains both NK cells mediating ongoing effector responses and NK cells rendered hyporesponsive due to chronic stimulation. Pancreases from mouse strains not prone to develop diabetes also contained NK cells. Compared to NOD mouse pancreatic NK cells, they displayed less killer cell lectin-like receptor G1 (KLRG1), a marker for mature NK cells that have undergone proliferation, and a lower rate of proliferation as measured by BrdU incorporation.
Conclusion: In conclusion we show that NK cells are present in the pancreas of several mouse strains but show distinct properties in the NOD mouse. This suggests that pancreatic NK cells may be sentinel cells, which under inflammatory conditions could become activated, proliferate and contribute to the development of organ-specific autoimmune diseases such as type 1 diabetes.
Supported by: the Swedish Diabetes Association

484

Expressions of phagocyte surface receptors for apoptotic cells in the bone marrow-derived dendritic cells from non-obese diabetic mice
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Background and aims: Dendritic cells (DC) are professional antigen presenting cells. In type 1 diabetes, DC play pivotal roles in the pathogenesis of this disease. When cells apoptose, they are engulfed by DC. Constituents of the corpse survive the intracellular processing and are recycled to the membrane of DC. The presentation of yielded antigens to T cells is a central event in the induction and the maintenance of peripheral immune tolerance. Conversely, errors in this pathway contribute to the pathogenesis of autoimmune diseases. A variety of signals distinguish apoptotic cells from those alive, and a significant number of phagocyte receptors have been shown to be involved in sensing the presence and the uptake of apoptotic cells, which include scavenger receptors (SR), Fc gamma receptors (FcgR), integerins, and C-type lectin-like receptors (Clec). To characterise molecular changes in $CD11c^{+}$ bone marrow (BM)-derived DC from non-obese diabetic (NOD) mice, we recently compared transcript profiles of BMDC with those from the sister strain, non-obese non-diabetic (NON) mice. In the present study, we analaysed our microarray data focusing on these receptors for apoptotic cells, which possibly play critical roles in triggering anti-islet autoimmunity.

Materials and methods: BM cells from 4-week-old female NOD and NON mice were cultivated in the presence of GM-CSF and IL-4 over 5 days, and of LPS for additional 2 days. The $CD11c^+$ DC were then sorted by magnetic beads-conjugated anti-CD11c antibodies (MACS, Miltenyi Biotech Inc). RNA extracted from the $CD11c^+$ DC from 3 independent mice (10 micrograms/ mouse) was pooled, and used to prepare targets for hybridization to the GeneChip Mouse Genome 430A Arrays (Affymetrix).
Results: Among the known receptors for apoptotic cells expressed on DC, CD36, MSR1/ CD204, integrin beta 5, Fcgr1, CD93/ C1qR, c-mer proto-oncogene tyrosine kinase (Mer), hepatitis A virus cellular receptor 2 / Tim-3, Clec4n/ dectin1, and Clec 4d/ dectin2 were noted to be more strongly expressed in the BMDC from NOD mice than those from NON mice (Table). On the contrary, the expressions of CD68, SRB1, integrin alpha M/ CR3, integrin alpha v, Fcgr2b, Fcgr3, low density lipoprotein receptor-related protein 1/ CD91, CD14, jumonji domain containing 6, TYRO3 protein tyrosine kinase 3, and Clec13b/ CD205 showed about equal intensity of expression between these strains.
Conclusion: In the present study, we showed that BMDC from NOD mice displayed elevated expressions of various receptors to uptake apoptotic cells compared to those from NON mice. Defects in apoptotic cell clearance reportedly contribute to the development of a lupus-like disease in the Mer-knockout mice. Contrarily in NOD mice with autoimmune-prone background, we propose that these receptors highly expressed in BMDC from NOD mice possibly lead to active uptake of self-antigens, and then to the predisposition to autoimmune diseases.

Table. Expressions of phagocyte receptors for apoptotic cells in DC

Surface receptors	NOD	NON	NOD/NON (%)
CD36	668.6	1384.1	207.0
macrophage scavenger receptor 1/ CD204	65.8	316.2	480.5
integrin beta 5	24.6	62.6	254.5
Fc receptor, IgG, high affinity I	25.2	115.0	456.3
CD93/ C1qR	78.4	192.5	245.5
c-mer proto-oncogene tyrosine kinase	45.4	154.7	340.7
hepatitis A virus cellular receptor 2 / Tim-3	29.1	68.4	235.1
C-type lectin domain family 4, member n/ dectin1	671	1552.6	231.4
C-type lectin domain family 4, member d / dectin2	154.3	365.8	237.1

485

The monocytes of type 1 diabetic patients (newly-diagnosed and long-standing) express significantly lower intensity of cell membrane HLA-DQ molecules compared to controls, while in long-standing patients very few monocytes express the surface markers CD122 (IL-2Rβ) and CD152 (CTLA-4)

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Background and aims: The monocytes are the main antigen-presenting cells in the body, playing an important role in autoimmunity. We have investigated the expression of key markers on monocytes (presence and intensity) that are important hereditary factors for type 1 diabetes (HLA-DQ, CTLA-4), and important in immune function (IL-2Rβ, CD122, TGFβ, and TGFβRII), particularly the generation and maintenance of Tregs, in type 1 diabetes (t1d) patients and controls.
Materials and methods: Peripheral blood from 13 newly-diagnosed type 1 diabetes patients (mean age 12.5 +/- 9.4 years, 9M/4F), including 5 followed-up 2 years later, 26 patients with long-standing disease (mean disease duration 13.8 years, mean age 26.7 +/- 9.2 years 12M/14F) and 32 healthy controls having no first or second degree relatives suffering from autoimmune diseases (mean age 25.3 +/-11 years,13M/19F), was analyzed via flow cytometry for the above characteristics of the monocyte population.
Results: Monocytes from all three groups uniformly expressed to high intensity HLA-DR molecules, with little differences in percent or intensity. By contrast, there was a significantly lower level of expression of HLA-DQ in the monocytes of t1d patients (MFI 111.53 +/- 40.02, 100.6 +/- 50.58, 157.70 +/- 104.41), of long-term, newly-diagnosed patients, and controls, respectively ($p=0.032$ and $p=0.02$, compared to controls, respectively). Very few monocytes of patients with long-lasting diabetes expressed IL-2Rβ (1.11 +/- 2.35 % vs. 23.12 +/- 22.52 % of newly-diagnosed patients and 23.56 +/- 25.90 % of controls; $p=0.006$ and $p<0.001$, respectively), or CTLA-4 (8.89 +/- 6.49 % vs. 25.31 +/- 28.79 % of newly-diagnosed patients and 23.67 +/- 27.17 of controls; $p=0.064$ and $p=0.007$, respectively). By contrast, cell surface expression of TGFβ, and TGFβRII was found in 23.77%, 32.39% and 36.42%, as well as 59.42%, 46.42% and 52.58% of long-term, newly-diagnosed t1d patients and controls, respectively (ns). In the 5 newly-diagnosed patients followed 2-years post onset, the surface expression of IL-2Rβ was significantly decreased to 0.24 +/- 0.19% of the monocytes ($p=0.005$), while CTLA-4 expression was non-significantly decreased.
Conclusion: The decrease in HLA-DQ monocyte cell membrane density in t1d patients may be due to the specific susceptibility alleles expressed by these patients and must be pursued along this line. By contrast, the significantly lower percent of monocytes bearing IL-2Rβ or CTLA-4 in long-standing t1d patients, appears to be manifested as early as 2 years post onset, and may signify decreased ability to participate in immune-regulatory networks. The documented presence of cell membrane TGFβ and TGFβRII, may signify additional factors in these networks.
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486

Association of adiponectin, IL-1ra, IP10 and number of islet autoantibodies with progression patterns of type 1 diabetes the first year after diagnosis

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Background and aims: Progression of type 1 diabetes after diagnosis is poorly understood. Our aim was to define distinct patterns of disease progression of juvenile-onset type 1 diabetes determined by preserved β-cell function, and further to assess the relation between disease progression and systemic cytokine concentrations and number of autoantibodies, the first year after diagnosis.
Materials and methods: Juvenile patients were subjected to a meal-stimulated C-peptide-test 1, 6, and 12 months after diagnosis with type 1 diabetes. On basis of C-peptide course from 1-6 months four progression groups were defined: patients with persistently low β-cell function ("stable-low"), patients with a rapid loss in β-cell function (rapid progressers), a slow loss in β-cell function (slow progressers) and patients with increased β-cell function (remitters). Serum concentrations of adiponectin, IL-1ra and IP10 were measured at 1, 6, and 12 months. GAD, IA-2A and ICA, were measured at 1 month.
Results: Serum adiponectin concentrations measured at 1 month predict disease progression at 6 months ($p=0.04$). Patients with adiponectin within the lowest quartile had a significant higher probability of becoming remitters than rapid progressers, OR 3.1 (1.3-7.6). At 6 and 12 months adiponectin differed significant between the groups ($p=0.03$ and $p=0.006$) with highest concentrations among stable-low and rapid progressers. IL-1ra and IP10 did not differ between the groups at any time point. Yet, rapid progressers showed significant decreases of IP10 from 1-6 months ($p=0.0007$). Number of autoantibodies differed significantly between the groups at 1 month ($p=0.05$), with rapid progressers having the highest number.
Conclusion: We defined distinct progression patterns distinguishing patients progressing rapidly from those diagnosed with low β-cell function, as well as patients with slow disease from those with actually improving β-cell function, pointing to different mechanisms of disease progression. We found that adiponectin concentration at 1 month predicts and associate with particular patterns of progression at 6 and 12 months. This indicates that adiponectin could be a potential biomarker for disease progression.

487

Latent autoimmune diabetes in adults vs recent-onset type 1 diabetes: differences in autoantibody levels, clinical and metabolic parameters were independent of the age of type 1 diabetes onset

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Background and aims: Previous studies have shown that Latent Autoimmune Diabetes in Adults (LADA) represents slowly developing form of Type 1 Diabetes (T1D), exhibiting similar as well as discrepant immunological, clinical and metabolic characteristics compared to recent-onset T1D. These similarities and differences between the two disorders were suggested to depend on the age of onset of T1D but they have not yet been clarified. Therefore the aim of this study was to compare: (a) the incidence of glutamic acid decarboxilase (GAD) and thyrosin phosphatase (IA2) autoantibody positivity, (b) clinical course and (c) insulin secretory capacity during the first year of the disease in: (A) LADA patients (group A) and (B) adult recent-onset T1D patients (group B) divided into subgroups in accordance to different age of onset (age < 20 yrs, group B1, n=22; age 20-25 yrs, group B2, n=39; age > 26 yrs, group B3, n=29).
Materials and methods: LADA was defined as diabetes without the need for insulin treatment during first 6 months after the onset of disease in patients aged > 35yrs. T1D was diagnosed in accordance to the WHO criteria. All patients were analysed in insulin requiring state. The GADA and IA2 positivity was determined by RIA. The clinical course was evaluated using the following parameters: duration of simptoms before diagnosis, frequency of ketosis, weight loss and incidence of clinical remission (CR). The CR was defined as optimal metabolic control without insulin lasting >30 days. The insulin secretion capacity was evaluated by determining C-peptide levels (RIA) before and after 1 mg i.v. glucagon stimulation (0/6 min).
Results: We found that percentages both of GADA and IA2 positive patients were lower in group A compared to all subgroups of the group B (A:3.7% vs B1:63.6; B2:59.3; B3:14.3%; p<0.001). However, the percentage of single positivity for GADA was higher in group A in comparison to all subgroups of the group B (A: 92.6% vs B1:4.5; B2:10.3; B3:65.3%, p<0.001). On the other hand, the percentage of single positivity for IA2 was lower in group A than in the B subgroups (A:3.7% vs B1:28.2; B2:27.3%, p<0.001, B3:3.6%, p=NS). Simultaneously, we detected significantly longer duration of simptoms (A:7.57±0.87 vs B1:2.73±1.67; B2 2.12±1.64; B3 2.83±197 months, p<0.01), lower frequency of ketosis (A:19.2% vs B1:61.9; B2:53.8; B3:50%, p<0.01), and smaller weight loss (A:4.03±2.18 vs B1:7.80±3.47; B2:6.34±3.26; B3: 6.73±3.64 kg, p<0.05) in group A than in all B subgroups. However, the basal and stimulated C peptide levels were significantly higher in group A than in all B subgroups (0 min A: 0.37 vs B1:0.18; B2:0.19; B3:0.20 nmol/l, p<0.01; 6 min A: 0.53 vs B1:0.2; B2:0.33; B3:0.35 nmol/l, p<0.01). In addition, the incidence of clinical remission was significantly lower in group A than in all B subgroups (A:13.8% vs B1:63.6; B2:59.0; B3:41.4%, p<0.05).
Conclusion: Our results have demonstrated that patients with LADA exhibited lower autoantibody response, together with milder clinical course and better preserved insulin secretion capacity, as well as less frequent CR in comparison to the recent-onset T1D. In additon, our results have shown that these differences were not dependent on the age of onset of T1D.

488

Association of immune reactivity with metabolic control in new-onset type 1 diabetes patients

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Background and aims: Previously, we reported an association of T-cell reactivity measured by ELISPOT with ß-cell function determined by stimulated C-peptide in the context of a prospective placebo controlled intervention trial in recent type 1 diabetes.
In a sub analysis, we have tested independently for additional associations of T-cell reactivity with metabolic control as determined by A1C and blood glucose.
Materials and methods: 50 adult (mean age 27.26 ±8.1 years) and 49 pediatric patients (mean age 10.9 ±2.8 years) with recent onset type 1 diabetes were included in the DiaPep277 Trial. We investigated secretion of IFN-γ (Th1), interleukin (IL)-5 and IL-13 (Th2) and IL-10 (Treg) by peripheral mononuclear cells (PBMC) upon stimulation with islet antigens peptide fragment of Islet Antigen 2 (pIA2), heat shock protein 60 (hsp60) or glutamic acid decarboxylase (GAD65) by ELISPOT according to the Peakman protocol. Cytokine responses entered regression analysis corrected for background signal as stimulation indices. Regression analysis included cytokine responses as dependent variable, A1c and blood glucose as independent variable. Analysis was adjusted for confounding factors such as sex, age, BMI, DiaPep277 treatment, HLA and AUC C-peptide.
Results: Cytokine secretion of PBMC upon islet antigen stimulation showed associations with A1C and blood glucose in adults and children.
In adults, response of Th1 related IFN-γ upon stimulation with GAD65 was positively associated with A1C (p=0.027) but negatively with blood glucose (p=0.002) at week 30. In addition, secretion of IL-10 upon stimulation with GAD65 was positively associated with A1C (p=0.042) at week 8.
In children, IL-13 showed negative associations with A1C but positive associations with blood glucose upon stimulation with GAD65 and pIA2 at week 8 (all, p<0.05). In addition, IL-13 showed negative associations with A1C upon stimulation with GAD65 at week 30 and 78 and hsp60 at week 78 (all, p<0.05). As in adults, IL-10 response showed positive association with A1C but negative association with blood glucose upon stimulation with hsp60 and GAD65 at week 0 and 8, respectively (all, p<0.05).
Conclusion: Cytokine secretion of PBMC upon stimulation with islet antigens was associated with metabolic control as determined by A1C and blood glucose in both adults and children. The association of A1C with immune response may give a basis for the observation by others that worse metabolic control leads to worse clinical outcome. Higher A1c was associated with increased response of Th1 related IFN-γ in adults and with decreased response of Th2 related IL-13 in children. IL-10 response was positively associated with A1c in paediatric and adult patients which may reflect a counter-regulatory attempt of the immune system to regulate immune reactivity.
We thank ANDROMEDA for providing DiaPep277

489

Positive correlation between the serum DPP-4 enzymatic activity and the CD3+ lymphocyte membrane bound CD26 expression in patients with type 1 diabetes mellitus

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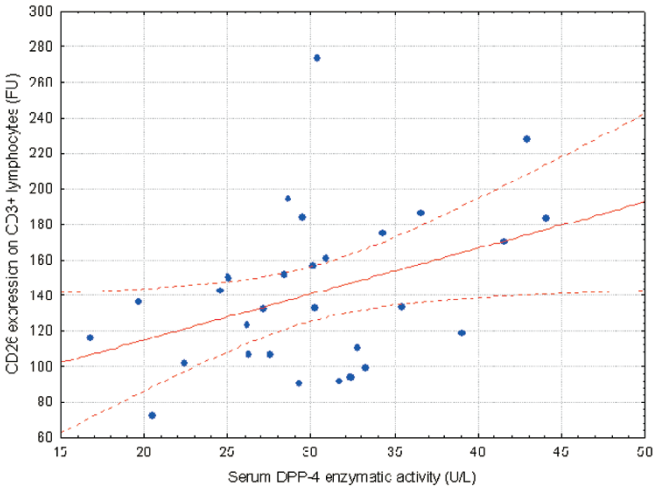
Background and aims: Serum dipeptidyl peptidase-4 (identical to CD26) has an important role in carbohydrate metabolism with the degradation of incretin-hormones (GIP, GLP-1). Membrane bound CD26 is widely present in the human body among many other cell types it is also expressed on the surface of lymphocytes. Both the soluble and the membrane bound form of CD26 can modulate in vitro T-cell proliferation and the serum enzymatic activity is also sometimes used as a marker of T-cell activation. DPP-4 inhibitors have convincingly demonstrated their beneficial effect in the treatment of type 2 diabetes, however little amount of data are available on the role of CD26 in type 1 diabetes mellitus.
Materials and methods: Thirty-five (F/M=17/18, mean age: 34.7yrs) patients with T1DM, and 27 (F/M=15/12 mean age:28.4yrs) young healthy volunteers were included in the study. Fasting serum DPP-4 activity was assessed in all the 62 individuals. Years from diagnosis of diabetes: 14.39 yrs, (95%CI: 10.6-18.2yrs). Serum DPP-4 activity was measured by microplate-based (Multiskan-Plus-MKII, Labsystem at 405 nm, 25°C for 30 min) kinetic assay. Gly-Pro-pNA (Bachem, Bubendorf, Switzerland) was used as substrate. Results are expressed in nmol/ml/min (U/L) pNA hydrolysis. Membrane bound CD26 expression was evaluated on CD3+ and CD4+ lymphocytes by Fluorescence Activated Cell Sorting method in a FACS Excalibur System. Results are expressed in fluorescens unit (FU). Kolmogorov-Smirnov normality test, two tailed T-test and Pearson correlation was used.
Results: We experienced a significant increase in the fasting serum DPP-4 enzyme activity in type 1 diabetes (30.35U/L, 95%CI: 28.1-32.6) compared to healthy individuals (23.18U/L, 95%CI: 21.26- 25.1, two-tailed p< 0.0001). CD26 expression on CD3+ and on its subpopulation CD4+ lymphocytes was not increased in T1DM compared to healthy subjects. However we found significant positive correlation between the CD26 expression on CD3+ lymphocytes and the serum DPP-4 enzyme activity within the T1DM group (r^2=0.14; r=0.38; p = 0.036; Figure 1).
Conclusion: The significant positive correlation between the CD3+ lymphocyte membrane bound CD26 expression and the fasting serum DPP-4 enzymatic activity in type 1 diabetes mellitus might support the hypothesis

that the excess DPP-4 activity in the serum of patients with T1DM might in part be the consequence of the autoimmune process. However the low r^2 value indicates that this correlation could not entirely be taken into consideration for the significant increase in serum DPP-4 activity in T1DM.



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490

Zinc transporter 8 autoantibody in Chinese type 1 diabetes

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Background and aims: Zinc transporter 8 (ZnT8) is a member of the SLC30A family that play a critical role in insulin maturation, storage and secretion in β cells. ZnT8 was recently identified as a major autoantigen in the development of Caucasian type 1 diabetes (T1D). The present study aimed to evaluate the diagnostic utility of ZnT8A for autoimmune T1D in Chinese patients.
Materials and methods: Radioligand binding assays based on human ZnT8 carboxytermini (amino acids 268-369) carrying 325Arg (CR) and a dimeric construct of 325Arg and 325Trp (CW-CR) were used to evaluate ZnT8A in a group of 429 T1D diabetic patients, 555 type 2 diabetic (T2D) patients and 405 healthy controls. T1D patients were divided into Ab+ and Ab- T1D subsets according to their glutamate decarboxylase antibody (GADA) and protein tyrosine phosphatase antibody (IA-2A) status and the ZnT8A distributions were analyzed in the two subsets. The clinical characteristics were also compared among GADA+, IA-2A+, ZnT8A+ alone and Ab- groups.
Results: The prevalence of ZnT8A in T1D patients of 24.2% (105/429), was markedly higher than that in T2D 1.9% (10/555) and controls 1.0% (4/405) (both P <0.001). ZnT8A and IA-2A in newly diagnosed Chinese T1D patients were less prevalent than in Caucasian populations (both P<0.001) , though not different from that in Japanese. Moreover, ZnT8A were more prevalent in the leaner cases in Ab-T1D subset. ZnT8A levels were correlated with IA2-A (R=0.211, P=0.001) but not with GADA (R= -0.107, P=0.093). The diagnostic sensitivity of autoimmune diabetes reached 65.3% when combining 3 antibodies (GADA, IA-2A and ZnT8A). About16 % (28/177) of T1D cases, who were negative for GADA and/or IA-2A, tested ZnT8A positive. Such individuals had longer duration of disease, a higher requirement for insulin (both P<0.05), yet presented with lower glycemia, higher fasting C-peptide and higher body weight than GADA+ or IA-2A+ subjects alone (P<0.05~0.001).
Conclusion: ZnT8A is an independent marker of ß-cell autoimmunity in GADA /IA-2A negative T1D cases and improved the diagnostic sensitivity of immune-mediated T1D when combined with GADA and/or IA-2A. Moreover, ZnT8A identified a milder T1D subset, intermediate between the phenotypes of GADA+/IA-2A+ alone T1D and Ab-T1D.
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PS 27 Immune modulation in diabetes

491

Epitope analysis of GAD65 autoantibodies in type 1 diabetes and LADA patients with thyroid autoimmunity

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Background and aims: Type 1 diabetes (T1DM) is frequently associated with autoimmune thyroid diseases (AITD). Latent autoimmune diabetes in adults (LADA) is a slowly progressive form of autoimmune diabetes, which also has high risk for thyroid autoimmunity. GAD65Ab is one important antibody in T1DM and LADA patients, but it also found in AITDs. This study was aimed to investigate the epitopes of GAD65 autoantibodies in T1DM and LADA patients with and without thyroid autoimmunity.
Materials and methods: The GADAb levels and their relative epitope reactivities to N-terminal (GAD65-N), Middle (GAD65-M) and C-terminal (GAD65-C) regions of human GAD65 were measured by radioligand immunoassay in 112 T1DM, 107 LADA patients. TPO-Ab and TG-Ab were measured by radioimmunoassay.
Results: Antibodies reactivity to N-terminal,Middle,C-terminal epitope of GAD65 was 11.6%,31.2%,68.8% in T1DM patients and 22.4%,31.8%,47.7% in LADA patients respectively. GAD65Ab reacting with the N-terminal epitope were more common in LADA than in T1DM patients (22.4% *vs* 11.6%, P=0.002). More T1DM patients recognized C- terminal of GAD65 than LADA patients (68.8% *vs* 47.7%, P=0.002). LADA patients with M+C-predominant GAD65Ab reactivity have higher risk for thyroid autoimmunity, lower C-peptide level and the requirement for insulin therapy compared with non-M+C-reactive patients (P<0.05). Compared with those without thyroid autoimmunity, more T1DM patients and LADA patients with thyroid autoimmunity had antibodies directed to both GAD65-M and GAD65-C (46.0% *vs* 14.5% and 51.6% *vs* 18.4% respectively, P<0.001).Multiple logistic regression shown that GAD65-M+CAb positivity was significantly associated with thyroid autoimmunity in T1DM and LADA patients (OR=5.016 and 4.724 respectively, P<0.05).
Conclusion: The majority of T1DM and LADA patients had antibodies directed to Middle and C-terminal epitopes of GAD65. LADA patients with M+C-predominant GAD65 reactivity have clinical features similar to those of T1DM patient. T1DM and LADA patients with GAD65-M+C epitopes specific reactivity showed an increase risk for thyroid autoimmunity.
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492

Common and unique environmental events associated with innate and adaptive immunity in type 1 diabetes

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Background and aims: Type 1 diabetes mellitus is caused by destructive adaptive and innate immune responses. Serum diabetes-associated autoantibodies, to glutamic acid decarboxylase (GADA), insulinoma-associated antigen-2 (IA-2A) and zinc transporter-8 (ZnT8A), reflect adaptive immunity, while carboxy-methyl-lysine (CML), an advanced glycation end-product, is associated with proinflammation and innate immunity. We assessed whether genetic or environmental factors determine serum autoantibodies and CML levels in type 1 diabetes.
Materials and methods: Serum autoantibodies and CML were determined in two prospective studies: a classical twin study of twins discordant for type 1 diabetes (32 monozygotic (MZ), 32 dizygotic (DZ) pairs) and a population study of 7,287 normal subjects. CML levels were determined by ELISA, autoantibodies by radio-immunoprecipitation,

Results: Baseline CML and autoantibodies predicted diabetes additively. CML levels were increased in diabetic and non-diabetic twins, population-based autoantibody positive and pre-diabetic subjects (all p<0.001). Twin CML correlations were strong, irrespective of zygosity: r(MZ) = 0.70, r(DZ) = 0.66; model fitting showed common environmental factors explained 66% of variance. Autoantibodies occurred more frequently in diabetic than non-diabetic twins (p<0.001); but twin correlations (r) were weak, irrespective of zygosity: e.g. IA-2A, r(MZ)=0.06, r(DZ)=-0.03; model fitting showed unique environment explained all variance.
Conclusion: In twin studies, environmental factors were strong determinants of two diabetes-associated features, which predicted type 1 diabetes additively in a large population study. Common environmental factors determined serum CML levels, while unique factors determined diabetes-associated autoantibodies. We postulate that distinct, additive, environmental events are associated with type 1 diabetes, common events activating innate immunity, unique events inducing adaptive immune responses.
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493

Modulation of innate immunity in enterovirus-infected human pancreatic islets
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Background and aims: Type 1 diabetes (T1D) is a chronic disease characterized by the selective destruction of insulin-producing cells in the pancreas. Enterovirus (EV) is the prime candidate to initiate this destruction and several inflammatory chemokines are induced by EV infection, potentially involved in the induction of insulitis. A protective effect of the female sex hormone 17β-estradiol on isolated human islets has been shown. The aim of this study was to evaluate the effect of 17β-estradiol on EV-induced chemokine secretion and cytolysis of human islet cells. In addition, the islet response to EV infection was compared to the response to the synthetic dsRNA analogue polyinosinic:polycytidylic acid (Poly I:C).
Materials and methods: Human islets, cultured with or without the addition of 17β-estradiol, were exposed to synthetic dsRNA (poly(I:C)) or infected with an EV strain isolated from a diabetic child at T1D onset. Secretion and gene expression of several cytokines were analyzed with ELISA and real-time quantative PCR (RT-qPCR) respectively. Virus replication was determined by tissue culture infectious dose 50 ($TCID_{50}$) titrations on green monkey kidney cells.
Results: The results confirm previous findings that EV infection induces high level IP-10 and MCP-1 secretion from human islets and show that this secretion is markedly reduced by addition of 17β-estradiol to the culture medium. In contrast, the IP-10 and MCP-1 secretion induced by treatment of islets with poly(I:C) is not affected by 17β-estradiol, suggesting that separate signalling pathways are responsible for the innate immune response triggered by EV and poly(I:C). 17β-estradiol did not affect viral replication but reduced the cytopathic effect of the virus on the islets. Exposure of the islets to poly(I:C) 24 h prior to EV infection either blocked (2 donors), reduced (3 donors), or had no effect (1 donor) on EV replication. A negative effect of poly(I:C) on EV replication correlated with a high induced expression level of genes involved in the innate immune response to virus infection (interferon-β, MDA-5, TLR3, and RIG-I). Surprisingly, interferon-α was not induced by any of the treatments. The antiviral effect of poly(I:C) could not be reduced by addition of 17β-estradiol, which is in line with the finding that 17β-estradiol had no effect on poly(I:C)-induced chemokine secretion.
Conclusion: The reported findings might have direct implications for the understanding of the etiology and forthcoming prevention of T1D. It also highlights the importance of genetic diversity in shaping the innate immune response to EV in islets, which most likely is involved in controlling the later antigen-specific adaptive immune response. Further studies on the genetically determined differences in response to acute EV infection need to be performed in order to explain the interplay between infection and genetic predisposition to T1D.
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494

Effects of metformin vs sulphonylurea on immune activity of Natural Killer (NK) cells in individuals with newly diagnosed type 2 diabetes
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Background and aims: Diabetic hyperglycemia may suppress the function of peripheral blood NK cells which represent the first line defense in the immune system. The earlier obtained results revealed significant impairment in activity of NK cells of diabetic patients in comparison to healthy control subjects (CS). Hypoglycemic drugs may influence and improve the immune activity of NK cells. In order to explore this hypothesis we evaluated the number and activity of NK cells obtained from newly diagnosed Type 2 diabetic patients (T2DP) before and after the administration of either metformin or sulphonylurea in comparison with CS.
Materials and methods: Incubation tests were performed at baseline in 25 newly-diagnosed T2DP, naive to any hypoglycaemic drugs and 20 carefully matched CS; in the diabetic patients, the tests were repeated after 6 and 12 months of therapy either with metformin (13 persons) or sulphonylurea - gliclazide (12 persons) accordingly to clinical indications. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll gradient centrifugation. The K562 human erythroleukemia cell line was used as the standard target for human NK cytotoxicity assay. K562 were labelled with DIO(3,3-dioctadecyloxacarbocyanine perchlorate). Target and effector cells were added to reach effector/target rations: 50:1 ,12:1. Dead target cells were stained with PI (propidium iodide). After 4 hours of incubation data were collected for analysis on the Becton-Dickinson FACScalibur flow cytometer. Immunofluorescent phenotyping of NK ($CD16^+$) cells in PBMC was performed using specific murine anti-human CD16 PE-conjugated monoclonal antibodies. The data was analyzed using Cell Quest software.
Results: The T2DP at baseline in comparison to CS had an increased number (14,27±6,2% vs 9,59±4,8%) but decreased activity (3,0±2,4% vs 9,1±3,8%) of NK cells (p<0,01). Treatment with metformin resulted in reduction in fasting plasma glucose (FPG) of 1.65 mmol/L (P<0.001) and in HbA_{1C} of 0.82% (P<0.001) after 6 months and further reduction of 0.48 mmol/L in FPG and of 0.34% in HbA_{1C} after 12 months of therapy. The T2DP treated 6 months with metformin demonstrated substantially decreased number and increased activity of NK cells in comparison to baseline values(10,91±4,7% vs 14,69±5,1% and 5,6±2,9% vs 2,9±2,0%)(p<0,01). Further 6 months of metformin therapy resulted in not statistically significant differences of these parameters (10,59±4,0% and 6,0±3,2%, respectively). Treatment with gliclazide resulted in reduction in FPG of 1,77 mmol/L (p<0,001) and in HbA1c of 0,69% (p<0,001) after 6 months and further reduction of 0.26 mmol/L in FPG and of 0.14% in HbA1c after 12 months of therapy. The T2DP after 6 months of gliclazide administration demonstrated decreased number and increased activity of NK cells in comparison to baseline values (12,13±3,9% vs 13,93±4,8% and 6,5±3,0% vs 3,1±2,2%)(p<0,01). This tendency became more intense after 12 months of therapy (10,12±4,2% and 7,3±3,5%, respectively)(p<0.05).
Conclusion: Both metformin and gliclazide improve immune activity of NK cells in Type 2 diabetes. The beneficial influence of metformin on NK cells number is accelerated and more pronounced than gliclazide. These findings constitute immune response as a new potential target of oral hypoglycemic drugs in type 2 diabetes mellitus.

495

Protection of the beta cell function by treatment with a rat specific CD3 antibody alone or in combination with the immunomodulatory agent FTY720 in the IDDM rat, an animal model of human type 1 diabetes
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Background and aims: The IDDM (LEW.1AR1-*iddm*) rat is an animal model of type 1 diabetes mellitus (T1DM) without lymphopenia. The aim of the treatment with CD3-antibody (AB) alone or in combination with the immunomodulatory agent FTY7220 was to prevent clinical manifestation of diabetes or to protect the remaining pancreatic beta cells from autoimmune destruction after onset of the disease. The CD3-AB interacts with the T-cell receptor to inhibit proliferation and cytokine production of T-lymphocytes, while the sphingosin-1-phosphate receptor antagonist FTY720 retains the lymphocytes in the organ draining lymph nodes.

Materials and methods: For the purpose of primary or secondary prevention the animals were treated with CD3-AB (0.5 mg/kg body weight) consecutively over 5 days and additionally in the combined therapy over 40 days with FTY720 (1 mg/kg body weight). Gene and protein expression of pro- and anti-inflammatory cytokines, MCP-1 and iNOS were analysed in pancreatic biopsies and the pancreas-draining lymph nodes at the time point of diabetes manifestation, at the end of therapy, and 60 days after the end of treatment.
Results: Primary prevention therapy with CD3-AB initiated before diabetes manifestation suppressed clinical diabetes manifestation. However, 60 days after CD3-AB treatment 60 % of the CD3-AB treated animals showed signs of immune cell infiltration, as documented by infiltration with macrophages and CD4 and CD8 T lymphocytes in the islets. But there was no increased beta cell apoptosis in the infiltrated islets. Ultrastructurally, the beta cells in the infiltrated islets showed signs of an unfolded protein response which were absent in beta cells of non infiltrated islets. The immune cells of the infiltrated islets in the treated animals expressed no pro-inflammatory cytokines, neither IL-1β, IFN-γ, nor TNF-α, on the gene and protein level. In the secondary prevention therapy with CD3-AB initiated after overt diabetes manifestation there was a sluggish increase of the blood glucose concentration over the first three weeks after treatment in comparison to the spontaneous development. The slower increase of the blood glucose was accompanied by a strong decrease of the pro-inflammatory cytokine protein concentrations in the peripheral blood. The remaining beta cells after diabetes manifestation could not be protected permanently against the autoimmune attack by CD3-AB alone. The combined treatment of CD3-AB and FTY720 induced a permanent normoglycaemia over 60 days after therapy. The remaining beta cells in the islets were ultrastructurally well preserved and the immune cell infiltration was restricted to the perivascular region.
Conclusion: The findings after CD3-AB treatment in the IDDM rat confirmed earlier observations in NOD mice and in humans with T1DM and illustrate the problem with a single compound therapy approach. In a combined therapy with CD3-AB and FTY720, however, the later compound retains the lymphocytes in the pancreas draining lymph nodes and thereby makes the therapy success permanent by prevention of pancreatic islet immune cell infiltration.

496

Preservation of residual beta cell function in newly diagnosed type 1 diabetes by treatment with atorvastatin: the DIATOR trial

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Background and aims: The lipid-lowering agent atorvastatin is also a potent immunomodulator. We therefore investigated the effect of atorvastatin on the progressive decline of residual beta cell function in patients with newly diagnosed type 1 diabetes.
Materials and methods: Adult patients with newly diagnosed type 1 diabetes and islet autoantibodies were randomly assigned to treatment with either atorvastatin or placebo in a multi-centre, outpatient, parallel group study. During a run-in period of 6 weeks the daily dose was increased from 20 mg to 80 g/day, which was to be reduced to 40 mg/day in case of side-effects (e.g. myalgia). Efficacy on beta cell preservation was assessed by means of two-hour standardised mixed-meal tests at baseline and after 12 and 18 months of treatment. A tight glycaemic control was aimed at with a target of HbA1c ≤ 6.5 %.
Results: Eighty-nine patients (mean age 30 years, median BMI 24 kg/m², 40 % females) participated in this study, 21 dropped out before the one year visit (8 placebo, 13 atorvastatin). The intention-to-treat analysis showed a significant drop in total serum cholesterol concentrations with atorvastatin treatment (median from 156 to 106 mg/dl). Median HbA1c levels at 18 months were 6.8 % in the placebo and 6.5 % in the atorvastatin group, the corresponding insulin doses were 0.34 vs 0.43 IU/kg (differences not significant). Median autoantibody levels (GADA, IA-2A, ICA) changed little during the study period. Median fasting C-peptide levels dropped from baseline to 12 and 18 months in the placebo group (from 0. 34 to 0.23 and 0.20 pmol/ml, $p < 0.001$) whereas they remained stable in the atorvastatin group (from 0.34 to 0.27 and 0.30 pmol/ml, ns). Median mixed-meal stimulated C-peptide concentrations dropped slightly between baseline and 12 months in both groups (placebo from 0.89 to 0.71, atorvastatin from 0.88 to 0.73 pmol/ml, $p < 0.01$ each) followed by a major loss in the placebo group (to 0.48 pmol/ml) but not in the atorvastatin group (to 0.71 pmol/ml, ns). Atorvastatin treatment was well tolerated and serious drug-related adverse events were not observed.
Conclusion: Daily treatment with atorvastatin slowed the loss of residual beta cell function in people with newly diagnosed type 1 diabetes over the study period of 18 months. Atorvastatin appears to interfere with mechanisms of autoimmune or inflammatory beta cell destruction.
Supported by: an unrestricted grant from Pfizer Pharma GmbH, Berlin, Germany

497

Adiponectin receptor expression on peripheral blood mononuclear cells is reduced in autoimmune diabetes and can be upregulated with lifestyle intervention

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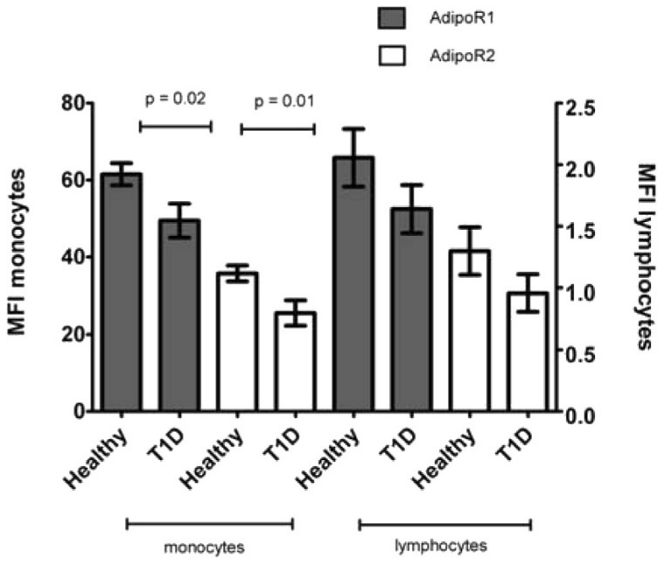
Background and aims: Studies of patients with genetic predisposition for T1D show that insulin resistance (IR) is an independent risk factor for T1D. We are interested in defining the mechanisms that link IR with progression to T1D, and in particular the potential role of adipocytokines such as adiponectin. Adiponectin is an anti-inflammatory and insulin sensitising cytokine whose levels are inversely associated with IR. It signals through receptors AdipoR1 & AdipoR2, whose expression levels are increased by manoevres that reduce IR. These receptors have previously been found on peripheral blood mononuclear cells (PBMC) where stimulation reduces their proliferation and cytotoxicity, and increases their secretion of anti-inflammatory cytokines. We hypothesise that adiponectin has an anti-inflammatory effect on human islet autoimmunity. Our initial aim was to assess the expression of adiponectin receptors on PBMC in healthy and T1D subjects, and whether these expression levels could be modulated by exercise.
Materials and methods: PBMC expression of AdipoR1 & AdipoR2 was quantified by flow cytometry and qPCR on 26 T1D subjects and 14 age and anthropometrically matched healthy male Caucasian volunteers. The proportion of cells expressing each receptor, and the mean fluorescence intensity (MFI) were calculated by subtracting from subject-specific isotype control staining. The estimated glucose disposal rate (eGDR) of T1D subjects was calculated using waist hip ratio, blood pressure and HbA1C as previously described. In addition, we measured the effect of 6 months of diet and exercise training on adiponectin receptor expression by PBMC using qPCR, in 9 subjects with autoimmune diabetes.
Results: AdipoR1 & AdipoR2 are expressed on monocytes and lymphocytes in both healthy and T1D subjects. On average, 47% of monocytes and 11% of lymphocytes expressed AdipoR1 and AdipoR2. AdipoR1 & AdipoR2 expression is decreased on monocytes (with a tendency to decrease in lymphocytes) in T1D compared to healthy subjects (figure). AdipoR1 & AdipoR2 expression by both monocytes and lymphcytes, measured by MFI, correlated significantly with eGDR in subjects with T1D (monocytes AdipoR1 $r = 0.475$, AdipoR2 $r = 0.512$; lymphocytes AdipoR1 $r = 0.292$, AdipoR2 $r = 0.420$). Lifestyle intervention increases AdipoR1 & AdipoR2 gene expression at 6 months (Fold change: AdipoR1 2.6 $p = 0.02$, AdipoR2 1.5, $p = 0.07$).
Conclusion: Adiponectin receptor expression is decreased on PBMC from T1D subjects. These findings are consistent with adiponectin playing an anti-inflammatory role on islet autoimmunity, and may explain the association between insulin resistance and the development of T1D. Furthermore, therapies that reduce IR may also modulate the development of this disease.



Supported by: NHS UK, Diabetes UK, Novo Nordisk

498

Protection against spontaneous immune-mediated diabetes in the IDDM rat by CD8+ T cell transfer correlates with an anti-inflammatory cytokine shift within the pancreas-draining lymph nodes

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Background and aims: The IDDM (LEW.1AR1-*iddm*) rat is an animal model of spontaneous human type 1 diabetes mellitus. This study analysed how adoptive transfer of selective T cell subpopulations affects the incidence of diabetes and the infiltration stages in the islets.
Materials and methods: $CD4^+$ or $CD8^+$ T cells were isolated from diabetic IDDM rats or the diabetes-resistant background strain LEW.1AR1. Lymphocytes were selectively transferred into athymic LEW.1AR1-*Whn*^{rnu} or prediabetic IDDM rats. The animals were monitored for blood glucose, islet infiltration and immune cell composition of pancreas-draining lymph nodes.
Results: After adoptive transfer of $CD4^+$ T cells from diabetic IDDM rats into athymic LEW.1AR1-*Whn*^{rnu} rats, 50% of the recipients developed diabetes. Transfer of $CD8^+$ T cells failed to induce diabetes. Only 10% of the athymic recipients became diabetic after co-transfer of $CD4^+$ and $CD8^+$ T cells. Adoptive transfer of $CD8^+$ T cells from LEW.1AR1 or diabetic IDDM rats into prediabetic IDDM rats halved the incidence of diabetes. In the protected normoglycaemic animals without any immune cell infiltration in the pancreatic islets regulatory $CD8^+/CD25^+$ and $CD4^+/CD25^+$ T cell subpopulations co-expressing FOXP3 accumulated in the pancreas-draining lymph nodes. Corresponding to the increased accumulation of both regulatory T cell subpopulations gene and protein expression shifted from a pro-inflammatory to an anti-inflammatory cytokine profile in comparison with the lymph nodes from diabetic rats after transfer of $CD8^+$ T cells.
Conclusion: Our results show that adoptive transfer of $CD4^+$ but not $CD8^+$ T cells from diabetic IDDM rats induced diabetes development. Importantly, $CD8^+$ T cells from diabetic IDDM rats and diabetes-resistant LEW.1AR1 rats provided protection against immune cell infiltration leading to beta cell destruction in the islets. The additional accumulation of regulatory T cells in the pancreas-draining lymph nodes from protected rats indicates that transferred $CD8^+$ T cells may have beneficial effects in the control of beta cell autoimmunity.

PS 28 Action of insulin and its analogues

499

A direct comparison of pharmacodynamics and pharmacokinetics of insulin detemir and neutral protamine lispro (NPL) insulin in subjects with type 1 diabetes

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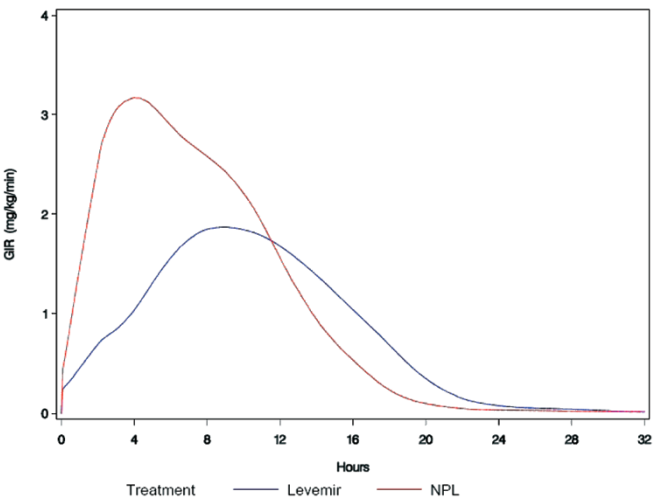
Background and aims: Basal insulin preparations attempt to recreate the low and constant plasma insulin levels seen between meals and overnight in normal physiology. For the insulin analogues detemir and glargine, comparable flat time-action profiles over 24 hours have been described. The aim of this study was to compare the pharmacodynamic and pharmacokinetic properties of a new insulin analogue, neutral protamine lispro (NPL) with those of insulin detemir in a euglycaemic glucose clamp trial.
Materials and methods: 30 subjects with C-peptide negative type 1 diabetes (42±11 years, BMI 25.4±3.0 kg/m², HbA1c 7.7±1.1 %, mean±SD) were randomly allocated to single dose administration of insulin detemir and NPL insulin. After normalization of plasma glucose with a variable insulin infusion, 0.4 U/kg BW of either insulin was administered on 2 separate dosing visits in a double-blind fashion. Insulin infusion was stepwise decreased and a euglycaemic clamp was initiated with a plasma glucose target of 5.5mmol/l over 32 hours. Duration of action was defined as the time from dosing until plasma glucose consistently exceeded 8.3mmol/l without glucose infusion.
Results: The primary endpoint "duration of action" was similar for insulin detemir and for NPL insulin (23.6±6.2h vs. 22.6±7.2h; p=0.557), with detemir showing a lower inter-subject variability. Compared to NPL insulin, insulin detemir exhibited a flatter pharmacodynamic profile reflected by significantly different glucose infusion rate (GIR) results [AUCGIR(0-32h) 1326 mg/kg (insulin detemir) vs. 1841 mg/kg (NPL insulin); p<0.01; AUCGIR(0-12h) 784 mg/kg vs. 1392 mg/kg; p<0.001; AUCGIR(12-32h) 455 mg/kg vs. 274 mg/kg; p<0.02) and a higher GIRlate(12-32)/GIRearly(0-12) ratio (0.33 vs. 0.04; p<0.01). Consequently detemir showed a lower peak action (GIRmax 2.0 mg/kg/min vs. 3.2 mg/kg/min, p<0.005) as well as a later peak of action (Tmax 7.2±2.4h vs. 6.2±4.5h)) when compared with NPL insulin.
Conclusion: The results of this study indicate that - using a single dose of 0.4U/kg - both basal insulin analogues have duration of action of approximately 24 hours and are hence both suitable for once daily dosing. That said, insulin detemir exhibited a flatter profile with a more even distribution of the metabolic effect over the 32 hours, whereas for NPL the main effect was observed in the first 12 hours. Furthermore, insulin detemir had lower GIRmax and less variability. Clinical trials with head to head comparison are thus needed to establish the superiority of one or more analogue preparations.



500

Impact of alternative splicing of insulin receptor to binding and signalling of insulin analogues

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Background & aims: The human insulin receptor is expressed as two isoforms that are generated by alternative splicing. The two mature proteins differ by the presence or absence of 12 amino acids encoded by exon 11 at the C terminus of the extracellular α-subunit. The aim of the present study was to explore the impact of the presence or absence of exon 11 to binding and signaling of the insulin analogs glargine, glulisine, and insulin AspB10.
Materials & methods: The affinity of insulin, insulin analogs, IGF-I, and IGF-II to the IR isoforms A and B was analysed in a competitive binding assay using SPA technology. IR autophosphorylation was measured via in-cell western (ICW) in CHO cells overexpressing either human IR-A or -B. Dephosphorylation kinetics were analysed using a Luminex XMAP System.
Results: Analysis of the binding of human insulin to the two IR splice variants A and B did not reveal a significant difference in the measured IC50 values (0.49±0.04 vs. 0.57±0.02 nmol/L, p=0.27). In contrast both IGF-I and IGF-II showed a higher affinity to IR-A. IGF-I binds 2.7x (64.5±5.1 vs. 171±50 nmol/L, p<0.05), and IGF-II 8.8x (6.2±0.34 vs. 46.6±7.8 nmol/L, p<0.05) better to IR-A, respectively. Measuring the binding of the insulin analogs glargine, glulisine and AspB10 resulted in the following IC50 values - glargine: 0.83±0.08 vs. 1.10±0.12 nmol/L, p=0.1015; glulisine: 0.88±0.07 vs. 1.08±0.25 nmol/L, p=0.5077; AspB10: 0.06±0.01 vs. 0.21±0.03 nmol/L, p<0.05.
Analysing IR autophosphorylation in CHO-IR-A or IR-B cells via ICW resulted in EC50 values as follows for IR-A and IR-B, respectively - insulin: 11.95±1.65 vs. 11.67±1.43 nmol/L; IGF-I: 449±61.7 vs. >1,000 nmol/L; IGF-II: 85.4±5.5 vs. 384±68.4 nmol/L; glargine: 19.03±3.39 vs. 26.39±3.34 nmol/L; glulisine: 49.6±6.05 vs. 51.7±7.22 nmol/L; AspB10: 5.12±0.69 vs. 3.32±0.78 nmol/L.
To assess dephosphorylation kinetics CHO cells expressing either IR-A or IR-B were stimulated for 10 min with insulin or analogs, then the medium was replaced and the IR phosphorylation was analysed at various time points. From these data half-lifes for IR-A were calculated: insulin 6.87 min, glargine 4.87 min, glulisine 6.54 min, and AspB10 17.7 min, and also for IR-B: insulin 12.04 min, glargine 10.74 min, glulisine 12.91 min, and AspB10 298 min.
Conclusion: Presence or absence of the 12 amino acids of exon 11 in the α-subunit of the IR results in different binding properties, especially IGF-I and IGF-II have a considerable higher affinity to IR-A. Analysing the binding properties of insulin analogs revealed that only insulin AspB10 has a significant unique profile with a 3.5x higher affinity to IR-A. Autophosphorylation of the IR kinase domain after ligand binding is the first step of the insulin signaling cascade. Analysis of this event via ICW reflected the observed higher binding of IGF-I/II to IR-A in a more potent activation of IR-A than IR-B. However, no special characteristics were found for insulin or the studied analogs. Dephosphorylation of IR-A occurs twice as fast as of IR-B after a stimulus with insulin, glargine or glulisine, respectively. Both IR splice variants show an extreme elongated dephosphorylation profile with AspB10. This protracted dephosphorylation of IR has been discussed as causative for increased mitogenic activity of AspB10 via the IR. Taken together these data show that in contrast to AspB10 the interaction of insulin glargine and glulisine with IR-A and IR-B is very similar to human insulin.

501

Glycaemic basis for reduced feeding by intact and diabetic rats in response to injections of insulin detemir

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Background and aims: Insulin detemir (DET) is a long-acting insulin analog that binds reversibly to albumin in the serum and interstitial fluid. Numerous studies indicate that the clinical use of DET in diabetic patients results in more stable long-term glycemia, fewer episodes of hypoglycemia, and decreased weight gain, in comparison with long-acting protamine (NPH) forms of insulin. The mechanisms for the weight-gain-sparing effect of DET are however unknown. We tested whether the glycemic effects of these two forms of insulin alter food intake (FI) in intact and diabetic rats in a manner that may influence body weight (BW) gain on a long-term basis.
Materials and methods: Groups of intact (INT) and mildly streptozotocin-diabetic (STZD) female Sprague-Dawley rats (n = 6-8) with 3-hr fasting glucose levels of 112±10.3 vs. 200±34.6 mg/dl, respectively, were maintained continuously in on-line feeding-monitoring chambers (BioDaq) with access to laboratory chow ad libitum. For testing, groups were administered a single s.c. injection of biologically equivalent doses of either 15U/kg NPH or 26.25U/kg DET at mid-morning (for dose equivalency see Diabetes 2008 V57: 746-756). Blood was sampled at 0 (Baseline), 2, 4, 6, 8, 10, 12 and 24 hrs post-injection, and FI was continuously monitored electronically.
Results: Results for STZD rats are shown in Fig 1. Significant differences of glucose level ($p < 0.05$) were seen between the groups at hrs 2, 4, 6, and 8 (Fig. 1A), with NPH rats displaying frank hypoglycemia during the first 4 hrs post-injection. In contrast, glucose levels of STZD DET rats remained moderate and stable over the first 8 hrs post-injection. Significantly greater cumulative FI ($p < 0.01$) was seen in NPH- vs. DET-injected STZD rats during each 2-hr interval from hrs 4 - 12 (Fig. 1B). Blood glucose levels did not differ between the groups from hr 10 onward, nor did cumulative FI from hr 14 onward. Similar significant differences in glucose level and cumulative FI were observed over the same intervals in DET vs. NPH-injected INT groups, and a tendency for decreased BW gain was seen in the INT DET group at 24 hrs (ns). At higher doses of DET and NPH that led to similar reductions of glycemia in INT and STZD rats, no differences of cumulative FI were observed.
Conclusion: Our results indicate that the more moderate and consistent glucose-lowering effects of DET eliminate episodes of hypoglycemia and glucoprivic feeding in INT and diabetic rats, and may predispose to reduced BW gain with repeated DET administration.

Fig. 1A. Glucose Levels Over Time

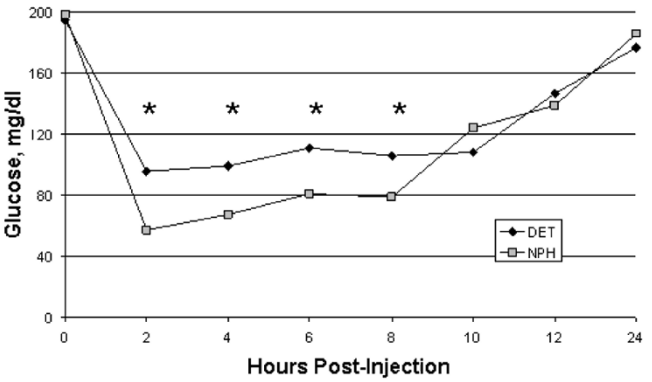
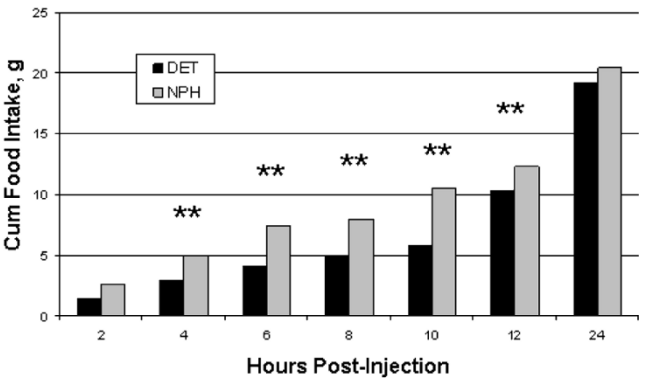


Fig. 1B. Cumulative 2-hr Food Intake



Supported by: Novo Nordisk

502

Effects of intranasal insulin on neuronal processing of food pictures in lean and obese subjects: a magnetoencephalographic study

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Background and aims: Insulin is known to be an anorexigenic hormone to the central nervous system (CNS) that contributes to the termination of food-intake in the postprandial state. In obese, however, diminished cortical activity was described both in animals and humans implicating insulin resistance in the brain. Intranasally administered insulin enters the brain via the olfactory nerve and bulb, and was shown to raise insulin concentrations in the cerebrospinal fluid without altering levels in the systemic blood circulation. Thereby, a potential malfunction of the blood-brain barrier as an underlying cause of insulin resistance in the brain in obese subjects can be disregarded, and insulin action in the brain can be monitored apart from effects in the periphery. In the present study, we therefore investigated whether neuronal activity modulated by intranasal insulin behaves differently in lean and obese subjects.
Materials and methods: We determined evoked potentials by magnetoencephalography measurements in 10 lean and 10 obese subjects for both in the basal state and after applying insulin or placebo spray. Neuronal stimulation was achieved by matched food and non-food pictures in randomized order, and multiple blood-samples were drawn to determine parameters of peripheral glucose metabolism.
Results: Intranasal insulin had no significant effect on glucose, insulin or C-peptide concentrations over a 60-minute period after application of insulin spray. However, insulin increased the amplitude of the evoked potentials in response to food pictures in lean subjects (10 ± 8 femtoTesla (fT)), while this effect was absent in obese (multivariate regression analysis: p=0.003). Moreover, in lean, the increase in the amplitude was observed in the components of evoked fields related to identification and categorization of incoming pictures at around 170 ms post-stimuli in the visual ventral stream.
Conclusion: Our data suggest that insulin-dependent processing of food pictures is decreased in obese subjects compared to lean, and therefore support the idea that insulin in the brain of obese is not sufficient to terminate food intake and might aggravate overeating and obesity.
Supported by: DFG (KFO 114)

503

Evidence of an impaired transcapillary insulin transport in obese females showing postprandial hyperglycaemia

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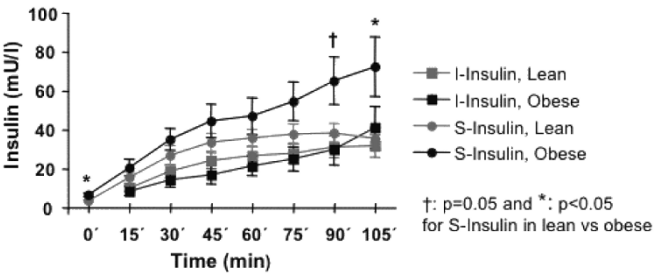
Background and aims: An endothelial barrier for the transport of insulin to the target tissues has been suggested to contribute to insulin resistance in obese subjects. However, the interstitial insulin concentration (I-Insulin) in adipose tissue and skeletal muscle has so far not been studied during physiological hyperinsulinemia in obese subjects characterized by postprandial hyperglycemia.
Materials and methods: Seven obese postmenopausal females without type 2 diabetes, but with signs of postprandial hyperglycemia (P-Glucose ≥9.7 mmol/l 60 min after an OGTT), and 9 postmenopausal lean glucose tolerant females were studied after fasting overnight (Age 61±2 vs 60±1 yrs, ns, (Mean±SEM); BMI 32.9±0.8 vs 22.8±0.6 kg/m^2, p<0.005; fS-Insulin 7.0±0.2 vs 3.9±0.5 mU/l, p<0.005; fP-Glucose 5.2±0.1 vs 4.7±0.1 mmol/l, p<0.05; P-Glucose 120 min after OGTT 9.0±0.4 vs 6.1±0.3 mmol/l, p<0.005 in obese and lean subjects, respectively) The microdialysis technique was applied to study I-Insulin in the subcutaneous (sc) adipose tissue and the brachioradialis muscle in the forearm, as well as to study the sc I-glycerol concentration. To achieve physiological hyperinsulinemia the measurements were performed during an OGTT. Adipose tissue blood flow (ATBF) was studied by ^{133}Xe-clearance.
Results: After the OGTT there were higher serum insulin concentrations (S-Insulin) in the obese, as compared to the lean group. However, I-Insulin did not differ significantly between the groups, neither in the sc adipose tissue (Fig 1), nor in the skeletal muscle (data not shown). Accordingly, the proportion of insulin passing from the circulation to the interstitial fluid in the sc adipose tissue was significantly lower in the obese as compared to the lean subjects (42±2% vs 79±4%, p<0.001) (mean during 2 hours after the OGTT). A similar relationship was seen in the skeletal muscle (37±3% vs 51±3%, p<0.005), although the difference between obese and lean subjects was more pronounced in the sc adipose tissue. As expected, the sc Interstitial-Arterial glycerol concentration difference (marker for lipolysis) (138±18 vs 81±9 µmol/l, p<0.05, at 60 min after the OGTT), as well as the P-Glycerol (38±5 vs 21±2 µmol/l, p<0.05, at 60 min), was significantly higher in the obese group, indicating insulin resistance. Furthermore, ATBF was higher in the lean group during the first 90 min after the OGTT (2.0±0.2 vs 3.8±0.6 ml x 100 g^{-1}x min^{-1}, p<0.05, at 90 min).
Conclusion: Obese females showing postprandial hyperglycemia need higher circulating insulin than lean controls to achieve similar interstitial insulin levels in sc adipose tissue and skeletal muscle, indicating a defective transcapillary transport for insulin. The endothelial barrier for insulin might counteract insulin´s ability to compensate for insulin resistance at the cellular level resulting in impaired sc antilipolysis and decreased muscle glucose uptake.



Supported by: Swedish Research Council, local research resources (ALF), Swedish Diabetes Association, Göteborg Medical Society

PS 29 Insulin sensitising agents - animal studies

504

The identification and characterisation of compounds with insulin-like properties

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Background and aims: Insulin resistance is a key hallmark of type 2 diabetes (T2D). Emerging evidence suggests that defects in information processing by signal transduction pathways may contribute to insulin resistance. If this is correct, drugs may be designed that can overcome and therefore restore signalling to downstream effectors such as the transcription factor FOXO1a and the key gluconeogenic regulatory enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase (G 6-Pase) that become poorly responsive to insulin in T2D. We recently identified three theaflavins as novel mimics of insulin/IGF-1 action on FOXO1a and PEPCK. Theaflavins are large molecules which contain a structural motif which is also present in much smaller molecules that I have recently discovered to have insulin-like signalling properties. Characterisation of one particular smaller compound and some of its derivatives is presented here.
Materials and methods: HEK293 cells were stimulated with various compounds and lysates underwent SDS-PAGE and Western blotting then probed with phosphospecific FOXO1a antibodies. RT-PCR assays of the genes PEPCK and G 6-Pase were carried out in HL1c cells. Statistical siginificance on average mRNA expression was calculated using one way ANOVA followed by Bonferroni's post hoc test.
Results: The compound elicits insulin-like effects on FOXO1a, PEPCK and G 6-Pase. The compound induces phosphorylation of FOXOs by PtdIns 3-kinase-sensitive and PKB-dependent phosphorylation of Thr24, Ser256 and Ser319, the latter residue then priming a further CK1 mediated phosphorylation of Ser322 and Ser325. The compound required a concentration of 50 μM to induce maximal phosphorylation and a minimum application of 15 minutes. The use of the inhibitors PI-103, wortmannin, PD98059, rapamycin and the PKB inhibitor, Akti, indictated the compound induced phosphorylation of FOXOs by a PtdIns 3-kinase-sensitive and PKB-dependent manner. The effect of derivitisation of the compound is discussed. The repression of hepatic gluconeogenesis by reduced expression of PEPCK and G 6-Pase is recognized as a key aspect of the anti-hyperglycaemic action of insulin so the effect of the novel compound was also investigated. The compound reduced average mRNA expression (as a % of Dex/cAMP expression) of both genes ($n=3$, $p<0.001$).
Conclusion: We have found a small compound that mimics the insulin-like action of black tea theaflavins on FOXO1a and gluconeogenesis. Further investigation might provide novel strategies for the treatment or deferral of T2D.
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505

VVP808 is a novel insulin sensitising agent that reduces endogenous glucose production

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Background and aims: We previously showed that VVP808 reduced fasting blood glucose concentration ($p=0.00004$) and HbA1c levels ($p=0.04$) in db/db mice, and improved glucose tolerance in diet-induced obese (DIO) mice ($p<0.05$). The aims of the current study were to determine the mechanism by which VVP808 exerts these antidiabetic effects.
Materials and methods: FAO liver cells were treated in glucose-free media with VVP808 (100 μM) or Metformin (200 μM), and known activators of glucose production (dexamethasone and CPT-cAMP), both in the absence and presence of sub-maximal insulin (0.5 nM). After 24 h, glucose concentration was determined in the media. 10-week-old male Sprague-Dawley rats ($n=10$ per group) were made diabetic by IV injection of streptozotocin (65 mg/kg). Male C57Bl/6 mice ($n=10$ per group) were given free access to a diet containing 45% energy from fat for 10 weeks to induce mild obesity and insulin resistance. Both rats and mice were administered VVP808 (50 mg/kg/d) or vehicle control by single daily oral gavage for 12 or 14 days. Clamps and insulin tolerance tests were performed using standard procedures.
Results: VVP808 inhibited activated glucose production both in the absence and presence of insulin by 35 and 45% respectively ($p<0.05$, $n=5$-7). Under the same experimental conditions, VVP808 decreased PEPCK and G6Pase gene expression levels by 20 and 27% respectively ($p<0.05$, $n=3$). Administration of VVP808 (50 mg/kg/d for 12 days) had no effect on fasting blood glucose concentration in STZ rats. However, VVP808 significantly enhanced the glucose-lowering effects of insulin (0.5U/kg) in an insulin tolerance test (by 2.2-fold after 30 min ($p=0.045$) and by 2.4-fold after 60 min ($p=0.038$)). Data from hyperinsulinemic-euglycemic clamp studies in DIO mice showed that treatment with VVP808 (50 mg/kg/d for 14 days) increased the glucose infusion rate by 27% ($p=0.005$), and this associated with a 23% decrease in endogenous glucose production ($p=0.04$).
Conclusion: In these studies we demonstrated that VVP808 acts as an insulin sensitising agent. VVP808 reduced PEPCK and G6Pase gene expression, and lowered glucose production from cultured FAO cells. VVP808 significantly enhanced the glucose-lowering effects of insulin in STZ rats, and increased the glucose infusion rate during the clamp procedure in mice. We also showed that VVP808 appears to act predominantly by reducing endogenous glucose production. These results confirm the potential of VVP808 for development as an anti-diabetic agent.

506

Malaysian herb *Labisia pumila var alata* improved insulin sensitivity and lipid profile in a rat model of polycystic ovary syndrome

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Background and aims: Labisia pumila var alata (LPva), a Malaysian herb thought to have phytoestrogenic effects, has shown promising results regarding body weight and insulin sensitivity in our previous study on ovariectomized rats. Here we investigated if LPva extracts have effects on metabolic features and body composition in rat model of polycystic ovary syndrome (PCOS) which incorporates ovarian and metabolic characteristics of the syndrome. PCOS is a complex endocrine and metabolic disorder associated with ovulatory dysfunction, hyperandrogenism, abdominal obesity and insulin resistance.
Materials and methods: Twenty-two female rats were given a continuous dose of dihydrotestosterone (7.5 mg/90days; PCOS groups) starting at 21 days of age, to induce PCOS. At 9 weeks of age, rats were randomly divided into 2 groups: i) PCOS Labisia pumila (50mg/kg body weight) and ii) PCOS control (1 ml deionised water) daily during 4 to 5 weeks by oral administration.
Results: Treatment with LPva in PCOS rats resulted in improved insulin sensitivity measured by euglycemic hyperinsulinemic clamp (1.4-fold increase, $p<0.05$). Total cholesterol and triglycerides levels were decreased in LPva treated rats (15% and 14% respectively, $p<0.05$). Plasma resistin levels increased in LPva (25.32 ± 0.92 vs. 20.72 ± 1.54 ng/ml for LPva vs. PCOS control, $p<0.05$). On the other hand, adiponectin levels showed a tendency of increased level in LPva group (24.02 ± 2.00 vs. 18.26 ± 2.02 μg/ml for LPva vs. PCOS control, $p= 0.07$). However, no effect on body weight, body composition or plasma leptin levels was observed. Despite no effects on plasma leptin level, relative mRNA expression in LPva treated group was significantly decreased (4.4 ± 0.7 vs. 8.4 ± 1.1 for LPva vs. PCOS control, $p<0.01$). Furthermore, uterine weight was increased by 29% ($p<0.05$), which indicated estrogenic effects of LPva.
Conclusion: Treatment with LPva extracts in PCOS rats improves insulin sensitivity and lipid profile without affecting body composition.
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507

The *Ginkgo biloba* extract EGb761 prevents palmitate-induced insulin resistance in C2 myotube via activation of 5' AMP-activated protein kinase (AMPK)

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Background and aims: EGb761, a standardized form of *Ginkgo biloba* leaf extract, was recently reported to mitigate various diseases, including cognitive dysfunction and Alzheimer's disease. But the effect of EGb761 on insulin resistance has not been intensively studied. In this study, we found a phenomenon that EGb761 prevented palmitate-induced insulin resistance and investigated the molecular and cellular mechanisms of the protective effect of EGb761 on palmitate-induced insulin resistance.
Materials and methods: We used differentiated C2 myotubes as an in vitro skeletal muscle model under the differentiation medium for 4 day. To investigate whether EGb761 ameliorates palmitate-induced insulin resistance in C2 myotubes, we treated palmitate, with or without EGb761, on C2 myotubes. After treatment of insulin, we investigated molecules of insulin signaling pathways and insulin stimulated glucose uptake. To elucidate the preventing mechanism of EGb761 on palmitate-induced insulin resistance in C2 myotube, we performed immunoblotting with IR(Insulin Receptor), IRS-1(Insulin Receptor Substrate), Protein kinase B (PKB/AKT), JNK(c-jun N-terminal kinase), p38, AMP-activated protein kinase (AMPK), acetyl-coenzyme A carboxylase (ACC) antibodies, and palmitate beta-oxidation assay.
Results: A 16 h exposure of C2 myotubes to palmitate, reduced insulin stimulated glucose uptake by 25%, IRS-1 tyrosine phosphorylation by 40%, and AKT phosphorylation by 30%. To determine the effect of EGb761 on insulin resistance, before palmitate treatment, we pretreated with EGb761 on C2 myotubes and then measured insulin-stimulate glucose uptake and insulin signaling. EGb761 prevented palmitate-induced reduction of insulin stimulated glucose uptake by 30%. But molecules of insulin signalling pathways, such as tyrosine phosphorylation of IRS-1 and phosphorylation of AKT were not changed. It has been well known EGb761 stimulate mitochondrial metabolism, so we measured palmitate oxidation, with or without EGb761, and monitored the activation of AMPK. EGb761 induced phosphorylation of AMPK in a time and dose dependent manner. Also EGb761 recovered palmitate-reduced beta-oxidation in a dose dependent manner. EGb761 prevented palmitate-reduced expression of glucose transporter 4.
Conclusion: Our data suggest that EGb761 prevented palmitate-induced insulin resistance through improvement of beta-oxidation by activation of AMPK.
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508

DSP-8658, a novel PPAR α/γ modulator, with favorable anti-diabetic profile distinct from that of thiazolidinediones

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Background and aims: Thiazolidinediones (TZDs), such as pioglitazone and rosiglitazone ameliorate insulin resistance, but cause side effects, including body weight gain, edema and congestive heart failure. In our search for new anti-diabetic agents with beneficial effects on insulin resistance-associated dyslipidemia and minimal side effects, we have found DSP-8658, a selective peroxisome proliferator-activated receptor (PPAR) α/γ modulator. DSP-8658 is currently under clinical development for the treatment of type 2 diabetes. Our aim in this study is to evaluate the beneficial effects of DSP-8658 on metabolic abnormalities in mice models and, characterize the mechanism of these effects and compare it to that of TZDs.
Materials and methods: Transactivation assays were conducted using COS-1 cells transiently transfected with a vector containing PPAR α or γ ligand binding domain as well as a vector containing a reporter gene. Mammalian-2-hybrid assays using HEK293 cells were also conducted to examine PPARγ interaction with cofactors, such as NCoR and P300. The effects of DSP-8658 on glucose metabolism were evaluated in diet-induced obese (DIO) and genetically diabetic KK-A^{y} mice. In addition, the anti-lipidemic action of DSP-8658 was evaluated in human ApoA1 transgenic mice. Finally, to examine regulatory effect of DSP-8658 on gene expression, mRNAs from white adipose tissue (WAT), peripheral blood leucocytes (PBLs), and adipose tissue macrophages (ATMs) in diabetic mice were subjected to RT-PCR analysis.
Results: In transactivation assays, DSP-8658 exhibited comparable EC_{50} values for human PPARα (1.08 μM) and human PPARγ (1.01 μM), and partial activation of human PPARγ (76% compared to that of pioglitazone). In mammalian-2-hybrid assays, DSP-8658 induced dissociation of the corepressor NCoR from PPARγ, but did not affect recruitment of the coactivator P300 to PPARγ. In oral glucose tolerance test, DSP-8658 reduced blood glucose level in DIO mice with an ED_{25} value of 6.2 mg/kg, but did not cause body weight gain or fluid retention even at a dose of 100 mg/kg. In addition, DSP-8658, but not pioglitazone, increased serum HDL-cholesterol concentration, possibly through PPARα activation, in human ApoA1 transgenic mice. In high-fat diet-fed KK-A^{y} mice, DSP-8658 decreased white adipose tissue (WAT) weight, while pioglitazone increased it. Pioglitazone also increased mRNA levels of genes related to both glucose metabolism (Glut4) and fat accumulation (glycerol kinase and FATP1) in WAT. In contrast, DSP-8658 increased mRNA level of Glut4 but had no effect on glycerol kinase and FATP1 in WAT. It is increasingly recognized that low-grade inflammation in metabolic tissues caused by imbalance between classically activated macrophages (M1) and alternatively activated macrophages (M2) underlies obesity-induced insulin resistance. Interestingly, DSP-8658 increased mRNA level of the M2 marker gene arginase 1 in PBLs of DIO mice, and decreased mRNA level of the M1 marker gene TNFα in ATMs, suggesting that DSP-8658 induces macrophages polarization toward M2 and suppresses inflammation.
Conclusion: Unlike pioglitazone, DSP-8658, a PPAR α/γ modulator, selectively modulates cofactor recruitment to PPARγ and exhibits differential gene regulation. DSP-8658 has therefore a highly promising therapeutic profile in the treatment of type 2 diabetes.

509

Fish oil minimizes insulin resistance and decreases food intake in Zucker fa/fa rats

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Background and aims: Dietary fish oil (FO) rich in n-3 polyunsaturated fatty acids, i.e EPA + DHA, can modulate insulin-resistance in rats depending on the dose given, the duration of its administration and on the cause of insulin resistance. High dose of FO (20% w/w) over 4 weeks prevents the decrease in phosphatidyl-inositol 3'-kinase (PI3K) activity and GLUT4 content in muscle and adipose tissue induced by a high n-6 PUFA diet (60% w/w). At low dose (7% w/w) over 8 weeks, FO reversed insulin resistance in sucrose-fed rats and increased plasma leptin and adiponectin levels. In contrast, lower dose of fish oil (4.4% w/w) over 4 weeks was unable to prevent dexamethasone-induced insulin resistance and defects of PI3K activity, probably because of too potent effects of dexamethasone.
In this study, we used Zucker fa-/fa- rats. These rats become spontaneously obese and insulin resistant because of a genetic mutation in leptin receptor gene. We aimed to study the effects of a low dietary intake of FO (7% w/w) on food intake and development of insulin resistance in Zucker rats.
Materials and methods: 24 male Zucker rats were fed over 9 weeks either a control diet containing 8 % of fat as corn oil (CO diet) or a n-3 diet containing 7% FO plus 1% CO (FO diet). Food intake was recorded daily and weight gain twice a week. In 12 rats, insulin sensitivity was estimated from both an oral glucose tolerance test (OGTT) and glycaemic response to IP insulin (IPITT). In 12 other rats, hyperinsulinemic-euglycemic clamp plus tritiated glucose infusion were performed to assess both hepatic glucose production and plasma glucose peripheral utilization. Glucose transport in peripheral tissues was determined from infusion of radiolabelled 2-deoxyglucose (2-DOG) during the clamp. Hypothalamus were harvested and analysed for ceramide and diacyglycerol (DAG) quantitation.
Results: At end of week 9, FO had decreased both food intake (- 8.4%, $p< 0.01$) and weight gain (- 6.6%, $p < 0.05$). During OGTT, area under the curve (AUC) of glycaemic response was not altered by FO (FO = 1643 ± 116 vs. CO = 1698 ± 210 mmol.l-1.2h-1) but AUC of insulinaemic response was decreased by 25% (FO = 3009 ± 216 vs. CO = 4006 ± 253 ng.ml-1.2h-1, $p<0.01$). During IPITT, FO induced a greater decrease in glycaemia after insulin injection (glycemia: FO = 8.24 ± 0.62 vs. CO = 11.06 ± 1.51 mM, $p < 0.01$). During clamp, FO did alter neither peripheral glucose utilization nor glucose

transport in tissues but decreased hepatic glucose production by 35% as compared to CO ($p< 0.02$). Ceramide and DAG content did not differ whatever the diet.
Conclusion: These results demonstrate that, in a genetic rat model, where insulin resistance and obesity develop progressively overtime, FO decreased both food intake and weight gain, probably independently from leptin action because Zucker rats are deficient for leptin receptor. Moreover, we demonstrated that FO minimized specifically liver insulin resistance.
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510

Possible effect of endogenous cannabinoids and rimonabant upon insulin resistance and beta cell function

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Background and aims: Obesity produces a decrease in insulin sensitivity and a compensatory increase in β-cell function. Anandamide (AEA, endocannabinoid) regulates appetite and other metabolic functions; thus, drugs interacting with its specific CB1 and CB2 receptors have been used to treat obese people. We studied the possible regulatory role of the general and local (pancreatic islets) cannabinoid system upon insulin secretion in rats with insulin resistance (IR) induced by administration of a fructose-rich diet (FRD).
Materials and methods: Normal male Wistar rats were divided into 4 groups and fed for 21 days as follows: *Control* (C): standard commercial powder diet and tap water *ad libitum*; *Fructose* (F): C plus 10% w/v fructose in the drinking water; *Rimonabant control* (RC): C plus 2 mg/rat Rimonabant; and *Fructose Rimonabant* (FR): RC plus the same amount of fructose as F. Blood samples were drawn at the time of sacrifice to measure glucose, insulin, thiobarbituric acid reactive substances (T-bars) and triglyceride levels. Serum glucose and insulin values were used to calculate insulin sensitivity (HOMA-IR). After sacrifice, pancreases from each animal were removed to isolate islets (collagenase digestion). Expression of insular CB1 and CB2 receptor genes was checked by RT-PCR and immunocytochemistry (confocal microscopy). We measured insulin secretion from isolated islets incubated with 2.8, 8.3 or 16.7 mM glucose with or without 0.1-200 μM AEA, 0.1-20 μM ACEA (CB1 agonist), or 0.1-20 μM JWH (CB2 agonist) (RIA).
Results: The presence of CB1 and CB2 receptors in normal rat islets was demonstrated by RT-PCR and immunocytochemistry. While CB1 receptors were selectively expressed in glucagon-producing cells, CB2 were expressed in both insulin- and somatostatin-producing cells. AEA at 10 μM enhanced significantly the release of insulin induced by 8.3 mM glucose (3.5 ± 1.0 *vs.* 7.4 ± 1.5 ng/islet/h; $p<0.05$), but these effect was observed with 100 μM at 16.7 mM glucose at (8.2 ± 0.7 *vs.* 10.8 ± 0.5 ng/islet/h; $p<0.05$). ACEA and JWH affected insulin secretion in the presence of 16.7 mM glucose in a dual way: they enhanced and inhibited the secretion at 1 μM and at 20 μM, respectively (ACEA: 9.7 ± 0.6, 11.8 ± 0.8 and 6.8 ± 0.8 ng/islet/h at 0, 1 and 20 μM, respectively. JWH: 10.4 ± 0.7, 15.1 ± 1.2 and 6.2 ± 0.8 ng/islet/h at 0, 1 and 20 μM, respectively; $p<0.05$ in all cases). FRD increased significantly daily caloric intake, body weight, serum glucose, triglyceride, T-bars, insulin concentration and IR state ($p<0.05$ in all cases). Rimonabant administration corrected significantly (except T-bars), all these abnormalities ($p<0.05$).
Conclusion: Islets have a specific distribution of CB1 CB2 receptors that modulate insulin secretion. Blockage of the endocannabinoid pathway *in vivo* corrected most metabolic abnormalities and FRD-induced IR.

Metabolic changes

	Body weight increment (g)	Calories intake (cal/rat/day)	Serum glucose (mmol/L)	Serum insulin (μU/ml)	HOMA-IR	Serum T-bars (pmol/mg prot)	Serum triglyceride (mg/dl)
C	47.7±11.9	59.1±1.5	5.0±0.1	10.5±0.8	2.3±0.2	233.1±8.1	56.0±5.3
F	72.0±9.5	86.2±1.8	5.5±0.1	15.9±0.9	3.9±0.2	312.9±8.9	73.7±3.5
CR	34.3±1.8	55.1±2.9	4.9±0.2	8.1±0.9	1.8±0.2	257.2±28.1	58.7±4.2
FR	48.3±10.9	60.1±3.5	5.2±0.3	11.1±0.9	2.6±0.3	325.2±9.8	63.7±1.6

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511

D-chiro-inositol acts as an insulin mimetic to attenuate epinephrine-stimulated hepatic glucose output in the isolated perfused rat liver

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Background and aims: D-chiro-inositol is an inositol compound present in comparatively low abundance in vivo compared to its stereoisomer, myo-inositol. Chronic administration of D-chiro-inositol in animal models of diabetes attenuates hyperglycaemia, and in polycystic ovary syndrome, a condition where insulin resistance is a primary feature, D-chiro-inositol administration also improves glucose tolerance and ovulatory function in human patients. The underlying mechanisms and sites of action involved are largely undefined, however, we hypothesised that attenuating effects on hepatic glucose output (HGO) by D-chiro-inositol could potentially explain these observations. Our aim was therefore to investigate whether D-chiro-inositol could in fact modulate HGO in an isolated perfused liver.
Materials and methods: Isolated rat livers (12-15g) from fed animals (220g-250g) were perfused with plasma-free buffer containing 5 mM glucose in a non-recirculating set-up. The perfusion protocol comprised a 40 minute equilibration period followed by a 30 min infusion with either insulin (1 nM), D-chiro-inositol (200 micromolar), myo-inositol (200 micromolar), or saline. Within the background of each of these experimental groups, an epinephrine infusion (50 nM) was then performed for a further 30 minutes. A final glucagon infusion (1.2 nM) was then performed to demonstrate hormonal responsiveness. Glucose, lactate and portal pressure were monitored continuously.
Results: Prior to epinephrine infusion there were no significant baseline differences in HGO between the four experimental groups. In the saline group (n=9), epinephrine evoked an acute (within 2 minutes) and approx. 3-fold increase in HGO that was largely sustained throughout the following 30 min infusion period. As expected, this increased HGO was substantially attenuated in livers co-infused with insulin (n=10). Co-infusion with D-chiro-inositol (n=10) also significantly attenuated epinephrine-stimulated HGO over 30 minutes to a degree intermediate between saline and insulin infusions. HGO in the myo-inositol-infused livers (n=8) also mimicked the attenuating effects of D-chiro-inositol over the first 10 minutes but then stabilised and by 30 minutes was not significantly different from the saline infusion.
Conclusion: These findings show that D-chiro-inositol has an intrinsic site of action on the liver and can reduce epinephrine-stimulated HGO. This insulin mimetic activity provides a potential mechanism by which D-chiro-inositol could mediate some of its observed therapeutic actions in diabetes and polycystic ovary syndrome.
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512

The $VO(dmpp)_2$, a promising insulin mimetic vanadium compound

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Background and aims: The importance of Vanadium Compounds (VCs) has greatly increased in the last years since they have shown pharmacological properties. In particular, their potential use as oral insulin mimetic has been demonstrated by in vivo and ex vivo studies, as well as in clinical trials. Thus, research has been carried out to develop VCs to be used in the treatment of Diabetes Millitus (DM) at an effective non toxic dose. Type 2 DM and other metabolic syndromes are characterized by insulin resistance originating high plasma insulin and glucose levels due to reduction of insulin action and glucose uptake. In this work we report some biochemical studies with a vanadium complex with a pyridinone ligand, the VO(dmpp)2, concerning glucose uptake in primary cultures of rat adipocytes, to prove its capacity to withdraw insulin resistance in these cells.
Materials and methods: The experiments were carried out with primary cultures of 6-8 weeks old Wistar rat adipocytes. Glucose uptake studies were performed using a radioactive assay by measuring the 14C-glucose in the extracellular medium after 45 minutes incubation with and without insulin, in the presence and absence of different concentrations of VO(dmpp)2 (0.1-

1mM). Alamar Blue test was used to assess cell viability when the adipocytes were incubated with the tested concentrations of the VC.
Results: The results obtained showed that when adipocytes were incubated with VO(dmpp)2 in concentrations of 0.1 mM and 0.5 mM, the increase in glucose uptake relative to basal value was respectively 85% and 131% ($p<0.001$), being the value obtained with insulin alone of 123% ($p<0.001$). Incubations in the presence of 10 nM of insulin, the concentrations of 0.1 mM and 0.5 mM of the VC originated an additional effect of 45% ($p<0.001$) and 32% ($p<0.01$), respectively, above the insulin value. Both concentrations of the VO(dmpp)2 tested are below acceptable cytotoxicity, as demonstrated by the Alamar Blue assay. These results were compared with those obtained with another VC with a similar structure, the BMOV, which is now in clinical trials. This compound also showed a dose dependent response, but none of the concentrations tested (up to 1.5 mM) showed a glucose uptake rate significantly different from basal or close to insulin values, In fact, for the concentration of 1.5 mM, which is the higher response obtained, the glucose uptake value is 33% below the insulin response ($p<0.001$).
Conclusion: Our results demonstrate that VO(dmpp)2, is able by itself to mimic insulin action on glucose uptake in adipocyte tissue. Moreover, when cells are treated with this VC along with insulin, an accumulative effect is observed, being effective at lower concentrations, thus minimizing the toxic effects on cells. Therefore VO(dmpp)2, may represent a major candidate as a powerful drug to treat DM.
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PS 30 Insulin sensitivity in adipose tissue and muscle - human studies

513

Adipose tissue expression of retinoic acid receptor-related orphan receptors gamma (RORg) is altered in morbid obesity and insulin resistance

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Background: Adipose tissue is an active endocrine and paracrine organ due to the production of secreted proteins and lipid indicators collectively called adipokines. These observations have spurred interest in the identification of the transcriptional and other regulatory pathways of adipocyte differentiation. The orphan nuclear receptor, peroxisome proliferator-activated receptor gamma (PPARg) and retinoic acid receptor-related orphan receptors gamma (RORg), are central mediators controlling adipocyte differentiation.
Aims: The aim of the study was to evaluate whether RORg and PPARg mRNA and protein expression levels in human adipose tissues and its relationship with insulin resistance in morbid obese versus non obese patients.
Material and methods: Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) biopsies were obtained from 26 morbidly obese patients undergoing bariatric surgery procedures. VAT and SAT samples were also obtained from otherwise non obese patients during surgical repair of hiatus hernias. RORg and PPARg mRNA and protein expression levels were measured in the tissues obtained by quantitative RT-PCR and western blotting respectively. The MO patients were divided into two groups: those with a low homeostasis model assessment of insulin resistance (HOMA-IR<5) (MO nonIR) and those with a high HOMA-IR (HOMA-IR≥8) (MO-IR).
Results: RORg mRNA and protein expression in VAT was significantly higher in the MO patients ($P<0.05$) than the non obese subjects, but there were no significant differences between the expression of PPARg in MO patients in comparison with non obese subjects. In addition, PPARg mRNA and protein expression levels were similar in VAT and SAT from the MO patients and not dependent on IR. In contrast, the RORg mRNA expression was higher in VAT ($P<0.05$), in the MO-IR than the MO nonIR group. Finally, the MO patients showed a significant positive correlation between mRNA and protein RORg expression and insulin resistance in VAT ($r=0.357$, $p=0.045$) and in SAT ($r=0.396$, $p=0.030$).
Conclusion: Expression of RORg, is up regulated in morbid obesity and showed to be related with the insulin resistance. Future studies are warranted to elucidate the role of this orphan nuclear receptor in the pathogenesis of insulin resistance in morbid obesity
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514

Investigating *PTEN* expression in tissues of insulin action illustrates reduced mRNA expression in adipose tissue of patients with type 2 diabetes

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Background and aims: Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is a critical regulator of cell growth and metabolism via its antagonisation of PI3K and control of the AKT/PKB pathway. Insulin signalling utilises the AKT pathway and this has generated interest in the tissue specific roles of PTEN in glucose homeostasis. Murine studies have shown that *Pten* is expressed in pancreatic islets, liver, muscle and adipose tissue. Tissue specific Pten deletion in both muscle and adipose tissue of mice leads to insulin hypersensitivity and protection against diabetes. We hypothesised that PTEN plays a critical role in tissues of insulin action in humans and that elevation of *PTEN* mRNA in both adipose tissue and muscle may contribute to the insulin resistance seen in patients with Type 2 diabetes (T2D).
Materials and methods: *PTEN* expression in human tissues (including liver, muscle, adipose tissue, pancreatic islets) was determined. Adipose tissue (omental & subcutaneous) was available for 16 patients (9 male, 8 female)

with T2D and 16 age, gender & BMI matched normoglycaemic controls. Muscle tissue was available from 3 patients with T2D and 3 age, gender & BMI matched controls. RNA was extracted from all tissues using TRI reagent, cDNA synthesised from a total of 2ug RNA and *PTEN* expression determined on an ABI7900HT system. Analysis was performed using the ΔΔCt method, normalising to 2 housekeeping genes (UBC & PPIA) and significance determined using a paired t-test.
Results: *PTEN* is highly expressed in human tissues of insulin secretion and action (liver, adipose tissue, muscle, islets). *PTEN* expression is reduced 1.48 fold (35%) in the subcutaneous adipose tissue of T2D subjects when compared to matched controls ($p = 7.9x10^{-4}$). Further analysis demonstrated that this reduction is more pronounced (1.57 fold, $p=4.7x10^{-3}$) in T2D males compared to T2D females (1.39 fold, p=0.07). *PTEN* expression was also reduced 1.3 fold in the omental adipose tissue of diabetic subjects, although this did not reach statistical significance (p=0.06). Gender specific analysis in this depot however reveals that there is a 1.55 fold (36%) reduction in *PTEN* expression in the T2D males (p=0.02) when compared to controls. Our data indicates there is only a minimal 1.06 fold reduction between T2D females and matched controls at the level of omental adipose tissue (p=ns). No difference in *PTEN* expression was seen in muscle, although numbers are currently limiting (n=3).
Conclusion: We have demonstrated that *PTEN* gene expression is reduced in adipose tissue from patients with T2D when compared with matched controls and suggests that the reduction in both fat depots may be more pronounced in males. Our data are in conflict with our initial hypothesis and suggests that there are compensatory mechanisms in adipose tissue whereby *PTEN* is down regulated to maximise insulin stimulated glucose uptake in this depot. Replication of our finding is required in further datasets along with studies to confirm that PTEN protein expression is also reduced.
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515

Insulin resistance is induced by endoplasmic reticulum stress in the human adipocytes
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Background and aims: Obesity is associated with insulin resistance and type 2 diabetes. However, the mechanisms linking these pathologies remain not completely understood. Recent studies in rodent models revealed endoplasmic reticulum (ER) stress in adipose and liver tissues and demonstrated that ER stress could cause insulin resistance. Morover, studies in human adipose tissue suggest a relationship between obesity-related ER stress and metabolic dysfunction in obese humans, such as insulin resistance. The aim of this study was to test the effect of ER stress on insulin resistance in human adipocytes.
Materials and methods: In this study we used a human preadipocyte cell strain derived from subcutaneous white adipose tissue of a patient with Simpson-Golabi-Behmel syndrome (SGBS). The SGBS cell strain exhibits a high capacity for adipose differentiation, resulting in mature fat cells which are biochemically and functionally similar to human adipocytes. Differentiated SGBS cells were incubated with (or without) thapsigargin for 1, 2, 4, 6, or 18 hours to induce ER stress. ER stress in our system was monitored by an increase in the protein expression of GRP94 and GRP78. Insulin sensitivity was measured as induction of Akt phosphorylation by 100 nM insulin for 15 min (at the end of 1, 2, 4, 6 or 18 hours incubations). The readout of insulin sensitivity is determined by the ratio phosphorylated AKT (phospho-Akt) vs Akt (ratio phospho-Akt/Akt).
Results: Thapsigargin induced ER stress, as measured by a 1.2 to 2.3 fold increase in GRP78 and 1.1 to 1.3 fold increase in GRP94. Insulin induced Akt phosphorylation at all time points (1.4 to 2.6 fold increase in phospho-Akt/Akt ratio). After 1, 2, 4, 6 hours of incubation with thapsigargin, insulin-induced Akt phosporylation was 1.3 fold higher compared to incubations without thapsigargin, suggesting improved insulin sensitivity. In contrast, a 18 hours incubation of cells with thapsigargin, reduced the effect of insulin on Akt phosphorylation compared to incubations without thapsigargin with 28%, indicating an induction of insulin resistance.
Conclusion: In human adipocytes (SGBS cells), short term induction of ER stress cells, resulted in an increase in insulin sensitivity, while adipocytes exposed to ER stress for a long period showed insulin resistance. This SGBS adipocyte model may be a useful tool to study the role of ER stress in human adipocyte biology.

516

Regulation of plasma metabolome and subcutaneous adipose transcriptome by insulin in the clamp study
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Background and aims: Insulin, the dominant anabolic hormone, suppresses endogenous glucose production, stimulates glucose utilization by insulin-sensitive tissues and inhibits the lipolysis in fat. Few studies describe insulin-dependent transcriptome changes and no data exist for metabolome. In this pilot study we examined acute insulin effects on plasma metabolites and subcutaneous adipose tissue (SAT) transcriptome under clamped euglycemia and hyperglycemia.
Materials and methods: Healthy obese men (n=14) underwent a placebo infusion test (0.9% NaCl-infusion for 4 h) and/or a hyperinsulinemic, euglycemic clamp (EC), and/or a hyperinsulinemic, hyperglycemic clamp (HC). SAT biopsy and plasma samples were taken at -40 min and 240 min of the tests. Full human genome Agilent chips were used for transcript profiling, and GC/MS analysis was applied to get the metabolome of plasma. An explorative analysis of insulin-induced changes with calculating 240 to basal ratio was conducted. We proposed "non-changed values" as range of 1.2 to 0.8 in these ratios with p>0.06. To evaluate a trend of changes, we estimated the proportion of up and down regulated genes and metabolites ("up-to-down ratio").
Results: The highest insulin and blood glucose levels were observed in the HC test (p<0.001). In EC, insulin levels were higher then these of placebo tests under similar blood glucose levels.

Plasma metabolome and SAT transcriptome changes

Tests	Metabolites changes (%), n_{total}=3199	Up-to-down ratio	Gene expression changes (%), n_{total}=38662	Up-to-down ratio
Placebo infusion	2.15	0.19	2.80	1.72
EC	16.30	0.025	3.50	2.82
HC	15.75	0.66	4.01	3.20

Conclusion: Insulin down regulates a big portion of plasma metabolome under euglycemia, while simultaneous expressional changes are only minor. This observation reflects possible prevalence of stoichiometric-driven changes in insulin-dependent metabolome.
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517

Level of TLR-4, IL-6, TNF-*a*, but not TLR-2 was elevated in skeletal muscles from non-obese subjects with impaired glucose tolerance (IGT) compared to normal subjects
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Background and aims: In obese subjects and type 2 diabetes, insulin resistance has been shown to be associated with chronic inflammatory states. Recent studies have suggested that Toll-like receptors, which are components of the innate immune system, and inflammatory signals may play a critical role in saturated fatty acid-induced insulin resistance in skeletal muscles of obese subjects and type 2 diabetes. However, the relation between inflammation and insulin resistance has not been intensively studied in non-obese patients with IGT. In this study, we investigated TLR gene expression, inflammatory cytokine, and insulin signaling in skeletal muscles from non-obese patients with IGT.
Materials and methods: Ten IGT subjects and 15 control subjects were recruited for this study. Whole-body insulin-mediated glucose uptake was de-

termined using a euglycemic hyperinsulinemic clamp test. Muscle biopsies were obtained from the vastus lateralis muscle before and after insulin stimulation. Next we determined TLR gene expression, levels of several inflammatory cytokine, and insulin signaling using immunoblotting.
Results: Subjects with IGT had significantly higher fasting blood glucose and HbA1c levels in comparison with control subjects. Total-cholesterol, triglyceride, and high-density lipoprotein levels were not significantly different between the groups. Insulin stimulated glucose infusion rates were 39% lower in the IGT group than the control group (control: 7.33 + 0.5 vs. IGT: 4.54+ 0.6, $p < 0.01$). Also the activity of insulin signaling molecules such as IR-β, IRS-1, AKT, and GSK-3β were reduced in the IGT group compared to the control group. To investigate involvement of inflammation in skeletal muscle from non-obese subjects with IGT, we measured levels of TLR-2, TLR-4, IL-6, and TNF-α. Interestingly, the level of TLR-4 significantly increased by 200% in the IGT group. But TLR-2 did not. Increased level of IL-6 and TNF-α were 600% and 200%, respectively, in the IGT group compared to the normal group. We then investigated intracellular signaling molecules related with inflammation. Phosphorylation of NFκB, mTOR, and STST3 were observed in the IGT group compared to the control group. But phosphorylation of JNK, p38 and ERK did not.
Conclusion: Levels of TLR-4, IL-6, and TNF-α were increased in skeletal muscles of non-obese subjects with IGT compared to normal subjects. Our data suggested that inflammation may associate with insulin resistance in non-obese subjects with IGT, as well as obese subjects.
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518

Histone variant H2A.Z is enriched in the vicinity of the transcriptional start site of insulin-modulated genes. A possible epigenetic link to insulin-dependent transcription
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Background and aims: Besides its metabolic effects, insulin controls gene expression in insulin-responsive tissues. In insulin resistance, extensive transcriptional alterations occur in skeletal muscle and adipose tissue, due to impaired insulin signalling. As insulin-dependent transcriptional control is regulated by the mutual interplay between the transcriptional machinery and its chromatin template, we sought to investigate whether known insulin-regulated genes bear specific chromatin signatures using L6 myotubes as a model system.
Materials and methods: Differentiation of L6 cells was carried out under normoglycaemic conditions (5.5 mM glucose). Fully differentiated myotubes were incubated for 6 hours in the presence of 100 nM insulin or 100 nM insulin in 25 mM glucose containing medium. Gene expression levels were compared to unstimulated myotubes in normoglycaemic conditions. Primer pairs around the hexokinase 2 (HK2) transcriptional start site (TSS) spanned a region from - 4000 bp to +500 bp. Primers for genes of the insulin signalling cascade were designed within 1 kb from the start codon. Chromatin immunoprecipitation (ChIP) was performed with antibodies directed to H2A, H2A.Z and acetylated H3K9/14. mRNA levels and DNA enrichment in ChIP were measured by real time PCR.
Results: As a paradigm for insulin-regulated genes in L6 myotubes we studied HK2, insulin receptor substrate 1 (IRS1) and IRS2. HK2 mRNA was increased > 2-fold following 6-hours insulin stimulation, while IRS1/2 mRNAs were downregulated. ChIP to H2A and H2A.Z showed that the histone 2A variant H2A.Z is specifically accumulated around the TSS of insulin modulated genes, including HK2, IRS1, IRS2. By contrast, H2A.Z enrichment was not observed distally to the TSS (i.e. 4 kb upstream) in the HK2 promoter, nor in the vicinity of the TSS in the HPRT1 housekeeping gene. The ratio between H2A and H2A.Z deposition was not sensibly affected by insulin treatment. However, H2A.Z deposition along the HK2 gene positively correlated with H3K9/14 acetylation levels, both in the basal and in the insulin-stimulated condition, in which H3K9/14 acetylation was further enhanced. Whether H2A.Z also coordinates the occurrence of other histone post-translational modifications on insulin-regulated genes, such as transcriptionally-promoting H3K4 methylation and transcriptionally-repressive H3K9 methylation, is currently under investigation.
Conclusion: Our findings on a selected subset of insulin-responsive genes in L6 myotubes suggest that they bear a common "epigenetic signature", consisting in enrichment of the H2A.Z histone variant around the TSS. We hypothesize that enriched H2A.Z deposition may be instrumental in conferring insulin-responsiveness to these genes, by facilitating the occurrence of histone post-translational modification. As an example we show a correlation between increased H2A.Z deposition along the HK2 gene and increased H3K9/14 acetylation following exposure to insulin. Further investigation is warranted to determine whether H2A.Z deposition may be dysregulated in insulin resistance and type 2 diabetes.
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519

Insulin and glucose promote expression of CD36 and phagocytosis of oxidized-LDL in monocytes from lean insulin sensitive and obese insulin resistant subjects
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Background and aims: Obesity and insulin resistance are atherosclerosis risk factors and commonly associate with hyperinsulinemia and hyperglycemia. Expression of CD36 on monocytes and phagocytosis of oxidized-LDL are an essential part of the process of atherosclerosis. The aim of the present study was to investigate whether insulin sensitivity associate with the atherosclerotic properties of monocytes in lean and obese with or without insulin resistance subjects in the environment of hyperinsulinemia and hyperglycemia.
Materials and methods: Monocytes were isolated from whole blood of 8 lean insulin sensitive, 6 obese insulin resistant and 6 obese insulin sensitive subjects. Insulin sensitivity was estimated with euglycemic hyperinsulinemic clamp. For the estimation of CD36 scavenger receptor density on monocytes surface, a fluorescein isothiocyanate (FITC)-linked monoclonal antibody was used. LDL was oxidised and labeled with DiI towards DiI-oxi-LDL and phagocytosis from monocytes was estimated through the measurement of fluorescence after 1h and 3h of incubation. All experiments were repeated after pre-incubation of monocytes with insulin or glucose. The SPSS 15.0 was used for the statistical analysis.
Results: The 3 groups did not differ in the age ($p=0.9$) but there was significant difference in BMI (Kg/m^2) (20.3± 0.9 lean, 32.1±0.9 insulin sensitive obese and 33.3±1.4 insulin resistant obese) and glucose disposal rate (mg/Kg*min) (10.9±1 lean, 7.1±1.5 insulin sensitive obese and 2.3±1.5 insulin resistant obese). The surface density of CD36 on monocytes after incubation with glucose was increased in the groups of the lean ($p=0.035$) and the obese insulin resistant ($p=0.002$) subjects compared to the control samples. The same result was observed when monocytes were pre-incubated with insulin ($p=0.004$, lean subjects and $p=0.006$, obese insulin resistant subjects). Phagocytosis of oxi-LDL in lean subjects increases after monocyte incubation for 1h and 3h with glucose ($p=0.02$ and $p=0.032$, respectively) and with insulin ($p=0.015$ and $p=0.041$, respectively) compared to the control samples. In the obese insulin sensitive subjects an increase was observed only after 1h of monocyte incubation with glucose and insulin ($p<0.001$) while in obese insulin resistant patients phagocytosis of oxi-LDL was increased after 1h of monocyte incubation with insulin ($p=0.027$) and after 3h with glucose ($p<0.001$). A correlation between insulin sensitivity and CD36 surface density (Spearman's rho=0.343, $p=0.026$) and oxi-LDL phagocytosis (Spearman's rho=0.578, $p<0.001$), after monocyte incubation with insulin, was observed.
Conclusion: Insulin and glucose can influence CD36 surface density and oxi-LDL phagocytosis on human monocytes and an association between these properties and insulin sensitivity was observed in the hyperinsulinemic state. The differences noted among obese insulin sensitive subjects could be attributed to a cofounding factor possibly associated with the maintenance of "inappropriate" (according to their obesity) insulin sensitivity.
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520

Insulin and GH signalling in human skeletal muscle *in vivo* following exogenous GH exposure: impact of an oral glucose load

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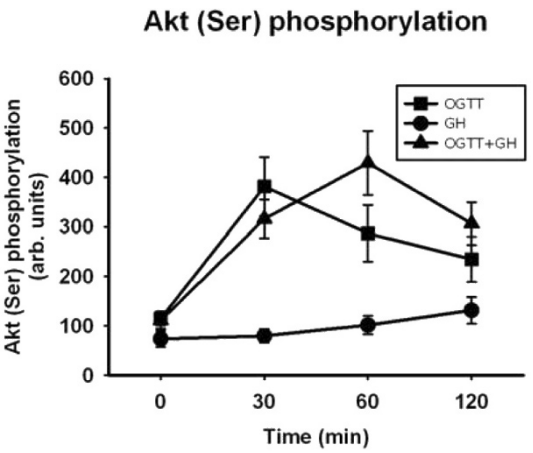
Background and aims: GH acutely induces insulin resistance in skeletal muscle in vivo but the underlying molecular mechanisms remain unknown. We tested the hypothesis that GH suppresses glucose-stimulated insulin signalling pathways
Materials and methods: Eight healthy young men (age 25 +/- 2 yrs) were studied in a single-blinded, randomised, crossover design. Each subject was studied in the morning after an overnight fast on three different days: 1) after an intravenous GH bolus (0.5 mg), 2) after an intravenous GH bolus plus and oral glucose load (75 g.), and 3) after intravenous saline plus oral glucose. Muscle biopsies were obtained at 0 min, 30 min, 60 min, 120 min and blood samples at regular intervals during each study day.
Results: GH increased $AUC_{glucose}$ (P= 0.05) without significant changes in blood insulin levels. GH induced phosphorylation of STAT5b independently of oral glucose intake. Conversely, oral glucose induced phosphorylation of the insulin signalling proteins Akt and AS160 independently of GH exposure.
Conclusion: 1) Insulin signalling pathways in human skeletal muscle in vivo are acutely activated by an oral glucose load. 2) A physiological GH bolus activates STAT5b signalling pathways acutely in skeletal muscle irrespective of ambient circulalting glucose and insulin levels.3) GH-induced insulin resistance in human subjects does not seem to be mediated by down regulation of insulin signalling pathways.



521

Influence of insulin kinetics on the post receptor signal transduction of insulin in patients with type 2 diabetes

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Background and aims: Next to its glucose lowering effects, insulin also exerts a specific activity when binding to insulin receptors on the endothelial cell. The nature of these effects is dependent on the insulin concentration and the degree of resistance of the insulin receptor. Dependent on the underlying conditions, insulin can lead to cardio-protective production of nitric-oxide or can induce atherogenic growth hormone effects. This pilot study was performed to investigate the impact of insulin pharmacokinetics on vaso-protective and atherogenic insulin signal transduction pathways in patients with type 2 diabetes mellitus.
Materials and methods: Seven patients with type 2 diabetes who participated in a prospective, randomized three-way crossover study could be included into this analysis (5 male, 2 female, age: 63±7 yrs., HbA1c: 7.1±0.6 %, BMI: 30.1±3.3 kg/m²). They received an individually calculated dose of regular human insulin (RHI) to cover a standardized liquid meal, or 90 % of this dose as the fast-acting insulin analog lispro (LP) and the ultra-rapid regular human insulin VIAject (VJ) on separate occasions. Next to glucose measurement, the mRNA of peripheral circulating monocytes, used as indicator cells for the endothelial insulin response, was isolated and subjected to quantitative determination of the expression of eNOS (anti-oxidative insulin effect) and MAPK-1 (atherogenic/growth hormone effect of insulin) by means of the LightCycler real-time PCR technology at 0, 30, 60, 90, 120 and 180 min.
Results: There was no difference in postprandial glycemic control between the three treatment arms. Treatment with RHI was associated with indications of atherogenic activity: increased postprandial expression of MAPK-1 (AUC of changes from baseline (0-240min): 80±124 AU), and decreased eNOS expression (-177±268 AU). In contrast, VJ showed a vasoprotective expression profile (MAPK-1: -50±78 AU, p<0.05 vs. RHI, eNOS: 98±134 AU, p=0.063). Intermediate results were obtained with LP (MAPK-1: 24±78 AU, p=0.084 vs. VJ, eNOS: -10±31 AU, p<0.05 vs. VJ).
Conclusion: In this pilot experiment, treatment with the ultra-fast regular human insulin formulation VIAject resulted in an improved endothelial function profile independent of glycemic control, while regular human insulin had atherogenic effects. These results suggest that the pharmacokinetic profile of insulin may have a major influence in the control of vasoprotective vs. atherogenic effects of insulin in patients with type 2 diabetes mellitus. Our results may be of help for the interpretation of recent outcome study results with insulin.
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522

AICAR stimulats insulin signalling pathway independent of AMPK activation in L6 myotubes

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Background and aims: AICAR is widely used as a pharmacological activator of AMPK and has been reported to increase glucose uptake and improve insulin sensitivity in skeletal muscle by activation of AMPK. Here, we observed the incremental effect of AICAR on insulin signaling pathway independent of AMPK activation in L6 myotubes.
Materials and methods: 2-deoxy [^{3}H] glucose uptake was performed in L6 myotubes to examine the effect of AICAR in the presence or absence of compound C or/and wortmanin under both basal and insulin stimulated states. Phosphorylation of AMPK and Akt were detected by western blot. Tyrosine phosphorylation of IR and IRS1 were detected by immunopricipitation. RNA interference was performed to silence AMPK gene expression.
Results: AICAR increased basal or insulin stimulated glucose uptake in L6 myotubes. The AICAR enhanced glucose uptake under basal condition could not be suppressed by preincubation with either compound C or wortmanin, but could be fully blocked by the combination of compound C and wortmanin. AICAR increased Akt phosphorylation in L6 myotubes with a time and dose dependent manner, and this effect could not be blocked by pretreatment with compound C or AMPK silencing using *si*RNA, which suggested that AICAR stimulated Akt phosphorylation independent on AMPK activation. Moreover, AICAR dose dependently increased tyrosine phosphorylation of IR and IRS1, which suggested that AICAR could activate insulin signaling pathway.
Conclusion: ACIAR could activate insulin signaling pathway independent on AMPK activation in L6 myotubes. The enhanced glucose uptake induced by AICAR might be not only due to the activation of AMPK, but also the activation of insulin signaling pathway.
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PS 31 Clinical physiology of insulin sensitivity

523

Gender effect on insulin sensitivity and secretion in different categories of glucose tolerance

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Background and aims: It is not yet clear whether parameters on insulin resistance and insulin secretion extracted from metabolic tests depend upon the sex of the subject. With this study, we aimed to evaluate the gender effect on insulin sensitivity and beta cell function in a large population of subjects divided according to main categories of glucose tolerance. Gender effect was assessed after having accounted for age and body mass index (BMI).
Materials and methods: A total of 2130 subjects of both genders (F=females; M=males) were studied with a 3h-75g oral glucose tolerance test (OGTT) with samples every 30 min. Subjects were divided into three categories according to their fasting and 2h glucose level: normal tolerance (NGT; 611/362 F/M; age 37±1/40±1 (mean±SE) [range 10-77] years; BMI=25.6±0.2/25.9±0.2 [16.3-57.3] kg/m^2; fasting glucose, G_b 4.7±0.02/4.9±0.02 mmol/l; fasting insulin, I_b 52±1/56±2 pmol/l); impaired fasting glucose or glucose tolerance (IGM; 301/441; age 55±1/54±1 [11-78]; BMI 28.6±0.3/27.5±0.2 [15.5-53.0]; G_b 6.1±0.03/6.2±0.02; I_b 74±3/73±2) and type 2 diabetes (T2D; 180/235; age 60±1/58±1 [25-79]; BMI 28.9±0.4/28.2±0.3 [17.3-62.9]; G_b 7.4±0.09/7.3±0.06; I_b 92±5/92±5). From OGTT data, we calculated the insulinogenic index as beta cell function [BCF=$(I_{30}-I_b)/(G_{30}-G_b)$, where I_{30} and G_{30} are insulin and glucose measured at 30 min], insulin sensitivity [IS=10000/$\sqrt{(I_b \times G_b \times \text{mean(I)} \times \text{mean(G)})}$, Matsuda Index] and glucose clearance (OGIS from mathematical modeling). Statistical analysis was a pooled analysis of 3 homogeneous data-sets performed using multiple regression, ANOVA and 2-sample t-test in suitably defined strata.
Results: BCF is reduced with age and increased with BMI independently of the tolerance category and sex ($p<0.0001$). Taken all together, BCF is higher in F than in M (103±6 *vs.* 79±10 pmol/mmol, $p<0.001$) after having accounted for age and BMI. Precisely, F have a significantly higher BCF only in NGT ($p<0.0001$), while BCF was not different by sex in IGM ($p=0.11$) and T2D ($p=0.30$). IS is markedly reduced with increasing BMI in every category ($p<0.0001$), while it does not change with sex ($p=0.46$) and age ($p=0.14$). When the gender effect is evaluated, after having accounted for age and BMI, F have a higher IS only in NGT (7.2±0.2 *vs.* 6.7±0.3, $p=0.015$), being $p=0.68$ in IGM and $p=0.21$ in T2D. OGIS depends upon age and BMI, decreasing when they augment ($p<0.001$), but only marginally related to sex ($p=0.081$). When dividing into categories, again, the difference between sex is observed only in NGT (F 473±3 *vs.* M 454±3 ml/min/m^2, $p<0.005$), being $p=0.66$ in IGM and $p=0.18$ in T2D.
Conclusion: Beta cell function is highly dependent on age and BMI, while sex related differences are present only in NGT. Also insulin sensitivity, highly dependent on BMI, is higher in F than in M only in NGT, when calculated accounting for age and BMI. In general, beta cell function and insulin sensitivity are influenced by gender only in individuals with normal glucose tolerance; with deterioration of metabolic control, as observed in impaired tolerance and type 2 diabetes, the effect of gender vanishes.
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524

Association of elevated serum ferritin concentration with insulin resistance and impaired glucose metabolism in Koreans

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Background and aims: Iron overload in patients with hemochromatosis or hematologic diseases, is reported to be associated with insulin resistance and development of diabetes. However, data concerning relationship between serum ferritin levels and glucose metabolism abnormalities in non-pathologic conditions are scanty. This study investigated the association of serum ferritin levels with insulin resistance and impaired glucose metabolism.
Materials and methods: We analyzed the clinical and laboratory data of 12,090 subjects (age 18-90 years, 6,387 men and 5,703 women) who underwent routine medical check-ups. Those included 1,084 subjects with type 2 diabetes (DM), 3,783 with impaired fasting glucose (IFG), and 7,223 with normal fasting glucose (NFG). Serum ferritin, HbA1c, fasting glucose, and insulin levels were measured. Insulin resistance (HOMA-IR) and beta-cell function (HOMA%B) indices were derived from homeostasis model assessment.
Results: Since the average serum levels of ferritin was higher in men than in women, we analyzed the data of men and women separately. Ferritin concentrations were the highest in DM group, followed by the IFG group, and the NFG group in both men and women (160 ± 98, 181 ± 116, 195 ± 139 ng/ml, respectively, in men; 59 ± 54, 76 ± 60, 91 ± 66 ng/ml, respectively, in women; $p < 0.01$ for all). In multiple regression analysis, serum ferritin levels were associated with BMI, waist circumference, fasting glucose, serum triglycerides, aspartate aminotransferase, alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), high sensitivity CRP (hsCRP), and HOMA-IR in men. In women, age, fasting glucose, triglycerides, ALT, GGT, and hsCRP levels were related to serum ferritin concentration. After adjustment for age, BMI, waist circumference, and blood pressure, the odds ratio (OR) for T2DM, comparing the fourth quartile of ferritin with the first quartile, was 1.60 (95% confidence interval 1.29-1.99) in men and 1.40 (0.98-2.01) in women. In men, after further adjustment for ALT, GGT, triglyceride, and hsCRP, the OR was attenuated to 1.28 (1.02-1.61). In non-diabetic subjects, after adjustment for age, BMI, waist circumference, and blood pressure, OR for IFG in the fourth quartile of ferritin was 1.64 (1.40-1.92) in men and 1.68 (1.39-2.03) in women. After further adjustment for ALT, GGT, triglyceride, and hsCRP, the OR was slightly attenuated to 1.36 (1.15-1.61) for men, and 1.52 (1.25-1.85) for women. In NFG subjects, the OR for metabolic syndrome in the fourth quartile of ferritin after adjustment for age and BMI, was 1.99 (1.36-2.92) in men and 1.19 (0.80-1.76) in women. In men, after further adjustment for ALT, GGT, and hsCRP, the OR was slightly attenuated to 1.61 (1.08-2.41).
Conclusion: Increased serum level of ferritin was associated with insulin resistance, type 2 diabetes, IFG, and metabolic syndrome in men, but only with IFG in women. These results suggest that iron overload is strongly associated with insulin resistance in men, but not clearly in women.

525

Ferritin concentrations, type 2 diabetes, metabolic syndrome and insulin resistance in a Chinese population

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Background and aims: Inflammation may play a key role in the development of Diabetes Mellitus (DM), metabolic syndrome and insulin resistance. High level of the acute phase reactant ferritin has been reported to positively correlate with DM and insulin resistance. In this study we examined the association among serum ferritin concentrations, DM, metabolic syndrome and insulin resistance in a Chinese population in Sichuan province.
Materials and methods: We conducted a cross-sectional study in 3040 subjects over 18 years old from three communities in Sichuan province. Serum ferritin concentrations, glucose tolerance status, plasma lipids, alanine aminotransferase, aspartate aminotransferase, uric acids and creatinine were determined in all subjects. Blood pressure, BMI and waist circumferences were also measured. Metabolic syndrome was diagnosed using the IDF criteria.
Results: Individuals with newly diagnosed diabetes, previously diagnosed diabetes and prediabetes (including IGT and IFG) had elevated serum ferritin concentrations compared with those of normal glucose tolerance (191.13±114.96μg/l vs. 184.63±109.51μg/l vs. 164.77±116.25μg/l vs. 119.58±97.57μg/l, $P<0.05$). After adjusting for age, race, BMI, smoking, alcohol consumption, activity, family history, education, residential district, waist circumferences, waist-to-hip ratio (WHR), blood pressure, plasma lipids and uric acids, (the odds ratio of prediabetes and DM was 1.54 (95% CI: 1.08-2.19) in men with ferritin concentrations ≥300μg/l and was 1.80 (95% CI: 1.36-2.39) in women with ferritin concentrations ≥150μg/l, respectively.) the odds ratio of prediabetes and diabetes was 1.46 (95% CI 1.05-2.03) in men with elevated ferritin concentrations in the highest quartile (≥271.1μg), and 1.76 (95% CI 1.34-2.33) in women with elevated ferritin concentrations(≥141.5μg).

The prevalence of metabolic syndrome in subjects with serum ferritin concentrations in the highest quartile was significantly higher than those whose serum ferritin concentrations were in the lowest quartile, either in men (32.2% vs 10.1%, P<0.001) or women (38.8% vs 10.7%, P<0.001). After adjusting for age, race, BMI, smoking, alcohol consumption, activity, family history, education and residential district, the odds ratios of metabolic syndrome in subjects with serum ferrtin concentrations in the highest quartile were 1.71 (95% CI 0.92-3.19) in men and 1.67 (95% CI 1.03-2.70) in women when compared with those in the lowest quartile. In all subjects, the serum ferritin concentrations increased gradually with the increasing numbers of co-existing metabolic components accordingly (P<0.001). This positive relationship between the numbers of co-existing metabolic components and serum ferritin concentrations remained significant when male and female subjects were analyzed separately (P<0.001). A positive association was found between serum ferritin concentrations and HOMA-IR. HOMA-IR (≥1.98) was associated with a higher level of ferritin and uric acid.
Conclusion: Serum ferritin concentrations increased remarkably in diabetic patients, and elevated serum ferritin concentrations were associated with an increased risk of prediabetes(including IGT and IFG) and diabetes. Furthermore, we found a positive association between serum ferritin concentrations and metabolic syndrome. Also elevated level of ferritin was positively associated with insulin resistance.

526

Independent association between the renin angiotensin aldosterone system and insulin resistance

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Background and aims: The nonsuppressed activity of the Renin Angiotensin Aldosterone System (RAAS) in the presence of high salt diet is increasingly implicated to play a key role in the development and progression of insulin resistance (IR). With the present analysis, we aimed to investigate the impact of plasma renin (PRC), aldosterone (PAC) and angiotensin 2 (ang2) concentrations on IR in a large cohort of patients (on usual western diet) of type 2 diabetic and nondiabetic individuals referred to coronary angiography.
Materials and methods: We performed multivariate adjusted statistical analysis to investigate the cross-sectional relations between mediators of the RAAS to IR. IR was assessed from fasting insulin and glucose levels and the validated homeostasis model assessment (HOMA-IR). We measured plasma renin (PRC), angiotensin2 (ang2) and aldosterone concentrations (PAC) reflecting systemic RAAS as wells as several circulating biomarkers reflecting inflammation (CRP), endothelial dysfunction (homocysteine), lipid metabolism (HDL-, LDL-cholesterol, free fatty acids, triglycerides, adiponectin and leptin), renal function (cystatin C), hemostasis (PAI-1 and fibrinogen) and neurohormonal activation (NT-pBNP).
Results: The mean age of the study population (n = 3153; 31.9% women) was 62.7 (± 10.6 years) and type 2 diabetes was present in 999 (31.7%) individuals studied. Fasting insulin (U/L) and HOMA-IR (U), were significantly different between diabetic and nondiabetic probands (11.9± 10.8 versus 15.8 ± 15.1 U/L, P<0.001; and 3.22 ± 4.5 U versus 5.1 ± 5.8, P<0.001, respectively). In regard of the RAAS, only PRC (pg/mL) was elevated in diabetic compared to nondiabetic individuals (46.7 ± 137.7 versus 39.8 ± 165.2; P=0.006). We performed (log-transformed) multivariable adjusted one-way analysis of variance to compare distribution of fasting insulin and HOMA-IR within quartiles of PRC, ang2 and PAC levels. Overall, we observed a steadily and significant absolute rise in mean fasting insulin across PRC-, ang2- and PAC-quartiles (Q) (mean increase: 3.24 U/L, P<0.001 (PRC-Q); 1.36 U/L, P<0.001 (ang2-Q) and 3.36 U/L, P<0.001 (PAC-Q), respectively). Accordingly, median HOMA-IR increased steadily from the bottom Q of PRC, ang2 and PAC-levels to the upper Q of each RAAS mediator (from 1.91 U (PRC-Q1) to 2.88 U (Q4), P<0.0001; from 2.19 U (ang2-Q1) to 2.55 U (Q4), P<0.001; and from 1.79 U (PAC-Q1) to 2.76 U (Q4), P<0.001). In a multivariate stepwise regression model after plasma leptin (β=0.299, P<0.001), PRC (β=0.170, P<0.001) and PAC (β=0.129, P<0.001) emerged as strongest predictors for fasting insulin. With this in line, plasma leptin, PRC, BMI, PAI-1, HDL-cholesterol and PAC (for all variables p<0.001) emerged as most important predictors for HOMA-IR. The overall models used explained 29.1% and 32.1% of the interindividual variability of fasting insulin and HOMA-IR, respectively.
Conclusion: Our findings suggest that inappropriate RAAS activity contributes considerably to an increased IR and may therefore provide a feasible mechanism for target organ damage in patients with impaired glucose metabolism.

527

The effect on insulin action of the angiotensin converting enzyme inhibitor captopril combined with either a calcium channel blocker, amlodipine, or low dose thiazide, bendroflumethiazide

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Background and aims: Combinations of antihypertensive agents are needed to meet stringent blood pressure (BP) targets in patients with diabetes. Concern exists regarding deleterious metabolic effects of some of these drugs. Angiotensin converting enzyme (ACE) inhibitors are neutral or maybe beneficial with regard to insulin action, whereas thiazide diuretics may cause insulin resistance unless used in low dose. Calcium channel blockers are often used first line or as part of combination regimens, but less is known about their metabolic effects. In this study we compared the effects on insulin action of two commonly used regimens; the ACE inhibitor captopril combined with low dose diuretic bendroflumethiazide, or captopril combined with the calcium channel blocker amlodipine.
Materials and methods: We performed a randomised cross over trial in 13 hypertensive patients with type 2 diabetes. Following six week placebo run in, patients were assigned captopril 50 mg twice daily combined with either amlodipine 10 mg once daily (CA) or captopril combined with bendroflumethiazide 1.25 mg once daily (CB) for 12 weeks. The primary outcome, insulin sensitivity, was assessed by the isoglycaemic hyperinsulinaemic clamp after placebo run in and following the two 12 week treatment periods. The power of the study, based on previous clamp data, gave a 90% chance of detecting a 20% difference in action of insulin at the 5% level of significance. Analysis was as recommended by Hills and Armitage for crossover studies.
Results: Both combinations led to a reduction in BP from baseline (CA 140/82 to 131/76 mmHg; p<0.05: CB 144/81 to 126/76 mmHg; systolic reduction p<0.05 and diastolic p=0.08). Systolic BP was lower following CB compared to CA (126 vs. 131 mmHg; p=0.004). Fasting glucose (CA 7.7 ± 0.4 mmol/l vs. 8.0 ± 0.5 mmol/l, p=0.35), HbA1c (CA 6.9 ± 0.4 vs. CB 7.2 ± 0.4 %; p=0.14) and fasting insulin concentrations (CA 9.4 ± 1.1 mU/l vs. CB 11.04 ± 1.42 mU/l, p=0.07) all tended to be higher following CB compared to CA, but none of these differences reached statistical significance. Exogenous glucose infusion rates required to maintain isoglycaemia during hyperinsulinaemia were similar after CA compared to CB (28.8 ± 4.2 vs. 25.3 ± 3.8 μmol/kg/min; p=0.12). There was also no difference between treatment periods in basal endogenous glucose production (CA 10.0 ± 0.7 vs. CB 10.9 μmol/kg/min; p=0.64), nor was there any change from baseline (10.0 ± 0.8 μmol/kg/min). Suppression of endogenous glucose production, an index of hepatic insulin sensitivity, was similar between therapies (p=0.74).
Conclusion: Whilst both combinations were effective in lowering BP from baseline, the captopril/bendroflumethiazide combination lowered BP to a greater extent than captopril/amlodipine. Although there was a tendency towards negative metabolic effects with the captopril/bendroflumethiazide combination, the differences did not reach the predetermined level deemed clinically significant. A small negative effect of captopril/bendroflumethiazide could be uncovered in a larger trial.
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528

A double-blind, placebo-controlled, crossover study to evaluate the effects of multiple doses of prednisone on insulin sensitivity in healthy lean males

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Background and aims: Supraphysiologic endogenous or exogenous glucocorticoid activity has been well established as a cause of impaired glucose

tolerance. To date, no single study has evaluated the dose-dependent effects of the widely used glucocorticoid, prednisone, on glucose disposal during short-term oral administration. The objective of the study was to use the hyperinsulinemic euglycemic clamp to assess the effects of commonly used prednisone doses on insulin action.
Materials and methods: Following one week of daily administration of 10 mg and 25 mg prednisone or placebo in a double-blinded, randomized, crossover study design of 17 healthy, nondiabetic, lean males (age 31.5 ± 9.2 years, BMI 23.2 ± 1.4 kg/m^2); glucose disposal was assessed using a two-step hyperinsulinemic euglycemic clamp with insulin infusion rates of 0.5 mU/kg·min for step 1 and 2.0 mU/kg·min for step 2. The amount of glucose infused during the final 30 minutes of each step ("steady state"), to maintain a stable serum glucose level (90 mg/dl) was used as a biomarker for insulin sensitivity and was used to calculate whole body glucose disposal (M), glucose metabolic clearance rate (MCR), M/I ratio and the insulin-sensitivity index ($S_{i)}$. The drug effect, as measured by a decrease in insulin sensitivity for each dose of prednisone as compared to placebo, was estimated and compared for the difference between doses and the associated one-sided P-value from an analysis of covariance (ANCOVA) mixed-effects model for 3 period crossover design. Results from each step of the clamp (i.e. the different insulin infusion rates) were analyzed separately.
Results: During Step 1 of the clamp, M and MCR were both reduced by 35% (p=0.003) while M/I was reduced by 29% (p=0.025) for 25 mg prednisone compared to placebo. No appreciable effect of 10 mg prednisone was observed. During Step 2 of the clamp, M was reduced by 33% (p=0.001) and by 15% (p=0.006), respectively for 25 mg and 10 mg prednisone compared to placebo; MCR was reduced by 32% (p<0.001) and 14% (p=0.008), respectively for 25 mg and 10 mg prednisone compared to placebo; M/I ratio was reduced by 31% (p<0.001) and 13% (p=0.026), respectively for 25 mg and 10 mg prednisone compared to placebo. Compared to placebo, S_i decreased by 33% (p<0.001) and 19% (p<0.014) for 25 mg and 10 mg prednisone, respectively. For 25 mg prednisone compared to 10 mg prednisone, S_i decreased by 17% (p=0.049).
Conclusion: Based on the analyses of these indices of insulin sensitivity, short-term prednisone administration results in dose-dependent inhibitory effects on insulin action in healthy, lean males, indicating that gluco-metabolic abnormalities may occur following treatment with even low to moderate doses of prednisone.

529

Evaluating effect of insulin resistance and beta cell function in a pioglitazone and metformin fixed-dose combination study
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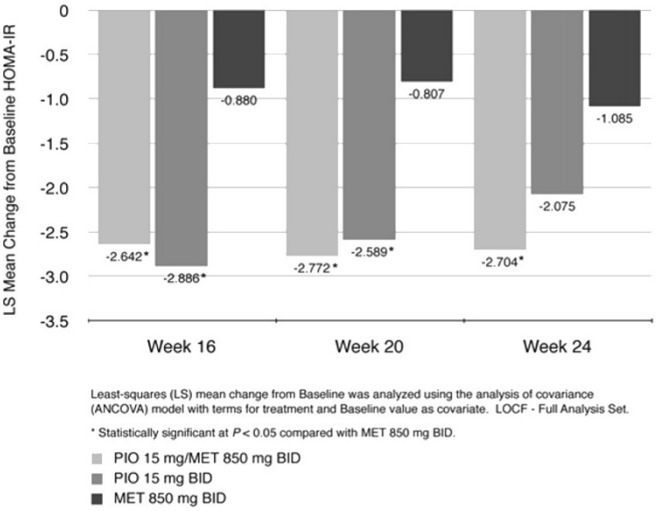
Background and aims: Insulin resistance and failure of the ß-cell to adapt to increasing insulin needs are the two core defects in type 2 diabetes mellitus (T2DM). Homeostatic model assessment (HOMA) is a method used to quantify insulin resistance or ß-cell function. Pioglitazone (PIO) improves glycemic control primarily by reducing insulin resistance, whereas metformin (MET) exerts its effect primarily by decreasing hepatic glucose output. In addition, thiazolidinediones may also have direct effects on ß-cell preservation and ß-cell function improvement.
Materials and methods: In this double-blind trial comparing fixed-dose combination (FDC) of PIO/MET vs respective monotherapies, the efficacy and safety of PIO/MET FDC therapy in T2DM patients during a 24-week trial period were studied. Patients were on no antidiabetes medications for 12 weeks and had a hemoglobin A1c (A1c) ≥7.5% and ≤10%. The primary endpoint was change from Baseline in A1c of PIO/MET FDC (N=201) vs PIO (N=189) or MET (N=210) monotherapy. Change from Baseline in HOMA-IR was evaluated as a secondary endpoint using fasting glucose and insulin levels.
Results: In all treatment groups, compared with Baseline (P<0.05), A1c was improved. A significant decrease in HOMA-IR was observed in Week 16 through 24 for the PIO/MET FDC, which was comparable to PIO monotherapy at Week 16 and 20, with a smaller decrease in HOMA-IR observed in the MET group. At Final Visit (Week 24), the greatest decrease in HOMA-IR was seen in those receiving PIO/MET FDC (Figure).
Conclusion: The improvement in HOMA-IR was most in PIO/MET FDC and PIO monotherapy, and PIO appeared to be the major contributing factor. Analysis of overall treatment-emergent adverse events showed no increase in the combination group with 50.7% for PIO/MET FDC and 52.1% and 53.1% for PIO and MET monotherapy, respectively.



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530

Pioglitazone and sitagliptin compared to pioglitazone and metfomin therapy on glycaemic control and on insulin resistance in type 2 diabetic patients
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Background and aims: our study aimed to compare the effects of pioglitazone (P) plus sitagliptin (S) vs pioglitazone (P) plus metformin (M) on glycemic control and on insulin resistance related-parameters in type 2 diabetic patients.
Materials and methods: ninety-nine type 2 diabetic patients with uncontrolled type 2 diabetes mellitus (HbA1c > 7.5 %) were randomised to P 30 mg o.d. and S 100 mg o.d. or to P 15 mg b.i.d. and M 850 mg b.i.d. All type 2 diabetic patients were resulted not well controlled with diet and physical activity and P at dosage of 30 mg/day. The treatment period had a 9 months duration. We evaluated BMI, HbA1c, FPG, PPG, FPI, Homa index and collected plasma samples of adiponectin (ADN), resistin (R), tumor necrosis factor-alpha (TNF-α), and high-sensitivity C reactive protein (Hs-CRP) at baseline, and after 9 months.
Results: ninety-two patients completed the study (46 in PS and 46 in PM group). BMI was significantly reduced by PM, but not by PS (from 27.8±1.4 to 27.4±1.1 Kg/m^2, ns vs baseline, and from 27.6±1.2 to 26.8±0.9 Kg/m^2, p< 0.05 vs PS, respectively). HbA1c was decreased by 1.1±0.07 % (p< 0.01), and by 1.3±0.09 % (p< 0.01); FPG was reduced by 17±3 mg/dl (p< 0.01), and by 23±4 mg/dl (p< 0.01); PPG was decreased by 29±5 mg/dl (p< 0.01), and by 31±6 mg/dl (p< 0.01), in PS and PM group, respectively. FPI was decreased by 3.0±0.2 μU/ml (p< 0.05), and by 3.9±0.3 μU/ml (p< 0.01 vs baseline, p< 0.05 vs PS), and Homa index by 1.6±0.5 (p< 0.05), and by 2.1±0.6 (p< 0.01 vs baseline, p< 0.05 vs PS) in PS and PM group, respectively. ADN was increased by 0.2±0.002 μg/ml (ns vs baseline), and by 1.2±0.2 μg/ml (p< 0.05 vs baseline, p< 0.05 vs PS), in PS and PM group, respectively. Resistin was reduced by 0.2±0.001 ng/ml (ns vs baseline), and by 2.2±0.3 ng/ml (p< 0.05 vs baseline, p< 0.05 vs PS), TNF-α by 0.2±0.001 ng/ml (ns vs baseline), and by 1.0±0.3 ng/ml (p< 0.05 vs baseline, p< 0.05 vs PS), and Hs-CRP by 0.7±0.005 mg/l (p< 0.05), and by 0.7±0.005 mg/l (p< 0.05), in PS and PM group, respectively. There was a significant correlation between Homa index decrease and ADN increase (r= -0.57, p< 0.01), R decrease (r= 0.55, p< 0.01), and TNF-α decrease (r= 0.53, p< 0.01).
Conclusion: both combinations ameliorated diabetes control, but only PM improved insulin resistance related-parameters. The ADN increase, R and TNF-α decrease seem to be related to Homa index improvement.

531

A comparison between metabolic and vascular effects of simvastatin and rosuvastatin in patients with type 2 diabetes

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Background and aims: Type 2 diabetes (T2D) is frequently characterized by metabolic and vascular abnormalities, like insulin-resistance, endothelial dysfunction and low-grade systemic inflammation, leading to an increased risk of coronary heart diseases. Statins have been largely reported to reduce cardiovascular events, with a distinctive benefit for patients with T2D. Beside variations in lipid profile, this beneficial effect might be attributable to a number of vascular actions of statins, including effects on endothelial dysfunction and inflammation. However, the potential effects of statins on insulin-resistance and glucose tolerance in diabetic patients are still controversial. The present randomized, parallel, single-blind, intervention study has been direct to compare the effects of treatment with rosuvastatin or simvastatin on glucose control, insulin-resistance and endothelial dysfunction in individuals with T2D**.**
Materials and methods: After a run-in period of 3 weeks, twenty-nine dyslipidemic T2 diabetic (non obese) patients (aged 56±8, mean±SD) treated with oral antidiabetic drug (OAD) were randomly assigned to rosuvastatin 20 mg/daily (Group R, n=14) or simvastatin 20 mg/daily (Group S, n=15) for six months. The following data were collected before and after treatment period: BMI, waist circumference, fasting glucose, HbA1c, lipid profile, hs CRP, fibrinogen, TNF-alpha, glucose disposal assessed by euglycemic hyperinsulinemic clamp and flow-mediated vasodilation (FMD) evaluated by brachial artery reactivity technique (BART). We used Student t-test for paired data to compare values (mean±SD) before and after treatment.
Results: No patients changed OAD therapy or modified their own life-style habits during the study. After six months of treatment, LDL decreased of 55% in Group R (56±13 mg/dl, p<0.001) and 40% in Group S (81±12 mg/dl, p<0.001). Faced to this improvement in LDL levels, we observed a change in glucose tolerance and insulin-resistance in both groups (baseline vs treatment): fasting glucose (Group R 135±20 vs 149±23 mg/dl, p=0.004; Group S 136±24 vs 142±19 mg/dl, p<0.05); HbA1c (Group R 6.6±0.7 vs 7.1±0.4%, p<0.001; Group S 6.7±0.2 vs 7.0±0.4%, p<0.05); peripheral glucose disposal (Group R 5.1±0.6 vs 3.8±0.9 mg/kg/min, p=0.02; Group S 5.9±0.7 vs 5.2±0.5 mg/Kg/min, p=NS). FMD improved from baseline both in patients taking rosuvastatin (9.2±3.4 vs 12.7±4.5%, p=0.01) and in patients taking simvastatin (6.7±2.4 vs 12.3±4.6%, p=0.002). Only subjects in Group R showed a significant change from baseline in hs CRP (3.4±1.5 vs 1.1±0.9 mg/l, p=0.03), fibrinogen (343±27 vs 287±26 mg/dl, p=0.04) and TNF-alpha (12.2±6.5 vs 8.9±4.3 pg/ml, p=0.02).
Conclusion: In T2 diabetic patients treated for six months with Rosuvastatin 20 mg/daily or Simvastatin 20 mg/daily it has been reported an increased defect in fasting glucose concentration and HbA1c levels. In patients taking rosuvastatin the alterations in glucose homeostasis are related to an impaired insulin sensitivity. By contrast, both statins improved vascular reactivity, with a significant change in inflammation biomarkers in the rosuvastatin arm. More data are requested to better elucidate the relationships between metabolic and vascular actions of different statins, in order to verify whether certain drugs could be more appropriate than others to treat T2D dyslipidemia.

PS 32 Insulin sensitivity and lipids

532

The relationship of liver fat content, glucose fluctuation and components of metabolic syndrome

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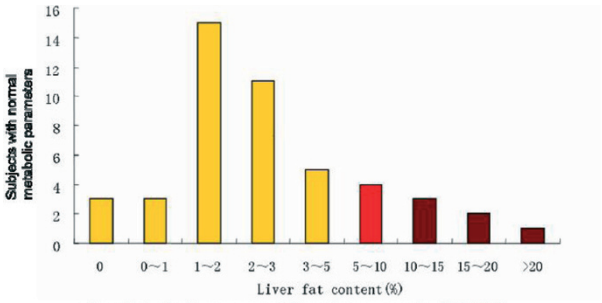
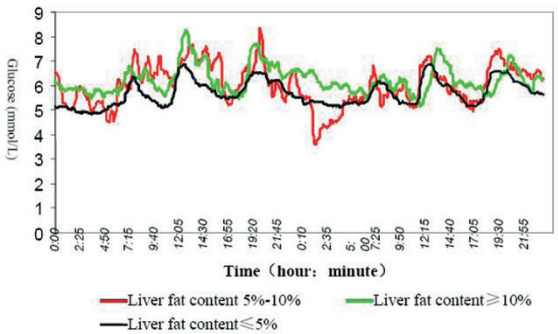
Background and aims: Both lean and obese insulin resistant individuals have an excess of fat in the liver. Liver fat is highly significantly and linearly correlated with all components of the metabolic syndrome independent of obesity. Excess accumulation of fat in liver increases the risk of type 2 diabetes and cardiovascular disease, but the association of liver fat content and character of glucose fluctuation is unknown. This study aims to observe the association of liver fat content, character of glucose fluctuation and main metabolic parameters in 47 subjects with normal metabolic parameters monitored using the Continuous Glucose Monitoring System(CGMS) and 34 patients with non-alcoholic fatty liver disease (NAFLD) tested the same metabolic parameters but without CGMS in China.
Materials and methods: 47 subjects with normal metabolic parameters were involved in the study by standard interview, anthropometrics (height, weight, waist circumference, hip circumference and blood pressure) measurement, Laboratory tests included liver enzymes, renal function, lipid profile, serum glucose and insulin in oral 75g glucose tolerance test. All subjects were monitored using the CGMS for 3 days.34 patients with NAFLD were tested the same metabolic parameters but the CGMS. The liver fat content was measured by proton magnetic resonance spectroscopy (^{1}H MRS).
Results: 37 subjects (78%) with liver fat content<5% (1.98%±1.17%), 10 subjects (22%) with liver fat content>5%(14.48%±12.22%). All subjects were divided into two groups according the median of liver fat content tested by ^{1}H MRS. The levels of serum triglyceride, fasting C peptide, insulin, HOMA-IR, area under curve of insulin and Insulin-plus of the 30th minute in OGTT in 23 subjects with liver fat content ≥2.21% were higher, but the HDL-c was lower than those of the group with liver fat content <2.21%.(P<0.05). Liver fat content was positive correlated with the level of BMI (r=0.547), ALT(r=0.676), AST(r=0.514), GGT(r=0.474), ALP(r=0.358), TG(r=0.577), fasting blood glucose (r=0.281) (all P<0.05), but was negative correlated with the level of HDL-c (r=-0.315, P=0.004) in all 81 subjects including 47 subjects with normal metabolic parameters and 34 patient with NAFLD. 35 valid data of CGMS were involved in the analysis. The MBG was 5.75±0.09 mmol/L, MAGE was 2.32±0.83 mmol/L. The liver fat content was positively correlated with MBG (r=0.338, P=0.047), AUC of blood glucose >5.6mmol/L(r=0.408, P=0.015), AUC of blood glucose>6.1mmol/L(r=0.409, P=0.015), and was not correlated with the highest blood glucose, the lowest blood glucose and MAGE.
Conclusion: 22% subjects with normal metabolic parameters have increased accumulation of liver fat and hepatic insulin resistance. Disorder of metabolism existed in the subjects with excess accumulation of liver fat. The glucose fluctuation was found in the subjects with liver fat content>2.21%.



Graph 1. the frequency of liver fat content by ^{1}HMRS



Graph 2. Curves of CGMS in three group according to liver fat content by ^{1}H MRS

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533

Lipid accumulation product is strongly associated with surrogates of insulin resistance, metabolic syndrome and cardiovascular disease risk in healthy men

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Background and aims: lipid accumulation product (LAP), a novel index of central lipid accumulation, has been associated with diabetes and cardiovascular risk. LAP is based on a combination of waist circumference (WC) and triglyceride (TG) [LAP = (WC - 65) × TG for men, and (WC - 58) × TG for women]. Because insulin resistance (IR) is extremely common and its prevalence is increasing, early detection of high-risk individuals could be useful to predict and prevent diabetes and cardiovascular disease (CVD). We performed a population cross-sectional study in order to identify simple parameters, as LAP, with high ability in predicting IR, metabolic syndrome (MS) and CVD risk.
Materials and methods: 601 unrelated healthy men were recruited at our hospital. All subjects were non-diabetic blood donors with normal findings on medical examination and blood count, and free from any medication. Their ages ranged between 18 to 65 years (36.9 ± 10.8 years). The study was carried out in accordance with the Declaration of Helsinki, and approved by the ethic committee of our hospital. MS was determined according to the International Diabetes Federation criteria (IDF-MS). Fasting insulin levels were measured using a human specific radioimmunoassay kit (cross-reactivity is less than 0.2% to intact human proinsulin and the primary circulating split form (des 31, 32)). HOMA-IR, QUICKI and LAP were calculated. Cardiovascular risk was determined using the Framingham risk score.
Results: LAP was positively correlated with HOMA-IR (P <0.0001) and fasting insulin (P <0.0001), and negatively correlated with QUICKI (P <0.0001). LAP showed high areas under the curves (AUCs) for ROC (receiver operating characteristic) curves for cutoff values of HOMA-IR (above 75th percentile, AUC 0.76 ± 0.01 [95% CI 0.72-0.81]), QUICKI (below 25th percentile, AUC 0.76 ± 0.01 [0.71-0.81]) and fasting insulin levels (above 75th percentile, AUC 0.76 ± 0.01 [0.72-0.81]). The prevalence of IDF-MS was 38.6%. LAP was strongly associated with IDF-MS (P <0.0001) and their individual components (P <0.0001 for every component). LAP showed the highest diagnostic accuracy for IDF-MS (AUC 0.89 ± 0.01 [0.86-0.92]) in comparison with other parameters such as HOMA-IR (AUC 0.71) among others. These findings were confirmed by internal validation using 32,000 bootstrap samples. The best cut-off value for prediction of IDF-MS was LAP 47.3 (sensitivity 0.76; specificity 0.82; predictive value of positive test 0.73; predictive value of negative test 0.85; correct classification rate 0.80). LAP was positively correlated with the cardiovascular Framingham risk score (P <0.0001).
Conclusion: This is the first report finding, in Argentinian healthy men, that LAP is strongly associated with IR, has the highest diagnostic accuracy for IDF-MS, and is strongly positively correlated with the cardiovascular Framingham risk score. LAP could be associated with a dysfunctional and lipolytic adipose tissue which is a central abnormality behind IR, MS, type 2 diabetes and CVD. The reliability of LAP in early identification of high-risk individuals for IR, MS and CVD requires further investigation using longitudinal designs.
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534

Effects of fish oil on metabolic alterations induced by carbohydrate overfeeding in healthy volunteers

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Background and aims: Long-term positive energy balance could induce metabolic alterations that lead to metabolic syndrome (MS). The aim of our study was to assess if fish oil (FO) supplementation could partially or completely prevent the metabolic alterations induced by 4-day carbohydrate overfeeding (O).
Materials and methods: 32 volunteers were randomized in 2 groups: one group without o (C) (10 W and 6 M, 44.1 ± 1.5 y, BMI: 22.30 ± 0.42 kg/m^2) and one group with O (7 W and 9 M, 44.1 ± 1.6 y, BMI: 22.63 ± 0.36 kg/m^2). Group C was studied once while absorbing usual diet; group O was studied 6-w apart while absorbing over 4-d prior to each experiment 75% energy from CHO/d in addition of their daily energy expenditure. One test was made on each volunteer from C. The volunteers from O have been submitted to 2 periods of O 6 weeks apart. During these 6 weeks, after randomization, 8 volunteers received 3g/d of FO (Epax 6000 TG QualitySilver®, Polaris, Pleuven, France: 1.7g/d of EPA + DHA), the other 8 received 3g/d of paraffin oil (placebo P). Parameters studied: Glucose Infusion Rate (GIR) (euglycaemic hyperinsulinaemic clamp: insulin infusion rate = 1 mU/kg/min), mean arterial blood pressure (MBP), heart frequency (HF), plasma FFA, HDL, triglycerides (TG), insulin concentrations and cutaneous endothelium independent vasodilatation.
Results: mean±SEM (ANOVA and Student's t-test). Comparison of O (n=16) vs. C (n=16): Basal state: O did not alter BMI (22.95 ± 0.31 vs. 22.63 ± 0.36 kg/m^2), increased basal insulinemia (5.5 ± 0.6 vs. 4.7 ± 0.3 mU/L ; p<0.05), TG (2.11 ± 0.29 vs. 0.72 ± 0.07 mmol/L; p<0.0001), decreased HDL cholesterol (1.02 ± 0.08 vs. 1.46 ± 0.09 mmol/L; p<0.001) and FFA (176 ± 35 vs. 446 ± 44 µmol/L ; p<0.0001). O increased DEE (1.08 ± 0.04 vs. 1.00 ± 0.03 kcal/min; p<0.05) and carbohydrates oxidation (3.09 ± 0.20 vs. 1.32 ± 0.09 mg/kg/min; p<0.0001) and decreased fatty oxidation (0.11 ± 0.04 vs. 0.62 ± 0.06 mg/kg/min; p<0.0001). HF tended to increase (65 ± 2 vs. 61 ± 2 bt/min; p= 0.055). Vasodilatation was not altered by O. During the clamp: O decreased the GIR (9.71 ± 0.47 vs. 11.45 ± 0.60 mg/kg/min; p<0.05). DEE was increased (1.20 ± 0.04 vs. 1.15 ± 0.05 kcal/min; p<0.05), carbohydrate oxidation was increased (4.02 ± 0.23 vs. 3.45 ± 0.18 mg/kg/min; p<0.0001) and fatty oxidation was decreased (0.00 ± 0.00 vs. 0.04 ± 0.03 mg/kg/min; p<0.0001). FFA were less inhibited (48 ± 6 vs. 17 ± 4 µmol/L; p<0.0001). MBP was increased (90.4 ± 1.4 vs. 87.0 ± 1.4 mmHg; p<0.05). Comparison of O + FO (n=8) vs. O + P (n=8): The P had no effect on each studied parameter. Basal state: FO decreased TG (1.58 ± 0.41 vs. 2.12 ± 0.58 mmol/L; p<0.05) without changing HDL cholesterol and LDL. FO decreased MBP (84.0 ± 6.8 vs. 86.4 ± 2.0 mmHg; p<0.0001) and increased vasodilatation (6.39 ± 0.59 vs. 5.45 ± 0.44 UA; p<0.05). During the clamp: FO increased the GIR (10.05 ± 0.70 vs. 8.84 ± 0.68 mg/kg/min; p<0.01), decreased MBP (85.3 ± 2.9 vs. 89.4 ± 1.7 mmHg; p<0.0001). FO had no effect on the other studied parameters.
Conclusion: A short term (4d) carbohydrate O reproduces without weight gain some metabolic alterations characteristic of the MS (increase in TG and of MBP, decrease in HDL cholesterol and insulin sensitivity). FO supplementation (1.7 g/d of EPA+DHA) over 6 weeks prevents the emergence of some

alterations: better insulin sensitivity, decrease in TAG and normalization of MBP.
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535

Paradoxal effect of chronic treatment with extended-release nicotinic acid on insulin sensitivity in patients with abdominal obesity and mixed dyslipidaemia

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Background and aims: Nicotinic acid (NA) is used to regulate lipid abnormalities by reducing triglyceride (TG) level and LDL cholesterol. It is the most potent available lipid regulating agent to increase HDL cholesterol. However, its global therapeutic benefit could be questioned as an increase in glycaemia has been reported after chronic treatment. Thus, we wanted to investigate the effects of NA on insulin sensitivity as well as on non esterified fatty acids (NEFA) evolution during both acute and chronic administration of extended release NA (ENA).
Materials and methods: We performed a cross over, randomized, double blind study of twice 8 weeks (wash out 3 weeks); 8 men, 18-65 years old, with abdominal obesity and mixed dyslipidemia (without diabetes and hypertension), received ENA at 2g/day after an escalation dose of 500mg/week during 4 weeks or placebo with 300mg of lysine acetylsalicylate. A euglycemic hyperinsulinemic clamp with 2 levels of insulin (0.2 and 1mUI/kg/min) associated to glucose tracer infusion was performed on day 53. Substrate oxidation was also measured by indirect calorimetry. Glycerol, NEFA, TG, β-hydroxybutyrate (β-OH), insulin and glycemia were monitored. Glucose, insulin, TG, NEFA, glycerol and β-OH kinetic were assessed for 8 hours on day 42 after a supplementary placebo administration and on day 56 after supplementary immediate release NA (INA) ingestion. This allows to appreciate acute and chronic effect of NA vs placebo.
Results: After 8 weeks of treatment, LDL cholesterol was decreased by 18.7% (3.63 ± 0.89 vs 2.99 ± 0.58 mM (p=0.028)) and HDL cholesterol increased by 5.9% (1.23 ± 0.20 vs 1.16± 0.22mM) but not significantly (NS). TG were significantly decreased during chronic treatment (3.03 ± 1.88 vs 1.31 ± 0.68 mM (p=0.012)). Chronic treatment with ENA significantly increase glycemia and insulinemia and HOMA was doubled (3.15 ± 0.52 vs 6.24 ± 2.24 (p=0.017)). During clamp, glucose utilisation tend to be decreased at the 2nd insulin dose with NA (4.44 ± 1.92 vs 3.73 ± 0.96 mg/kg/min (NS)). No change was observed on basal hepatic glucose production which was similarly inhibited during the clamp. Baselines NEFA were slightly increased with ENA (437 ± 267 vs 501 ± 264 µM (NS)) and similarly inhibited during the clamp as were glycerol and β-OH. After INA ingestion, we observed a decrease in NEFA followed by a rebound effect (basal value: 464 ± 217 µM; p=0.61 - nadir: 177 ± 123 µM; p=0.012 - peak: 987 ± 246µM; p=0.017). During chronic treatment, we observed a non significant increase in NEFA. Same kinetics were observed for glycerol and β-OH.
Conclusion: We demonstrate that chronic administration of ENA was associated with dramatically changes in glucose metabolism and in insulin resistance profile. This occurs despite a significant decrease in TG but with a trend to increase in NEFA. This insulin resistance seems to occur at the peripheral level (muscle) as hepatic glucose production inhibition was not altered. Relative risk-benefit of extended release NA should be assessed taking into account the HDL increase and deleterious change in glucose metabolism.
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536

The role of lipid subfractionation in predicting insulin sensitivity in normal glucose tolerant subjects

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Background and aims: Insulin resistance (IR) is associated with alterations of lipoprotein particle size especially with small-dense LDL, preceeding all other lipoprotein modifications and is associated with increased cardiovascular risk. The predictive value of lipoprotein particle size for IR is unknown. In the present study, we analyzed the predictive value of lipoprotein size-subfractions to hyperinsulinemic normogycemic clamp (HNC) measured glcuose disposal rate adjusted for muscle mass (M_m) in both normal and impaired glucose tolerant subjects compared to other fasting parameters well known to predict IR.
Materials and methods: 159 previously healthy (H), medically non-treated individuals (83 males and 76 females, mean age: 44 ± 2 years) underwent a HNC to measure M_m. Individuals were categorized as healthy (NGT, n=80) or glucose intolerant (GI: n= 79; IFG/IGT or drug-naïve 2DM) based on an OGTT. Lipoprotein size-subfractions were determined electrophoretically with the lipoprint system (Quantrimetrix). Basic anthropometric and metabolic parameters (fasting insulin, glucose, conventional lipids), free fatty acid (FFA), adiponectin, leptin, resistin, TNF-alpha, IL-6, apoprotein B and A1 were measured, percent body fat (PBF) and muscle mass were determined by DEXA.
Results: In H subjects, after adjusting with BMI, age, gender and PB, bivariate correlations with M_m stayed significant only in case of adiponectin (r=+ 0.407, p=0.006), leptin/adiponectin ratio (r = -0.268, p = 0.01), large and small IDL subfractions (IDL-A and IDL-C, r = +0.232, p = 0.014 and r = +0.247, p = 0.033) but not of conventional lipid and other parameters. According to a multivariate regression model BMI (p<0.0001), adiponectin (p<0.0001), VLDL% (p=0.0009), large (buoyant) LDL (LDL-1%, p=0.003), small-dense LDL (LDL2-7%)-to- total-LDL (LDL1-7%) ratio, total-LDL-to-HDL ratio (p<0.0001) and small-dense LDL% (p<0.0001) were independent predictors of M_m (R^2=0.51), but not fasting insulin, which only had a week bivariate correlation with the M_m (r = -0.259, p=0.018), similar to HOMA-IS (r= +0.28, p=0.014). In the GI group independent predictors of M_m (R^2=0.50) were fasting insulin (p<0.0001), FFA (p=0.005), adiponectin (p=0.005) and triglyceride (p=0.031). HOMA-IS showed week bivariate correlation with the M_m value in GI subjects (r=+0.36, p=0.003).
Conclusion: In healthy subjects adiponectin and lipoprotein subfractionation predicted clamp measured IS better than the HOMA model. Decreased number of large buoyant LDL and IDL particles along with the increase of small-dense LDL may indicate IR before hyperinsulinemia and dyslipidemia occurs. Conventional lipid panel (hypertriglycerdemia) and hyperinsulinemia (i.e. the HOMA model) only indicated IR in glucose intolerant subjects.

537

The antioxidant N-Acetyl-L-cysteine does not reverse hepatic insulin resistance induced by prolonged (48h) lipid infusion

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Background and aims: Our group has previously demonstrated that the antioxidant N-Acetyl-L-Cysteine (NAC) and the anti-inflammatory sodium salicylate completely prevent hepatic and peripheral insulin resistance induced by short-term (7h) i.v. lipid infusion. To determine if the mediators of hepatic insulin resistance differ depending on the duration of lipid infusion, we also established a prolonged (48h) lipid infusion protocol. The prolonged lipid infusion model mimics the chronically elevated plasma free fatty acids (FFAs) and increased FFA tissue availability characteristic of obesity. We have previously found that i.v. infusion of sodium salicylate at the same dosage as for the short-term lipid infusion model prevents peripheral but not hepatic insulin resistance. We wished to determine whether NAC is effective at the same dosage as in the short-term lipid infusion model.
Materials and methods: Wistar rats (250-310g, n=7-13/group) were chronically cannulated and given i.v. saline control or Intralipid plus heparin (IH; 20% Intralipid plus 20 U/ml heparin at 5.5 µl/min), with or without NAC

(0.35 mg kg^{-1} min^{-1}) for 48h; the rate of IH infusion was the same as for the 7h protocol. During the last 2h of treatment, a hyperinsulinemic (5 mU kg^{-1} min^{-1}) euglycemic clamp with concomitant tritiated glucose methodology was performed to determine hepatic and peripheral insulin sensitivity.
Results: IH administration resulted in hepatic insulin resistance, as indicated by blunted insulin-mediated suppression of endogenous glucose production (EGP) (% change of EGP from basal: Saline, -69.4±10.6%; IH, -21.0±6.7%, p<0.05), and peripheral insulin resistance, as indicated by a modest reduction in clamp glucose utilization (GU) (Saline, 30.9±2.0 mg kg^{-1} min^{-1}; IH, 26.0±1.1 mg kg^{-1} min^{-1}, p<0.05). Similar to sodium salicylate, NAC co-infusion completely prevented peripheral insulin resistance (GU: IH+NAC, 31.9±1.2 mg kg^{-1} min^{-1}, p<0.05 vs. IH alone), but did not prevent IH-induced hepatic insulin resistance (% change of EGP from basal: IH+NAC, -13.5±9.6%, not significant vs. IH alone).
Conclusion: Similar to sodium salicylate, NAC, which completely prevented hepatic insulin resistance in the 7h lipid infusion model, is not effective in preventing IH-induced hepatic insulin resistance caused by prolonged lipid infusion. Unlike the short-term lipid infusion model, which is characterized by hepatic and peripheral insulin resistance, prolonged lipid infusion is a more selective model of hepatic insulin resistance, with minimal peripheral insulin resistance. Our findings indicate that either a greater dosage of IKKbeta inhibitor (sodium salicylate) or antioxidant is required in this model, perhaps in combination, or more likely that unknown mediators, other than IKKβ and oxidative stress, are important. These results highlight the difficulties in target identification for the treatment of fat-induced insulin resistance in obesity.
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538

The lipid peroxidation product 4-hydroxynonenal uncouples insulin-stimulated Akt phosphorylation from its activation, resulting in adipocyte insulin resistance
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Introduction: Although it is accepted that diabetes and obesity are associated with increased oxidative stress, and oxidative stress as an isolated factor can cause insulin resistance, the exact oxidative mediators and molecular mechanisms are poorly understood, particularly in fat cells. 4-hydroxynonenal (HNE) is a major peroxidation end-product of unsaturated fatty acids, which has been shown to be elevated in patients with diabetes and obesity. Moreover, its conjugation to proteins was suggested to constitute the major carbonyl modification in adipose tissue in these conditions. The aim of the present study was to characterize whether and how HNE impairs insulin signaling in adipocytes, with emaphasis on activation of Akt/PKB, a step particularly affected in adipocytes by other oxidants.
Methods: Differentiated 3T3-L1 adipocytes were exposed to 0-100 μM HNE for up to 4h, rinsed, and insulin signaling and action were studied after stimulation with 100 nM insulin. GLUT4 translocation and fusion was studied using confocal microscopy after introducing (by electroporation) a GFP-GLUT4myc plasmid, and assessing externalization of the myc epitope in non-permeabilized cells using anti-myc Ab.
Results: Treatment of 3T3-L1 adipocytes with HNE increased total protein corabonylation, as assessed by a Protein Oxidation Detection Kit (COMPANY). Consistent with previous reports, HNE impaired insulin actions including the stimulation of glucose uptake in 3T3-L1 adipocytes, in a time and dose dependent manner. This was mediated by impaired capacity of the hormone to stimulate GLUT4 fusion with the plasma membrane, though translocation (GFP rims) seemed to be less affected. Intriguingly, insulin-stimulated phosphorylation of Akt on both Ser473 and Thr308 residues was increased 3-4 fold (p<0.05) following HNE pre-treatment, despite a time and dose-dependent decrease in total Akt content (p<0.05). HNE increased Akt phosphorylation by 3 fold also in the absence of insulin (p<0.05). To determine whether insulin resistance induced by HNE occurred downstream of normal or even exaggerated Akt activation by the hormone, phosphorylation of Akt substrates was assessed. Although basal phosphorylation of GSK3 on residues 9/21 mirrored basal Akt phosphorylation, it suggested a discrepancy between Akt phosphorylation and activation at higher doses in response to insulin. We next utilized the anti-phospho-Akt substrate (PAS) antibody generated against the phosphorylated Akt consensus sequence on target proteins. The notable increase in basal Akt phosphorylation was associated with a mild elevation in phosphorylation of various Akt substrates. Yet, despite the robust elevation in insulin-stimulated Akt phosphorylation induced by HNE, Akt-mediated phosphorylation of its various target proteins, including AS160, was markedly diminished.
Conclusion: These results suggest that the lipid peroxidation product 4-HNE can cause adipocyte insulin resistance by uncoupling Akt phosphorylation from the enzyme's activity in response to insulin stimulation.
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539

N-acetylcysteine reduces gluconeogenesis without altering hepatic lipid accumulation or mitochondrial superoxide production in an *in vitro* model of NAFLD
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Background and aims: Reactive oxygen species and insulin resistance are implicated in the progression of simple hepatic steatosis to steatohepatitis. Free radical scavenger, N-Acetylcysteine (NAC) is a thiol compound that has been proposed to prevent oxidative stress, thus protecting hepatocytes from injury in steatohepatitis. The aim of this study is to elucidate the effect of NAC on hepatic lipid accumulation, hepatic mitochondrial superoxide production and hepatic endogenous glucose production (EGP) as a marker of insulin resistance.
Materials and methods: C3A cells, a clonal derivative of HepG2 cell line, were incubated for 3 days with Lactate (L), Pyruvate (P), Octanoate (O) and Ammonia (N), as previously described with or without NAC (0.1mM). After a 3 day preconditioning, the presence of intracellular lipid was determined by oil red O staining. Triglyceride concentrations were measured using an enzymatic assay. Mitochondrial superoxide were detected by fluorescent microscopy using MitoSox Red and quantified by FACS. Total glucose production in the supernatant and glucose concentrations after 4 hour incubation with gluconeogenic substrates were measured using Bergmeyers method. Cell viability was assessed using propidium iodide staining followed by FACS. Experiments were done for n=3 in triplicate.
Results: Incubation with LPON and NAC did not affect cellular viability in all groups. LPON induced significant triglyceride accumulation when compared with control (Control (mean±SEM) 89.3±9.6 vs LPON 248.8±11.04 μg/g protein, P<0.01). LPON cells had higher mitochondrial superoxide formation (peak intensity LPON 31.5±2.9 vs control 16.5±4.1, P<0.05). Addition of NAC to LPON cells did not alter triglyceride concentrations (P=0.25) or superoxide production (P=0.40). In contrast, NAC caused a significant reduction in EGP (LPON:6799±231 vs LPON+NAC:5896±127.1 μmol/gTP; P=0.003), and this was also associated with a lower gluconeogenic capacity (LPON:7.1±0.8 vs LPON+NAC:4.9±0.6 μmol/gTP; P=0.04).
Conclusion: NAC treatment caused reduction in EGP, and presumably insulin resistance. This decrease was not associated with alteration in intracellular lipid accumulation or dependent on reduction of mitochondrial superoxide production. However, it is possible that the effect of NAC on mitochondrial hydrogen peroxide (H_2O_2) may underlie the observed reduction in EGP. In addition, our data showed that the degree of intracellular lipid accumulation was not related to the EGP or gluconeogenic capacity, hence hepatic insulin resistance.

540

Dysregulation of intracellular triacylglycerol breakdown in fatty liver contributes to the development of hepatic insulin resistance
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Background and aims: Hepatic steatosis is associated with the development of hepatic insulin resistance (HIR). This study was designed to test the hypothesis that steatosis-associated HIR is causally linked with dysregulation of endogenous triacylglycerol (TAG) degradation in fatty liver.
Materials and methods: Hepatic steatosis was induced by 2-week high-fat diet (HFD, 69 cal% as lard) administration to male Wistar rats (300±15 g). Hepatic lipase, (HL), triacylglycerolhydrolase (TGH)and lysosomal acid lipase (LAL) were identified according to their different pH optimum and their activity was measured on either emulgated ^{3}H-labeled triolein (^{3}H-TO) or

endogenous TAG in liver homogenate. Activated phagolysosomes (PhL) were prepared from 20% liver homogenate (H) by differential centrifugation.
Results: All three lipases exhibited measurable activities on 3H-TO substrate LAL > HL >> TGH (not shown) but the only lipase capable significantly degrade endogenous TAG was LAL. In rats fed SD total LAL activity in homogenate was higher in fasted animals and lower in fed ones. Corresponding changes were found in mRNA expression. HFD administration resulted in significant stimulation of LAL activity in homogenate in fed animals but LAL mRNA and total LAL protein expression were not significantly different compared with SD group (tab-H). This contradiction could be explained by specific intracellular localisation of LAL that is functional only in lysosomes activated by substrate ingestion - fagolysosomes. While in homogenate no correlation between LAL protein expression and LAL activity was found, in phagolysosomal fraction these two variables changed in accordance with each other. We propose an additional mechanism of LAL activation independent on mRNA and protein synthesis when the increased amount of intracellular fat droplets in steatosis makes their contact with lysosomes more frequent and the probability of fagolysosomal formation increases. The tissue concentration of DAG was elevated in fatty liver (56±12 vs 154±21 nmol. g-1). The data obtained in experiments designed to measure the production of DAG from endogenous TAG by LAL in vitro (SD vs HFD fasted: 85±9 vs137±14 p<0,01; fed: 56±8 vs129±10 p<0,01 nmol.mg-1.60 min-1) indicate that stimulated lipolysis in HFD group is an important one source of liver DAG. HFD feeding led to the impairment of insulin signal transduction in liver (attenuated insulin-stimulated IRS, PKB and mTOR phosphorylation) and to the increased activation of PKCε.
Conclusion: The lipolysis of intracellular TAG is increased in fatty liver due to the substrate-dependent stimulation of LAL activity and leads to the overproduction of DAG. Recent data indicate that PKCε activation is an important mechanism in HIR development. DAG, as a recognized activator of PKCε, may represent the causal link between fat-induced hepatic TAG accumulation and hepatic insulin resistance via the PKCε activation.

		standard diet		high-fat diet	
		fasted	fed	fasted	fed
H	TAG content (μmol.g^{-1}	3,2 &plsmn 0,2[a]	2,9 &plsmn 0,2[b]	14,6 &plsmn 1,4[a]	16,2 &plsmn 2,5[b]
	TGH activity (nmol FFA. mg^{-1}.60 min^{-1})	0,01	0,01	0,01	0,01
	HL activity (nmol FFA. mg^{-1}.60 min^{-1})	0,9 &plsmn 0,15	1,5 &plsmn 0,25	0,7 &plsmn 0,1	1,2 &plsmn 0,13
	LAL activity (nmol FFA. mg^{-1}.60 min^{-1})	24 &plsmn 2,2[c]	13,5 &plsmn 1,2[c,d]	29,4 &plsmn 4	35,5 &plsmn 4,9[d]
	LAL protein expression (arb. units)	18 &plsmn 2,5	16,9 &plsmn 2,2	20 &plsmn 0,8	21,4 &plsmn 1,5
	LAL mRNA (pg cDNA. ml^{-1})	27,2 &plsmn 3,6[e]	11,2 &plsmn 2,8[e]	33,6 &plsmn 3[f]	17,2 &plsmn 2,4[f]
PhL	LAL protein expression (arb. units)	9,8 &plsmn 0,9[g]	4,5 &plsmn 1[g,h]	10,4 &plsmn 1,2	11,2 &plsmn 1,8[h]
	LAL activity (nmol FFA. mg^{-1}.60 min^{-1})	18 &plsmn 1,8[i]	10,8 &plsmn 0,9[i,j]	22,7 &plsmn 3,3	29 &plsmn 3,8[j]

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541

Relationships of TNF-alpha system with glucose and lipid oxidation in lean and obese subjects

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Background and aim: One of the key actions of insulin is the regulation of glucose and lipid oxidation. Disturbances in substrate oxidation play an important role in the development of insulin resistance. Insulin action is inversely associated with circulating pro-inflammatory cytokines such as soluble TNF-α receptors (sTNFR1 and sTNFR2). The aim of the present study was to analyze the associations between serum sTNFR1 and sTNFR2 concentrations and glucose and lipid oxidation and non-oxidative glucose metabolism in lean and obese subjects with normal glucose tolerance.
Materials and methods: We examined 42 subjects (30 females and 12 males), 22 lean (BMI<25 kg x m-2, 16 females and 6 males) and 20 with overweight or obesity (BMI>25 kg x m-2, 14 females and 6 males) with normal glucose tolerance. Insulin sensitivity was measured with the hyperinsulinemic euglycemic clamp technique. Glucose and lipid oxidation was evaluated with indirect calorimetry in the baseline state and during the last 30 minutes of the clamp. Non-oxidative glucose metabolism in the hyperinsulinemic state was calculated by subtracting glucose oxidation from the total glucose metabolism. Metabolic flexibility was assessed as an increase in respiratory quotient (deltaRQ) in response to insulin.
Results: Serum sTNFR1 concentrations were higher in the obese in comparison with the lean group (p<0.0001). Insulin sensitivity was negatively related with serum sTNFR1 (r=-0.38, p=0.014), whereas the relationship with sTNFR2 was approaching the level of significance (r=-0.30, p=0.057). Serum sTNFR1 concentration was positively associated with the baseline glucose oxidation (r=0.32, p=0.043) and negatively - with the increase in glucose oxidation in response to insulin (r=-0.40, p=0.01) and with non-oxidative glucose metabolism (r=-0.35, p=0.022). Significant negative correlation between serum sTNFR1 and metabolic flexibility was observed (r=-0.36, p=0.019). Serum sTNFR2 was positively related to baseline respiratory quotient (r=0.36, p=0.018).
Conclusion: Our data suggest that TNFα system is associated with multiple metabolic pathways regulated by insulin.
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PS 33 Pathophysiology of insulin resistance

542

Evaluating the insulin sensitivity and secretion function from the 2-hour OGTT by mathematical model

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Background and aims: To assess insulin sensitivity and secretion function from the 2-hour oral glucose tolerance test (OGTT), the mathematical model LMM (linear minimal model) was established from five physiological assumptions in this paper.
Materials and methods: The insulin sensitivity index LMM-ISI and secretion function index LMM-BCI could be calculated simultaneously from the values of plasma glucose and insulin at four time points (0, 30, 60 and 120 min) during the 2-hour OGTT by model LMM. .Eighty Chinese female volunteers in Chongqing area (50 subjects for NGT and 30 subjects for IGR) were involved in this study, aged 27.04±4.77 and BMI 22.59±3.59. Using the data from the Botnia clamp test and the 2-hour OGTT, the feasibility of LMM-ISI and LMM-BCI was studied in this paper.
Results: The Pearson's linear correlation coefficient between LMM-ISI and GIR (glucose infusion rate at steady state of the Botnia clamp test) was 0.740 (P<0.001) , which was larger than those corresponding values calculated from QUICKI, HOMA-ISI, HOMA2-%S, Cederholm-ISI and Composite-ISI, the respective values of which were 0.452, 0.493, 0.503, 0.610 and 0.678. After removing the effect of GIR, the partial correlation between LMM-BCI and G_{120} (plasma glucose concentration at 120 minute after ingestion of the oral load) in the OGTT was most significant (r=-0.642, P<0.001) among the six insulin secretion function indices (HOMA-β, HOMA2-%B, $\Delta I_{30}/\Delta G_{30}$, $\Delta I_{60}/\Delta G_{60}$, MBCI and LMM-BCI) derived from the OGTT.
Conclusion: So LMM-ISI and LMM-BCI is a relatively good pair of insulin sensitivity index and secretion function index derived from the 2-hour OGTT.

Correlation Coefficients between Sensitive Index and lnGIR

Partial Correlation Coefficients between Secretion Function Index and G120 after Removing the Effect					
lnHOMA-ß	lnHOMA2-%B	$\ln\Delta I_{30}/\Delta G_{30}$	$\ln\Delta I_{60}/\Delta G_{60}$	lnMBCI	lnLMM-BCI
-0.231*	-0.225*	-0.457#	-0.636#	-0.620#	-0.642#

Supported by: Tianjin schoolboard

543

Inflexion of the glucose disposition vector among insulin resistant children may be an early sign of beta cell insufficiency

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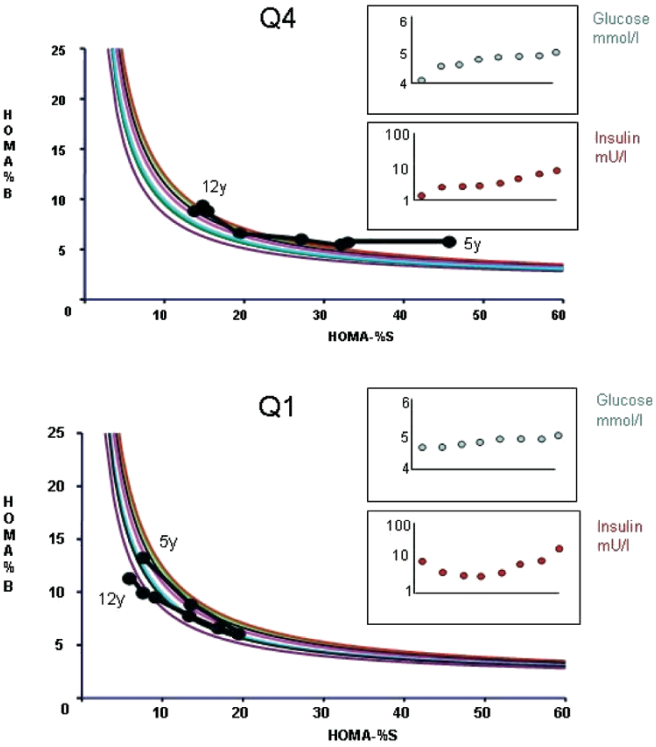
Background and aims: Blood glucose is controlled by the interaction between insulin sensitivity (S) and insulin secretion (B), whose vector can be plotted over time. We tracked the vector of children who were insulin sensitive, and compared it with that of children who were insulin resistant.
Materials and methods: BMI (kg/m2), fasting glucose, insulin, HOMA-%S and HOMA-%B were recorded annually from 5-12y in 258 healthy children (144 boys) from the EarlyBird cohort, grouped according to quartile of insulin sensitivity at 5y (highest Q4, lowest Q1).
Results: BMI SDS (+0.16 Kg/m^2, p<0.01) and glucose (+0.6 mmol/l, p<0.001) rose progressively from 5-12y. The BMI of Q4 was lower than Q1 (p<0.02), HOMA-%S (predictably) higher (p<0.02), and HOMA-%B lower (p<0.03) throughout. The vector of Q4 shifted only slightly in response to the loss of S associated with puberty. The vector of Q1, on the other hand, suggested early loss of B cell function (with compensatory increase in insulin sensitivity), followed by an acute switch in direction at 7y to indicate falling insulin sensitivity (with only partial beta cell compensation).
Conclusion: The inflexion appears to be a feature of early insulin resistance, and may correspond to a weakening of glucose control associated with adjustment to beta cell loss. Importantly, it does not appear to be a normal event, and could represent early beta cell pathology among contemporary children.



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544

Insulin resistance is associated with metabolic syndrome but not with angiographically determined coronary artery disease

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Background and aims: Insulin resistance (IR) is the key feature of the metabolic syndrome (MetS) and in prospective studies predicts atherothrombotic events. Its association with directly visualised coronary atherosclerosis is unclear. We hypothesised that IR is associated with both angiographically determined coronary artery disease (CAD) and with the MetS.
Material and methods: We enrolled 986 consecutive patients undergoing coronary angiography for the evaluation of suspected or established stable CAD; significant CAD was diagnosed in the presence of ignificant coronary stenoses with lumen narrowing ≥50%. IR was determined by the HOMA index; the MetS was defined according to ATPIII criteria.
Results: HOMA IR scores were significantly higher in MetS patients than in subjects without the MetS (6.4 ± 2.1 vs. 2.2 ± 2.0; p <0.001). In contrast HOMA-IR did not differ significantly between patients with significant CAD and those who did not have significant CAD (3.9 ± 14 vs. 3.2 ± 4; p = 0.490). When both, the presence of MetS and of significant CAD were considered, HOMA-IR was significantly higher in patients with the MetS both among those who had significant CAD (7.2 ± 28 vs. 2.3 ± 2.1; p <0.001) and among those who did not have significant CAD (5.3 ± 5.7 vs. 2.1 ± 1.4; p <0.001) whereas it did not differ significantly between patients with significant CAD and subjects without significant CAD in patients with the MetS (7.2 ± 28 vs. 5.3 ± 5.7; p = 0.679) nor in those without MetS (2.1 ± 1.4 vs. 2.3 ± 2.1; p = 0.411). Similar results were obtained with the IDF definition of the metabolic syndrome.
Conclusion: IR is significantly associated with the MetS but not with angiographically determined coronary atherosclerosis.

545

Relation between cardiac autonomic dysfunction and insulin resistance in non diabetic obese patients

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Background and aims: Obesity is often associated with cardiac autonomic dysfunction (CAD). The role of insulin resistance in CAD has been suggested in diabetic patients. The aim of the present study was to evaluate the role of insulin resistance in CAD in non diabetic obese patients and whether metabolic disorders are more common and blood pressure higher in patients with both insulin resistance and CAD.
Materials and methods: 394 overweighed or obese patients, aged 38,1 ± 14,4 years, BMI 38;5 ± 7 kg/m², were included. An OGTT was performed, HOMA insulin resistance index was calculated and metabolic syndrome was assessed according to IDF criteria. CAD was defined by at least one abnormal out of 3 cardiac autonomic function tests (deep breathing, lying-to-standing, Valsalva).
Results: CAD was present in 213/394 patients. Insulin resistance was defined by HOMA index ≥ 2.93 (median value). Insulin resistance was associated with a higher rate of CAD (63% vs 49% in the patients with HOMA < 2.93 ; p = 0.006) and severe CAD as defined by 2 or 3 abnormal tests (23% vs 14% ; OR 1.86 [1.10-3.15] ; p = 0.02). The population was divided in 4 groups : HOMA < 2.93 with either 0-1 abnormal CAD test or 2-3 abnormal CAD tests (groups 1 and 2), HOMA ≥ 2.93 with either 0-1 abnormal CAD test or 2-3 abnormal CAD tests (groups 3 and 4). There was a regular increase in BMI, waist and hip circumferences, waist/hip circumference ratio, systolic and pulsed blood pressure, blood glucose and serum insulin 2 hours after glucose intake, HbA1c, triglycerides from group 1 to group 4 (p = 0.02 to 0.0001). Similarly, the prevalence of impaired glucose tolerance and type 2 diabetes according to OGTT and the prevalence and severity of metabolic syndrome increased regularly from group 1 to group 4 (p < 0.0001 for all comparisons).
Conclusion: These data strongly suggest that in non diabetic obese patients insulin resistance is associated with a higher prevalence of CAD and a more severe CAD and that CAD aggravates the consequences of insulin resistance on metabolic disorders including glucose dysregulation and on blood pressure elevation.

546

Metabolic flexibility in the postprandial phase: a global assessment of metabolic defects in type 2 diabetes

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Background and aims: Impaired metabolic flexibility has been highlighted in type 2 diabetes using a euglycemic-hyperinsulinemic clamp. Our aim was to assess such an impaired metabolic flexibility in more physiological insulin-induced conditions following a meal and to connect it with the postprandial metabolic pattern.
Materials and methods: Using indirect calorimetry and the percent relative cumulative frequency curve (PRCF) analysis for respiratory quotient (RQ), metabolic flexibility (as defined by H: slope of PRCF curve of RQ) and nutrient oxidation were evaluated postprandially after an oral glucose load in 11 type 2 diabetic subjects (DT group) and 9 control healthy subjects (C group). In parallel, insulinemia, glycemia, non-esterified fatty acids (NEFA) concentration and total and endogenous glucose kinetics were assessed.
Results: Metabolic flexibility was significantly lower in the DT group (p<0.0001). Insulin concentration kinetics differed dramatically in the DT group, with no first acute phase but a longer-lasting response associated to a prolonged inhibition of lipolysis. In the DT group, $EGP_{0\text{-}120min}$ (Endogenous Glucose Production) was significantly higher, $RaT_{120\text{-}300min}$ (Appearance of Total glucose) significantly lower and $RdT_{120\text{-}300min}$ (Disappearance of Total glucose) significantly higher (p<0.05). H was significantly correlated to the insulinemic response, the glycemic peak, the total and endogenous glucose kinetics and to NEFA suppression.
Conclusion: H is an original marker of metabolic flexibility in the dynamic postprandial conditions and presently characterized the impaired ability to match fuel availability and utilization in type 2 diabetes. H integrates the interaction between metabolic flexibility and the defects in both insulin-sensitivity and insulin kinetics and showed that nutrient availability pattern significantly determine metabolic flexibility.
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547

Association of restrictive ventilatory dysfunction with insulin resistance and type 2 diabetes

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Background and aims: It has been suggested that early life exposures which affect lung growth could also be associated with insulin resistance. This study was performed to investigate the association of ventilatory dysfunction with insulin resistance and type 2 diabetes.
Materials and methods: We cross-sectionally examined clinical, laboratory and pulmonary function data on 35,456 Korean adults (age 18-93 years, 40% women) which were recorded for routine health check-ups. Insulin resistance was estimated by fasting serum insulin concentration and homeostasis model assessment (HOMA IR).
Results: Type 2 diabetes group and pre-diabetes (impaired fasting glucose) group showed higher prevalence of restrictive ventilatory dysfunction (18% and 11%, respectively, vs. 8%; p < 0.01) and obstructive ventilatory dysfunction (4.3% and 3.2%, respectively, vs. 2.3%; p < 0.01) than normal glucose group. Compared with subjects with normal ventilatory function, the subjects with restrictive or obstructive ventilatory dysfunction had older age, higher fasting glucose, HbA1c, systolic and diastolic blood pressure, and greater proportion of smokers. Serum triglyceride, fasting insulin levels, and HOMA IR were higher only in restrictive ventilatory dysfunction group than in normal or obstructive ventilatory dysfunction group. On logistic regression analysis, age and sex-adjusted odds ratio (OR) of type 2 diabetes for restrictive ventilatory dysfunction was 1.59 (95% confidence interval, 1.43-1.78). After controlling exercise, drinking, and smoking habits, presence of hypertension, BMI, and waist circumference, the OR was 1.38 (1.23-1.55). However, further adjustment for HOMA IR attenuated the OR (1.12 [0.97-1.29]) to become insignificant. In contrast, obstructive ventilatory dysfunction was not independently related with type 2 diabetes.
Conclusion: Our results indicate that restrictive ventilatory dysfunction is independently associated with type 2 diabetes, probably via insulin resistance.

548

Algorithm based on HMW multimers of adiponectin strongly correlates with parameters of insulin resistance in non diabetic severe obese patients who are candidates for bariatric surgery

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Background and aims: Because insulin resistance is associated with an increased risk of metabolic and cardiovascular morbidities, its identification in severe obese patients can argue in favour of bariatric surgery. The homeostasis model assessment (HOMA-R) is often used to evaluate insulin resistance, although suffering from limitations due to the specificity of plasma insulin assays and impairments of insulin secretion in case of abnormal glucose tolerance. We investigated the correlation between the various isoforms of adiponectin (LMW, MMW, HMW), total adiponectin, leptin and parameters of insulin resistance in non diabetic severe obese patients in order to assess whether these proteins (or a combination) could represent valuable markers of insulin resistance.
Materials and methods: In this study, 112 severe obese subjects (41 men and 71 women, BMI range: 33-87 kg/m²) with normal glucose tolerance were investigated as candidates for bariatric surgery. Blood glucose and plasma insulin assays allowed the calculation of HOMA-R value. The patients were considered as insulin sensitive when HOMA-R was < 2.6 (n=53) and insulin resistant when HOMA-R was > 2.6 (n=59). The serum concentrations of each isoform of adiponectin and leptin were measured by ELISA (Daiichi Pure Chemicals, Tokyo, Japan; Alpco, Salem, United States, respectively). All statistical analyses were performed with the R software for statistical computing.
Results: Differential statistics using Wilcoxon test revealed clinical or biochemical parameters able to discriminate between insulin resistant (IR) subjects and insulin sensitive (IS) subjects. The most significant parameters

were a) leptin/ HMW isoforms of adiponectin ratio (p=0.0001; Nfoldmedian (IR/IS subjects = 2.09)); b) leptin/total adiponectin ratio (p=0.0001; Nfold median (IR/IS subjects = 1.82)); c) HMW isoforms of adiponectin (p=0.0001; Nfold median (IS/ IR subjects = 1.82)). Analyses of univariate ROC curves showed that leptin/HMW ratio allowed a discrimination between IS and IR patients with a sensitivity of 74.6 and a specificity of 71.7 (Area under curve (AUC) = 0.775). Besides, AUCs were 0.746, 0.739 and 0.732 for leptin/total adiponectin ratio, HMW isoforms of adiponectin and BMI, respectively. Finally, analyses of multivariate ROC curves based on the association of clinical and biochemical parameters showed that the combination of BMI, adiponectin HMW and leptin is the best method to discriminate between IR and IS subjects. Thus, we found an algorithm based on these three parameters allowing a significant discrimination between IR and IS subjects, with 80% sensitivity and 74% specificity (AUC = 0.803). Omission of leptin led to a slightly reduced AUC of 0.790, but sensitivity was increased to 85% while specificity remained at 70%.
Conclusion: Our data disclose the specific value of assessing circulating HMW isoforms of adiponectin in order to identify insulin resistance in non diabetic severe obese patients. The measurement of this parameter, combined to BMI in our algorithm, could represent a useful tool to further estimate the metabolic and cardiovascular risk of overweight patients; it should support the development of easier methods of specific assays to be extended in routine practice.

PS 34 Insulin sensitivity - animal studies

549

Opposing effects of ROCK1 deficiency in liver and adipose tissue on insulin action
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Background and aims: Rho-kinase (ROCK) isoforms have been shown to modulate insulin signaling and glucose metabolism. Deficiency of ROCK1 causes systemic insulin resistance by impairing insulin signaling in skeletal muscle. The current study was designed to further determine the mechanism(s) for insulin resistance induced by absence of ROCK1.
Materials and methods: Mice with global knock out of ROCK1 ($ROCK1^{-/-}$) were injected with insulin (10U/kg) or saline and sacrificed 10 min later. Molecular parameters of insulin signaling in liver and adipose tissue were measured.
Results: Insulin-stimulated PI3K activity associated with IRS-2 was reduced ~35% without any alteration in tyrosine phosphorylation of insulin receptor in liver of $ROCK1^{-/-}$ mice. ROCK1 deficiency also resulted in impaired insulin action on Akt, S6K and S6 in liver. In addition, $ROCK1^{-/-}$ mice displayed dysregulation of liver glycogen metabolism, as evidenced by the decreased expression of glycogen synthase and the defective phosphorylation of GSK3. Despite the fact that $ROCK1^{-/-}$ mice are insulin-resistant, the ability of insulin to activate PI3K was dramatically enhanced in adipose tissue of ROCK1 deficient mice compared with wild type mice. This increase corresponded closely with an elevation in IRS-1 tyrosine 612 phosphorylation, the binding site for the p85 subunit of PI3K, in $ROCK1^{-/-}$ mice. These effects are mainly due to increased insulin action at the level of insulin receptor. Insulin-stimulated phosphorylation of S6K and S6 in adipose tissue was unaltered by ROCK1 deletion.
Conclusion: These data suggest, combined with our previous data on impaired insulin action in skeletal muscle, that defective IRS/PI3K signaling could contribute to the pathogenesis of insulin resistance caused by ROCK1 deficiency. However, increased action of insulin in adipose tissue could not mask whole body insulin resistance.

550

RANKL affects insulin signalling in vascular smooth muscle cells
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Background and aims: Osteoprotegerin (OPG), a soluble decoy receptor for receptor activator of nuclear factor-κB ligand (RANKL), accumulates in the arterial wall in patients with diabetes. RANKL is almost absent in normal vasculature but is upregulated in atherosclerotic and calcified lesions. Insulin has been shown to affect OPG production and calcification of vascular smooth muscle cells. However, possible interactions between the OPG-RANKL system and insulin have not previously been considered. The aim of the present study was to investigate the effects of RANKL and OPG on gene expressions in vascular smooth muscle cells, and particularly putative interactions with effects of insulin.
Materials and methods: Primary human vascular smooth muscle cells (HVSMCs) were used for global gene expression studies using Affymetrix microarrays. Three individual studies were performed: Study one: HVSMCs were treated with RANKL (six hours) or vehicle. Study two: Cells were treated with negative control or OPG siRNA for reduction of the OPG production (30 hours). Study three included four experimental groups: HVSMCs treated with RANKL (nine hours), insulin (three hours), RANKL (nine hours) and insulin (three hours), or vehicle.
Results: To investigate the effects of RANKL on gene expressions in HVSMCs, global pathway analysis was applied to study one. Surprisingly, it was found that RANKL downregulated the insulin signaling pathway. OPG knock down (study two) did not affect the insulin signaling pathway but upregulated the

electron transport chain and the ubiquinone biosynthesis pathway. Next, it was investigated whether the observed RANKL-mediated downregulation of the insulin signaling pathway attenuates insulin's effects on HVSMCs (study three). It was found that insulin upregulated 52 genes in HVSMCs (P<0.05, FC >1.2). RANKL inhibited the insulin-mediated upregulation of 16 of the 52 genes. RANKL itself did not downregulate the 16 genes. Therefore, the result showed that RANKL attenuates some of insulin's effects in the arterial wall.
Conclusion: These data demonstrated that RANKL disturbs insulin signaling in HVSMCs. Since insulin upregulates genes through different pathways we suggest that RANKL only has an impact on one or some of these pathways. These results indicate that RANKL has an impact on insulin signaling in the arterial wall and this may be important in relation to the development of vascular calcifications, observed with a high frequency in patients with diabetes.
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551

Palmitate affects insulin signalling in pancreatic alpha cells
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Background and aims: Under physiological conditions high glucose or insulin levels inhibit glucagon secretion, but this regulation is altered in T2DM. Since insulin directly affects glucagon secretion by binding to specific receptors in alpha cells, an alteration in insulin receptor function or signaling could impair this regulatory effect. In this study we investigated the effects of chronic exposure to palmitate on insulin receptor (IR) phosphorylation and insulin signaling in pancreatic alpha cells.
Materials and methods: We cultured α-TC1 (a mouse glucagonoma cell line) with palmitate (0,5 mM) for 48 h. After chronic palmitate exposure, we acutely stimulated the cells with insulin (10-9 M) for different times (from 1 to 20 minutes). At the end of insulin stimulation the IR downstream was investigated. In particular, we studied the insulin receptor phosphorylation (IR-P), the IRS-1 and IRS-2 phosphorylations and the expression of AKT.
Results: After 48 h of palmitate exposure, insulin (10-9 M for 5 minutes) determined an increase of whole IR-P/IGF-1R-P (654±5 AU) significantly higher than in control cells (227±2 AU, n=5, p<0.05). In contrast, the specific immunoprecipitation of IR and the subsequent blot with anti-phosphotyrosine showed a decrease of phosphorylation in palmitate groups (28±2 vs. 229±5 AU in control cells, n=5, p<0.05). To investigate IRS-1 we measured its phosphorylation in Tyr-612 (the most active form). In cells cultured with palmitate we found a decrease of IRS-1-P (Tyr-612) (46 ± 4 AU vs 420 ± 2 AU in control cells; n=5, p<0.01). In addition, we found an increase of IRS-2-P in palmitate groups (102±2 AU in control cells vs 279±3 AU in palmitate cells, n=5, p<0.05). The AKT phosphorylation levels were also decreased in palmitate groups (64 ± 3 AU vs 182 ± 6 AU in control cells; n=5, p<0.05).
Conclusion: In α-TC1, a mouse pancreatic alpha cell line, the chronic exposure to palmitate affects the intracellular insulin pathway. In particular, the phosphorylation of IR and IRS-1 (Tyr-612) were impaired, thus suggesting that trough this mechanism FFA might induce insulin resistance in alpha cells. The increased IRS-2 phosphorylation might drive the insulin signals through the mitogenic pathway.

552

Post-natal diet-induced programming of insulin resistance in rats alters *in vitro* aortic reactivity
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Background and aims: Endothelial dysfunction, an independent predictor of cardiovascular events, is associated with the metabolic syndrome in part in relation with insulin resistance. We have shown that immediate postnatal overfeeding in rats, obtained by reducing the size of the litter, programs insulin resistance and adipose tissue metabolism in adulthood. These alterations being further enhanced by high-fat diet. The purpose of the present study was to characterize in this experimental model the endothelial function in relation with the hemodynamic actions of insulin.
Materials and methods: We investigated, using a technique of isolated organ, the dynamic properties of thoracic aorta obtained from adult control (C) or postnatally programmed (P) rats subsequently fed low- (LF) or high-fat diet (HF). Organ were isolated, pretreated with 10^{-6} M epinephrine, and the vasoreactivity to acetylcholine (Ach, 10^{-9}-$10^{-4\,M}$), in the absence or presence to N^{ω}-nitro-L-arginine methyl ester (a nitric oxyde synthase inhibitor, LNAME, 100 μM) or Wortmannin (a PI3-kinase inhibitor, 50 μM) was determined. The effects of insulin alone or during Ach-induced relaxation with or without Wortmannin were also studied.
Results: P or HF induced insulin resistance, this effect being more marked when both paradigms were associated. Inhibition of nitric oxide synthase significantly decreased ACh-induced relaxation, with lower potency in arteries from rats fed HF as compared to LF ($P < 0.01$). Aorta of PHF rats exhibited lower maximal relaxation to ACh and higher EC_{50} for ACh than did those of the other groups ($P < 0.02$). Inhibition of PI3-kinase reduced Ach-induced relaxation in C, but not in P animals ($P < 0.03$), whatever the diet, indicating the existence of a P-induced impairement of PI3-kinase. Insulin (0.035-35 nM) alone was devoid of effect either under a basal state or after epinephrine-induced vasoconstriction. Pre-incubation with insulin (150 nM) decreased ACh-induced aortic relaxation, this effect being less potent in P rats ($P < 0.005$ vs C rats). Pre-incubation with Wortmannin did not affect the above mentioned inhibitory effect of insulin.
Conclusion: Whereas HF alone was ineffective, its association with postnatal programming reduced the ACh-induced vasodilatation partly due to ineffective PI3-kinase pathway. Interestingly insulin reversed Ach-induced vasodilatation whatever the group, and this effect was not affected by PI3-kinase inhibition. This indicates that adult rats exhibit an insulin resistant state with defective PI3-kinase pathway leading to a prominent vasoconstrictive effect of insulin. Surprisingly this vasoconstrictive effect was less pronounced in P rats suggesting the development of compensatory mechanism(s). The reduced effect of NO synthase blockade in high fat-fed animals is in favor of regimen-induced endothelium-independent alterations. The expression level and phosphorylation status of the various proteins involved in insulin and ACh pathways and smooth vascular cells function are currently investigated.

553

Glucosamine-induced endoplasmic reticulum stress causes insulin-resistance in L6 skeletal muscle cells
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Background and aims: Endoplasmic reticulum (ER) stress might play an important role in the pathogenesis of insulin-resistance and Type 2 Diabetes. Hyperglycaemia (HG) causes insulin resistance and this process appears to be linked to ER stress. Glucosamine (GlcN), generated by the hexosamine pathway (HP) during HG, also causes insulin resistance and disturbances similar to glucose toxicity. In this work, we sought to evaluate the role of HG- and GlcN- induced ER stress on the insulin-resistance in L6 skeletal muscle cells.
Materials and methods: L6 skeletal muscle cells were incubated either with 10mM GlcN or with 0.5μM thapsigargin (Thap), a well known ER stressing agent, pretreated or not with either 10mM 4-Phenyl Butyric Acid (PBA) or 5mM Tauroursodeoxycholic Acid (TUDCA) for 1 hour.
Results: In L6 cells, 24 hours incubation with either 25mM glucose (HG) or 10mM GlcN, increased the mRNA levels of the ER stress marker *BiP* by 2- and 6-fold, respectively. Azaserine (20μM), an inhibitor of the HP, was able to prevent HG-induced ER stress, suggesting that HG effect on *BiP* was mainly due to the rate of glucose converted into GlcN through the HP. GlcN caused a 2-fold increase of *ATF-6* mRNA ($p < 0.001$), a 3-fold increase of *ATF-3* mRNA levels ($p < 0.001$) and an increase of *XBP-1* spliced form. Furthermore, GlcN significantly impaired insulin-induced 2-deoxy-D-[^{14}C]-glucose (2DG) uptake. These effects were paralleled by a 70% decrease of both mRNA and protein levels of the insulin-sensitive glucose transporter GLUT4. Time-course experiments showed that *GLUT4* mRNA levels were reduced by 30% after 4 hours ($p < 0.001$), and by 70% after 8 hours ($p < 0.001$) of GlcN treatment. *GLUT4* regulation occurred, very likely, at the transcriptional level, as demonstrated by the decrease of the mRNA levels of the two transcription factors known to regulate its expression, MEF2A and PGC1. Moreover, the reduction of *MEF2A* and *PGC1* mRNA observed upon GlcN treatment was paralleled by a 50% and a 45% decrease in their binding to *GLUT4* promoter respectively . Interestingly, the use of the chemical chaperones PBA and TUDCA, known to alleviate ER stress, prevented both *BiP* induction and

GLUT4 downregulation. As for *GLUT4*, also *MEF2A* and *PGC1* mRNA levels did not show any appreciable variation when L6 cells were treated with GlcN in the presence of PBA or TUDCA. Furthermore, PBA or TUDCA pre-treatment restored insulin-induced 2DG uptake in L6 cells treated with GlcN to levels comparable to those measured in L6 control cells. To understand the molecular mechanism involved in the transcriptional inhibition of *GLUT4* induced by ER stress, we analyzed the expression of the inhibitory transcription factor *SHP* that is induced upon stress conditions. *SHP* expression was increased by 2-fold upon GlcN treatment suggesting that it could be involved in *GLUT4* regulation.
Conclusion: In L6 skeletal muscle cells GlcN induces insulin resistance by inhibiting insulin-stimulated glucose uptake and by decreasing *GLUT4* expression through ER stress activation.
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554

Leptin infusion in lateral ventricle enhances the insulin sensitivity in hypothalamus and liver despite the induction of PTP1B
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Background and aims: Leptin and insulin increase energy expenditure and reduce food intake by act on the hypothalamic arcuate nucleus. However, a leptin resistance state has been described in obese humans as mirrored by an increase of appetite, a reduced energy expenditure and high leptin plasma levels. Leptin resistance is frequently associated to other metabolic diseases such as type 2 diabetes characterized by an insulin-resistance. The molecular mechanisms underlying the onset of the two resistances at the central level are still misunderstood. Recently in rat hypothalamus, we have shown that leptin is able to induce the expression of phosphotyrosine phosphatase 1B (PTP1B), which plays a crucial role in leptin and insulin signalling by dephosphorylation of JAK-2 (Janus kinase) and insulin receptor. The present study aims to investigate the consequences of a chronic central overexposure to leptin on the PTP1B expression and on insulin sensitivity in hypothalamus and liver.
Materials and methods: Adult male Wistar rats were stereotaxicaly implanted in lateral ventricle with Alzet osmotic pumps and received for 2 weeks saline or leptin (0, 25 µg/µl). At the end of the infusion, animals were treated with saline or insulin (1U/kg/rat) by intraperitoneal (IP) injection, 30 minutes before euthanasia. Hypothalamus and liver were removed and solubilised proteins were subjected to Western-blots analysis using adequate antibodies.
Results: A 2-weeks central leptin infusion increases significantly the basal STAT-3 phosphorylation (+250%) but not MAPK in hypothalamus. In controls rats, insulin treatment is not able to activate insulin signalling pathways in the hypothalamus but interestingly and despite PTP1B induction, leptin infusion potentiates insulin action on the phosphorylation of STAT-3 (+450%), and MAPK (+180%). In liver of leptin-infused rats, the insulin-dependent phosphorylation of Akt was also strongly potentiated (+300%) as compared to control rats (+200%). This increase of insulin sensitivity could be attributed to the overexpression of IR and/or to the leptin-dependent decrease of potential negative regulators of insulin signaling such as serine phosphorylated IRS1/2.
Conclusion: A chronic central leptin infusion for 14 days induces the hypothalamic PTP1B expression which was unable to alter insulin action. This chronic leptin treatment seems to potentiate the insulin action at both hypothalamic and hepatic levels. This suggests that during early stage of leptin-resistance onset and despite PTP1B increase, insulin signaling pathways are not yet impaired and probably leptin-resistance will lead to insulin-resistance later when the leptin resistance will be clearly established with weight gain and increased food intake.

555

Postprandial glucose disposal is subject to hepatic parasympathetic control
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Background and aims: In the last few years it has emerged a new post prandial mechanism suggesting that besides insulin from the pancreas, the liver produces a humoral factor known as Hepatic Insulin Sensitizing Substance - HISS, which doubles total glucose uptake. The major site of action for this factor is still unknown, but it was observed that changes in response to insulin before and after parasympathetic denervation resulted in unaltered effect of insulin on net glucose balance across the hepatic and splanchnic region. However, significant changes were observed in hindlimb responses. These results highlighted the importance of a new study to evaluate the specific site of action of HISS, which is the aim of this work. Our hypothesis is that HISS acts selectively at the skeletal muscle.
Material and methods: Male fed Sprague-Dawley rats (9weeks-old) were used. To measure insulin sensitivity the Rapid Insulin sensitivity Test (RIST) was used. The RIST is an euglyceamic clamp that quantifies the glucose infused after an insulin bolus (50mU/kg, 5 min). The rate of glucose infusion is adjusted in order to maintain euglycemia and it is the parameter used to evaluate insulin sensitivity/resistance. This study was carried out in the presence and absence of HISS (denervated vs non-denervated animals). Insulin sensitivity was determined before and after each intervention (denervation or sham), using the RIST. The plasma [3H]2-deoxy-glucose ([3H]2DG) rate of disappearance was also quantified during the RIST and incorporation of [3H]2DG uptake into the following individual tissues was measured: skeletal muscle (gastrocnemius, extensor digitorium longus (EDL) and soleus), liver, adipose tissue, heart and kidney. For this purpose, a bolus of 250µCi/kg [3H]2DG was injected at t=9 min of the second RIST.
Results: In the non-denervated group, the first RIST was 228.9±15.3 mg glucose/kg and the second RIST was 221.2±12.3mg glucose/kg (n=5). In the denervated group the control RIST (before denervation) was 251.6±27.5 and after the denervation process (after blocking the HISS pathway) the RIST decreased to 153.5±12.3 ($p<0.01$, n=6). The denervated animals showed a decreased rate of plasma [3H]2DG disappearance, and when we evaluated the tissues we observed that the skeletal muscle, specifically the soleus and the EDL, were affected by 35% ($p<0.05$) and 48% ($p<0.01$), respectively, in the denervated group. The cardiomyocytes also were affected in the denervated group, in this case the decrease was 46% ($p<0.05$) when compared to the sham group.
Conclusion: When we block the HISS pathway, there is a significant decrease in insulin action (observed by the RIST Indexes). In the *in vivo* incorporation of [3H]2DG uptake into individual tissues we observed that the tissues that were affected by the HISS manipulation was the skeletal muscle, mainly the soleus and the EDL muscles and the cardiomyocytes. These observations suggest that when the hepatic parasympathetic nerves are compromised glucose uptake is only decreased in the skeletal muscle and cardiomyocytes.
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556

Knockdown of CHOP, an inducer of cellular apoptosis upon excessive endoplasmic reticulum stress, in liver ameliorates insulin resistance and glucose homeostasis in mice model of type 2 diabetes
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Background and aims: Transcription factor CHOP (C/EBP homologous protein), is known as a strong inducer of cellular apoptosis in response to excessive endoplasmic reticulum (ER) stress. CHOP is also associated with pancreatic β-cell apoptosis and insulin secretory dysfunction in diabetic condition. CHOP is specifically induced upon severe ER stress, when cells can no longer adapt to survive using unfolded protein response pathways, and contribute to cell death to eliminate dysfunctioning ones. However, it is yet known the function of CHOP in insulin resistance, especially in liver.

Materials and methods: We utilized younger age db/db (BKS.Cg-m +/+ Leprdb/J; 5w old~) mice to determine the impact of CHOP on insulin resistance. CHOP expression was down-regulated by CHOP siRNA expression plasmid which was delivered using in vivo jetPEI intraperitoneally. Injection of CHOP siRNA and control siRNA were performed once a week from 5 to 15 weeks old mice.
Results: The followings were observed in CHOP siRNA treated group (Control v.s. CHOP siRNA group).
1) Suppression of body weight increments (43.2g v.s. 39.1g at 10 weeks old, $p< 0.05$),
2) Reduction of fasting and random fed glucose levels (Fasting: 644 mg/dL v.s. 309 mg/dL at 15 weeks old, $p< 0.05$; Random fed: 721 mg/dL v.s. 505 mg/dL at 13 weeks old, $p< 0.05$),
3) Comparable fasting insulin levels (8.2 ng/mL v.s. 9.4 ng/mL at 11 weeks old),
4) Significant reduction of HOMA-IR (13.03 v.s. 7.17, $p< 0.05$),
5) Improvement of glucose tolerance upon GTT and insulin resistance upon ITT,
6) Suppression of glucose elevation upon pyruvate loading test,
7) Strong suppression of CHOP induction in liver by 95%,
8) Attenuation of ER stress marker, Bip expression in liver,
9) No significant alteration of CHOP expression in muscle and pancreatic islets.
These observations suggest that CHOP silencing in liver is associated with amelioration of insulin resistance and glucose homeostasis in vivo. It is known that CHOP activates interleulin (IL)-1β via caspase-11 induction. Systemic administration of IL-1 β can cause insulin resistance.
Conclusion: CHOP may have a significant role in deterioration of insulin resistance by the induction of inflammatory signals in db/db mice.
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557

Regulatory role of mouse SIRT5 in metabolism
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Background and aims: The nuclear protein SIR2, an NAD dependent deacetylase, is known to be involved in life span extension in yeast. In mammalian, there are seven SIR2 homologues, SIRT1-7. SIRT1 is localized in nucleus and regulates the activity of critical transcriptional regulators of metabolism, including FOXO1 and PGC1α. SIRT3, SIRT4 and SIRT5 are localized in mitochondria. SIRT3 deacetylates and regulates acetyl CoA synthetase 2. SIRT4 ADP-ribosylates glutamate dehydrogenase and inhibits its activity in pancreatic beta-cells. However, the function of SIRT5 is still unknown. In this study, to understand the function of SIRT5 in metabolism, we examined the expression level of SIRT5 in different nutrient status, and determined the molecules which are regulated by SIRT5 protein.
Materials and methods: Eleven-week-old C57/BL6 mice were fed *ad libium*, fasted for 24 hours, and re-fed for 24 hours, sequentially. Total RNA was extracted from various organs including liver. SIRT5 mRNA level was determined by qutantitive RT-PCR. SIRT5-overexpressing transgenic (SIRT5 Tg) mice were generated, in which mouse SIRT5 cDNA fused with FLAG tag was expressed by CAG promoter. Overexpression of SIRT5 protein was confirmed by western blotting with anti-FLAG antibody. The liver mitochondrial protein samples from the SIRT5 Tg mice and the littermates were applied for isoelectric electrophoresis, and then separated by SDS-PAGE. The obtained gels were stained with SYPRO Ruby strain kit, and the images were captured with a laser scanner. Then the protein whose position was altered between SIRT5 Tg mice and the littermates was identified using MALDI-TOF-MASS.
Results: SIRT5 mRNA level was increased in liver at fasting condition and decreased at re-feeding up to *ad libium* condition. We established two independent SIRT5 Tg mouse lines on the C57BL/6 inbred background. The SIRT5 Tg mice of both sexes were normally fertile and showed normal appearance. No histological abnormalities were observed in light microscopic analysis in all organs examined. Two-dimension electrophoresis of liver mitochondrial protein was performed and the protein whose position was altered was identified using MALDI-TOF-MASS. The protein is known to be an acetylated enzyme which is involved in metabolism, and its activity was increased in liver of the SIRT5 Tg mice.
Conclusion: SIRT5 may be involved in the regulation of metabolism by modulating the activity of liver enzyme through deacetylaion.

558

S-nitrosothiols as insulin resistance reversers
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Background and aims: Hepatic parasympathetic nerves mediate peripheral insulin action. In the post prandial state the hepatic parasympathetic nerves (HPN) are activated leading to an increase in glucose uptake by insulin action. This HPN activation seems to occur through a new mechanism to which optimal levels of hepatic glutathione (GSH) and nitric oxide (NO) are essential.
Prandial stages are related to different insulin sensitivities. In the fasted state the response to an insulin bolus is decreased when compared to the response of the same insulin bolus in the fed state. Administration of S-nitrosothiols to fasted animals increased the response of an insulin bolus to similar values as observed in the fed state. Our aim was assess the effect of RSNOs in the insulin action dependency on the HPN pathway. Our hypothesis is that blockade of the hepatic parasympathetic nerves leads to an insulin resistance state, which is reversed by RSNOs administration.
Material and methods: Male Wistar rats (9 weeks-old) were used. Insulin action was measured by the Rapid Insulin Sensitivity Test (RIST). The RIST is an euglycemic clamp that quantifies the amount of glucose necessary to infuse after an insulin bolus (50mU/kg over 5 min). The quantity of glucose infused to maintain the euglycemia is refered as the RIST Index and is the parameter used to evaluate insulin sensitivity/resistance. The animals were fasted for 22h and refed for 2h. Three RISTs were done. In the first test, insulin sensitivity was measured in the post prandial state. In a second RIST, insulin sensitivity was evaluated after denervation of the hepatic parasympathetic nerves. The third RIST was done after the administration of a nitrosothiol, the S-nitroso-N-acetylpenicillamine (SNAP). The SNAP was administered intravenously in a dose that was shown previously to be effective in fasted animals (15 mg/kg).
Results: The control RIST Index was 265.3±15.8 mg glucose/kg. After the ablation of the hepatic nerves the RIST Index decreased to 153.9±16.1 mg glucose/kg ($p<0.01$). This decrease was reversed by the administration of SNAP to values similar to the ones observed in the control RIST, 274.3±24.2 mg glucose/kg ($p<0.01$).
Conclusion: In this study it was observed the involvement of the hepatic parasympathetic nerves in insulin action. Moreover the insulin resistance observed with surgical denervation of the HPN was totally reversed by the administration of RSNOs. This observation suggests RSNOs as potential tools to treat dysfunctions related to insulin resistance.
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PS 35 Glucagon - secretion and effects

559

Preserved inhibitory potency of glucagon-like peptide-1 on glucagon secretion in type 2 diabetes mellitus

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Background and aims: The incretin glucagon-like peptide-1 (GLP-1) stimulates insulin secretion and inhibits glucagon release from pancreatic alpha cells. A dose-response relationship for GLP-1's insulinotropic properties has been established; whereas the effect on alpha cells remains less well described. We investigated the dose-response relationship for GLP-1-induced glucagon suppression in healthy subjects and in patients with type 2 diabetes mellitus (T2DM) during hyperglycaemia and after normalization of plasma glucose (PG) levels.
Materials and methods: Ten patients with T2DM (duration of DM: 4±1 years; HbA_1c: 7.1±0.3%; fasting plasma glucose (FPG): 10.3±1 mM; BMI: 31±1.1 kg/m^2) were studied on 2 separate days, after 1 week wash-out of oral antidiabetic drugs, with stepwise increasing GLP-1 infusions (0.25, 0.5, 1.0 and 2.0 pmol/kg/min-each infusion was maintained for 45 minutes) (Day 1) or saline infusion (Day 2) with PG clamped at fasting level. On a Day 3 PG was normalized over-night ($FPG_{t=-30}$: 5.9±0.3 mM) using a variable intravenous insulin infusion. The insulin infusion was stopped 30 minutes before initiation of a stepwise GLP-1 infusion ($FPG_{t=0}$ 6.7±0.2 mM) as on Day 1. Ten matched glucose tolerant subjects were examined with the same protocol as the patients with T2DM on Day 1 and 2 including clamping at FPG levels.
Results: We observed a dose-dependent stepwise suppression of glucagon secretion in both patients and control subjects. Significant suppression was observed at a infusion rate resulting in physiologic GLP-1 plasma levels (0.25 pmol/kg/min) as early as time 15 minutes in control subjects and time 30 minutes in patients with T2DM (Day 1 and Day 3). Area under the curve (AUC) for glucagon during the 3-hour GLP-1 infusion was significantly reduced on Day 1 and Day 3 (1096±109 and 1116±108 pmol/l·3h; P=NS) as compared to Day 2 (1733±193 pmol/l·3h; P<0.01) in the patients. A similar reduction in AUC was observed in control subjects 1122±186 pmol/l·3h (Day 1) vs. 1733±312 pmol/l·3h (Day 2) (P<0.001).
Conclusion: We conclude that the alpha cells in patients with T2DM appear to be fully sensitive to the inhibitory action of GLP-1 both during high and near-normalized PG levels, but respond with a significant delay compared to glucose tolerant control subjects.
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560

Hepatic knockdown of glucagon receptor via siRNA improves glucose homeostasis in mice

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Glucagon is an important counter-regulatory hormone of insulin that plays a key role in the maintenance of glucose homeostasis by stimulating glycogenolysis and gluconeogenesis in liver. The physiological effects of glucagon are mediated by the glucagon receptor (GCGR), a G-protein coupled receptor predominantly located on the plasma membranes of hepatocytes. Pharmacological and genetic interventions to suppress glucagon receptor signaling have been reported to reverse hyperglycemia in animal models. Here, we examine the extent to which siRNA lipid nanoparticle (LNP)-mediated knockdown (KD) of *Gcgr* in the livers of high fat diet-fed C57BL/6N (DIO) and *db/db* mice results in an improvement in glucose control. Intravenous (IV) administration of GCGR siRNA LNP at a dosage of 3 mg/kg resulted in a 65% KD of *Gcgr* expression in the livers of DIO mice 4 days post-injection, concomitant with 1) significant reductions in ambient and fasted glucose levels, 2) ~2-fold increases in circulating glucagon, 3) improved oral glucose tolerance, and 4) diminished hyperglycemic response to glucagon. In *db/db* mice, two independent *Gcgr* siRNA oligos also achieved significant hepatic KD of *Gcgr* (~65% versus control siRNA LNP) 4 days following an IV injection at a dosage of 3 mg/kg, accompanied by a 75-80% normalization of ambient glucose levels relative to lean controls with no effect on plasma insulin levels (glucose levels were 480.4 +/- 23.0 mg/dl in the control group and 271.0 +/- 18.0 mg/dl or 286.7 +/- 13.0 mg/dl for each of the two GCGR siRNA treatment groups). Elevations in circulating levels of glucagon (3.7-fold) and total GLP-1 (1.9 to 2.3-fold) were also observed, consistent with a 5-fold elevation in the mRNA levels of *preproglucagon* in islets. Together, these data confirm that the attenuation of glucagon signaling improves glucose homeostasis in diabetic mouse models. Importantly, the use of siRNA provides an alternative modality and a powerful tool to interrogate the role of hepatic pathways in glucose control.

561

Role of glucagon receptor in energy metabolism and body weight regulation

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Glucagon, the counter-regulatory hormone of insulin, plays a key role in maintaining glucose homeostasis. In contrast to its role in glycemic control, the relevance of glucagon receptor (GCGR) in the regulation of body weight is less clear. Our recent studies of GCGR knockout (KO) mice demonstrated GCGR deletion improves metabolic control by multiple mechanisms, including inhibition of hepatic glucose output as well as elevation of GLP-1 and FGF21 in circulation. These findings suggest that GCGR signaling is also important for the control of adiposity and insulin sensitivity. To further investigate its role in energy metabolism, GCGR homozygous (KO) and heterozygous mutant mice of both sexes were evaluated for changes in their metabolic phenotype while the mice were maintained on a chow diet or a high fat diet (HFD). As reported previously, male KO mice on chow diet were slightly leaner but maintained similar body weight as wild type (WT) littermates. These KO mice showed a partial resistance to diet induced obesity (DIO). In comparison with their male counterpart, the female KO mice exhibited a lean phenotype to a greater degree. When fed a chow diet, female KO mice displayed significantly increased body weight than their WT littermates (27.5±0.7 vs. 24.5±0.6 g, week 21 of age), largely due to a >10% increase of lean mass. On HFD, female KO mice were completely resistant to DIO during the study period (5-28 weeks of age). Reduced body weight gain in KO mice under HFD was solely due to a decrease of body fat content (lean mass 23.6±0.9 vs. 25.0±1.0 g, p=0.15; fat mass 3.4±0.8 vs. 29.7±1.4 g, p<0.001; week 28, KO vs. WT, n=16). Accompanying reduced adiposity under HFD, ambient glucose levels, plasma and liver triglyceride contents were significantly decreased in KO mice of both sexes. These correlated well with enhanced glucose tolerance and insulin sensitivity seen in KO mice. In contrast to complete GCGR deletion, heterozygous mice of both sexes were non-distinguishable from their WT littermates with respect to body weight and metabolic parameters. In summary, these results demonstrate that GCGR plays a critical role in body weight regulation in rodents, which is more apparent in female mice.

562

Dual effects of low and high concentrations of cAMP on glucagon secretion from mouse pancreatic A-cells

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Background and aim: Hyperglycemia is a result from a combination of impaired insulin secretion and insulin resistance at the target tissue. In addition it is associated with oversecretion of glucagon. Normally glucagon is secreted from the pancreatic alpha-cells (A-cells) in response to low glucose levels and it is under neuronal and hormonal control by agonists such as adrenaline, GIP and GLP-1. They all increase the cAMP-concentration of the A-cell, but still GLP-1 inhibits and adrenaline and GIP stimulates glucagon secretion. The aim of this study is to investigate the cellular mechanisms behind cAMP-dependent inhibition and stimulation within the pancreatic A-cells.
Material and methods: Glucagon secretion was measured on freshly isolated mouse islets and analyzed using radioimmunoassay (RIA). The electrical

properties of the A-cells such as ion channel currents and electrical activity was measured on single A-cells using the perforated patch configuration of the patch clamp technique.
Results: First we confirmed that glucagon secretion stimulated at 1 mM glucose (10±0.5 pg/islet/h, n=38) is inhibited by GLP-1 (6±0.6 pg/islet/h; P<0.0001; n=16) and stimulated by adrenaline (34±7 pg/islet/h; p=0.0001; n=10). The inhibitory action of GLP-1 was almost totally reversed in the presence of the PKA-inhibitor 8-Br-Rp-cAMPS (11±1 pg/islet/h; P< 0.0001 vs GLP-1 alone; n=8) and the stimulatory effect of adrenaline was partially antagonist by Rp-cAMPS (19±2 pg/islet/h; P<0.05 vs adrenaline alone; n=10). The observed effects by GLP-1 and adrenaline on glucagon secretion could be mimicked by low (1-10 nM) and high (10 μM) concentrations of forskolin. Likewise Rp-cAMPS counteracted the inhibitory effect of 3 nM forskolin (P<0.01; n=6-11) and did not reduce (n=8-10) the potentiating effect of 10 μM forskolin. Measuring the cAMP concentration ([cAMP]) in presence of different concentrations of forskolin revealed that 1 nM forskolin increased [cAMP] to 7±1 fmol/islet compared to 91±8 fmol/islet in the presence 10 μM forskolin. Together these data suggests that low [cAMP] is responsible for the inhibitory action on glucagon secretion and the high [cAMP] for the potentiating effect. Interestingly, there exist two isoforms of PKA, PKAI and PKAII, where PKAI have been demonstrated to be activated at a lower [cAMP] than PKAII. Using immunocytochemistry we could demonstrate the presence of both these PKA-isoforms in A-cells. Patch-clamp experiments on single A-cells revealed that the PKA-dependent suppressive action of GLP-1 on glucagon secretion act through inhibition of the N-type Ca^{2+}-currents (P<0.001; n=7) and reduced electrical activity (P<0.01; n=4).
Conclusion: We conclude that GLP-1 and adrenaline increase [cAMP] inside the A-cell to a low and high level, respectively. We further suggest that 1) low concentrations of cAMP activates PKAI leading to inhibition of N-type Ca^{2+}-currents and electrical activity and 2) high [cAMP] activate PKAII and PKA-independently the cAMP-binding protein Epac2 resulting in amplification of glucagon secretion.
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563

Glucose inhibits glucagon secretion from mouse pancreatic islets independently from insulin release and by mechanisms that are distinct from those elicited by K_{ATP} channel modulators
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Background and aims: Glucagon secretion by pancreatic α-cells is normally inhibited by hyperglycemia and stimulated by hypoglycemia. The mechanisms by which glucose (G) inhibits glucagon secretion are still unclear. In particular, it is unknown if G influences glucagon secretion by modulating K_{ATP} channels as in β-cells and if a β-cell derived factor is responsible for the G-induced inhibition of glucagon secretion. These questions were studied here.
Materials and methods: NMRI mouse islets were isolated by collagenase digestion of the pancreas and cultured overnight in RPMI 1640 medium containing 7 mM G. Glucagon and insulin secretion from islets were measured in perifusion experiments. A 5 mM mixture of amino-acids (1 mM alanine, 1 mM leucine, 1.5 mM glutamine and 1.5 mM lysin) was added to all perifusion media to better mimic the physiological conditions and have a detectable glucagon secretion. Because glucagon secretion is Ca^{2+}-dependent, the free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) of α-cells was also measured in some experiments by using a Ca^{2+}-sensitive probe (D3cpv) specifically targeted to α-cells within islets.
Results: Increasing stepwise the glucose (G) concentration of the perifusion medium from 0 to 20 mM (2, 5, 7, 10, 15, 20 mM) showed that inhibition of glucagon secretion was already of large amplitude at G2 (64%), and maximal at G7 (69%), whereas stimulation of insulin secretion started at G5. A high concentration of the K_{ATP} channel opener diazoxide (250 μM) decreased glucagon secretion without affecting insulin release during perifusion with G0, whereas it did not affect glucagon secretion but decreased insulin release during perifusion with G7. By contrast, a high concentration of the K_{ATP} channel blocker tolbutamide (500 μM) stimulated glucagon secretion during perifusion with G7 and did not affect it in G0, whereas it strongly stimulated insulin secretion at both [G]. Low concentrations of diazoxide (5μM) and tolbutamide (2μM) did not affect glucagon and insulin secretion, except for a small stimulation of insulin release by tolbutamide at G7. To test if G inhibits glucagon secretion by decreasing the efficacy of $[Ca^{2+}]_c$ on exocytosis, the effect of G was tested during perifusion with a medium containing 30 mM K^+ and 250 μM diazoxide, a condition where α- and β-cell $[Ca^{2+}]_c$, and glucagon and insulin secretion were steadily elevated. Under this condition, increasing the [G] from 0 to 7 mM did neither affect α-cell $[Ca^{2+}]_c$ nor glucagon release whereas it strongly stimulated insulin release by the well documented amplifying pathway of G-induced insulin secretion.
Conclusions: The lack of association between inhibition of glucagon secretion and stimulation of insulin secretion under various experimental conditions refutes the proposal that insulin or a β-cell derived factor coreleased with insulin is the paracrine factor responsible for G-induced inhibition of glucagon secretion. The distinct effects of G and K_{ATP} channel modulators on glucagon and insulin secretion suggest that G does not inhibit glucagon secretion by modulating K_{ATP} channels in α-cells as in β-cells. The lack of effect of G on glucagon secretion when α-cell $[Ca^{2+}]_c$ was steadily elevated demonstrates that G does not inhibit glucagon secretion by decreasing the efficacy of Ca^{2+} on exocytosis.
Supported by: JDRF

564

***In situ* α-cell electrophysiological characterizations using a novel whole pancreas tissue slice approach**
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Background and aims: The mechanism underlying glucagon secretion remains elusive. Despite a recent surge in α-cell studies, large body of reports have been conflicting because selected α-cell populations or properties could have been inadvertently induced or selected during the conventional islet isolation procedures. An assay specifically designed to overcome these technical limitations is therefore urgently required to reveal insights into α-cell biology and to provide therapeutic targets for treatment of type 1& 2 diabetes.
Material and methods: A novel pancreas slice preparation was developed to surmount enzymatic and mechanical perturbations inherent in conventional islet cell isolation procedures. Mice pancreata were agarose gel-infused and sectioned into slices, wherein intact islets were embedded. Intra-islet cellular communication and islet architecture can be well-preserved and α-cells within slices are easily reachable by a patch pipette. Along with standard patch clamp techniques, we developed electrophysiological protocols to rapidly distinguish α-cells, thereby, enabling large sampling and unbiased examination of high quality α-cells.
Results: Mouse α-cells in slices revealed classical features including readily-activated A-type I_K, voltage-gated I_{Na}, small size (~4 pF), low resting membrane conductance (<0.5nS), and inducible low/high voltage-activated I_{Ca} at -80mV. I_{Ca} influx correlated with glucagon exocytosis as either train of depolarization (train) or UV photo-release of intracellular-loaded caged-Ca^{2+} stimulated C_m increase. N-type Ca^{2+} channels modulated α-cell secretion predominantly because ω-conotoxin GVIA (GVIA) pre-treated α-cells revealed diminished C_m increase in response to a train. Consistently, GVIA-sensitive I_{Ca} became significantly reduced after applying a train to α-cells without pretreatment, which also likely suggests cycling of N-type Ca^{2+} channels on the membrane from active to inactive states or between insertion and internalization. We frequently encountered widely-deviated α-cell parameters, including I_{Na} amplitude (100-900pA), amount of K_{ATP} conductance (0-22 nS), specific subtypes of I_{Ca} in different α-cells, and multiple phases of train-induced C_m change (endo-, no change and exocytosis) in a pool of α-cells. Furthermore, some of these parameters displayed a bell-shaped distribution along with a "window" of maximal activity. More remarkably, when applying multiple trains on individual α-cells at different time points, C_m changes revealed an oscillatory pattern of glucagon secretion.
Conclusion: α-cell ion channel properties examined in slices were largely consistent with previous reports, validating the feasibility of using pancreas slice approach to specifically investigate α-cells. The pattern of wide-range data distribution, cycling of ion channel activities, multiple secretory responses (endo-, no change and exocytosis) within a pool of α-cells, and windows of maximal channel activities within a bell-shaped curve led us to postulate that these properties collectively contribute to the waveform-like oscillatory pattern of glucagon release observed in α-cells. Within this secretory pattern, glucagon secretes maximally when K_{ATP}, Na^+, Ca^{2+} channels reached their optimal "window" of activities (at the peak of a wave cycle). Outside this window, at the upstroke or downstroke of the wave, glucagon secretion declines.
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565

Structure function studies with glucagon related peptides reveal glucagon receptor antagonist activities *in vitro* and *in vivo*

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Background: The impact of abnormally elevated postprandial glucagon responses has been shown to contribute to the increased hepatic glucose output and overall hyperglycaemia associated with type 2 diabetes. Glucagon receptor antagonists have the potential to alleviate this unwanted hyperglycaemia.
Aims: In this study we examined the effects of structural modification of glucagon on peptide stability in the presence of dipeptidylpeptidase-4 (DPP-4). Furthermore, the functional ability of glucagon peptide analogues to block glucagon mediated effects in cultured rodent pancreatic beta cells and in normal mice was examined.
Methods: Two structural analogues of glucagon, namely desHis^{1}Glu^{9}glucagon-amide and desHis^{1}Glu^{9}Lys^{30}Pal-glucagon were synthesised. The stability of these two analogues and native glucagon to DPP-4 was examined *in vitro*. Peptides were incubated with DPP-4 (pH 7.8, final peptide concentration 2 µmol/l) at 37°C for 0, 2, 8 and 24 h. Enzymatic reactions were stopped by the addition of 10% (v/v) trifluoroacetic acid and degradation products were then analysed using reversed-phase HPLC. Acute insulin secretion studies (20 min) were performed with control (5.6 mM glucose), glucagon alone or glucagon in the presence of either desHis^{1}Glu^{9}-glucagon or desHis^{1}Glu^{9}Lys^{30}Pal-glucagon in pancreatic BRIN-BD11 cells. *In vivo* studies were performed with groups of fasted mice (n=9-10, aged 10-14 weeks) injected i.p. with saline, glucagon alone (25 nmol/kg) or desHis^{1}Glu^{9}-glucagon in the presence or absence of glucagon. Blood samples were collected by tail venepuncture at 0, 15, 30, 60 and 105 min.
Results: Glucagon was progressively degraded following incubation with DPP-4 and the primary degradation product was identified as glucagon(3-29) by MALDI-MS. After 8 h incubation only 20% of glucagon remained intact, whereas 60% of desHis^{1}Glu^{9}-glucagon remained intact. In contrast, 100% of desHis^{1}Glu^{9}Lys^{30}Pal-glucagon remained intact up to and including 24 h. Glucagon dose-dependently (10^{-12} to 10^{-7} M) increased insulin secretion (P<0.05-P<0.001, Students t-test) compared to 5.6 mM glucose controls in BRIN-BD11 cells. In contrast, both desHis^{1}Glu^{9}-glucagon and desHis^{1}Glu^{9}Lys^{30}Pal-glucagon failed to promote insulin secretion in these cells over the entire range of concentrations tested (10^{-12}-10^{-7} M) at 5.6 mM glucose. Both desHis^{1}Glu^{9}-glucagon and desHis^{1}Glu^{9}Lys^{30}Pal-glucagon effectively antagonised 10^{-7} M glucagon-induced insulin secretion over the concentration range (10^{-12} to 10^{-7} M) causing a 65-78% decrease in glucagon stimulated insulin secretion (P<0.001). *In vivo* studies demonstrated that plasma glucose responses to glucagon were significantly enhanced (P<0.001) compared with saline (Area Under Curve, AUC 0-105 min) in normal mice. DesHis^{1}Glu^{9}-glucagon showed a reduced overall glucose excursion (P<0.01) compared with glucagon alone. In addition, when combined with glucagon, desHis^{1}Glu^{9}-glucagon effectively antagonised glucagon-induced glucose production, 0-105 min AUC excursion (P<0.001).
Conclusion: These studies demonstrate that desHis^{1}Glu^{9}-glucagon and desHis^{1}Glu^{9}Lys^{30}Pal-glucagon are more stable than native glucagon and are specific glucagon receptor antagonist candidate molecules with potential in alleviating glucagon-induced hyperglycaemia in normal mice. Further studies are required to investigate the efficacy of these peptide analogues in animal models of diabetes.
Supported by: University of Ulster RCSF

566

Impaired glucose-induced glucagon suppression after 50% partial pancreatectomy in humans

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Background and aims: The glucose-induced decline in glucagon levels is often lost in patients with type 2 diabetes. It is unclear, whether this is due to an independent defect in alpha-cell function or secondary to the impairment in insulin secretion. We examined, whether a 50% partial pancreatectomy in humans would also impair post-challenge glucagon concentrations, and if so, whether this could be attributed to the reduction in insulin levels.
Materials and methods: 36 patients were studied before and after an ~50% partial pancreatectomy with a 240 min oral glucose challenge, and the plasma concentrations of glucose, insulin, C-peptide, and glucagon were determined.
Results: Fasting glucose concentrations increased significantly after the partial pancreatectomy (p<0.0001). This was accompanied by a reduction in both fasting and post-challenge insulin and C-peptide levels (p < 0.0001). Fasting glucagon concentrations were not altered by the intervention (p = 0.11). Oral glucose ingestion elicited a decline in glucagon concentrations before surgery (p < 0.0001), but this was lost after partial pancreatectomy (p < 0.01 vs. pre OP values). Likewise, the absolute mean difference in glucagon levels from baseline, and the reduction in the integrated incremental glucagon concentrations were lower after partial pancreatectomy (p<0.01). The loss of glucose-induced glucagon suppression was found after both pancreatic head (p < 0.001) and tail (p < 0.05) resection. The glucose-induced changes in glucagon levels were closely correlated to the respective increments in insulin and C-peptide concentrations (p < 0.01).
Conclusion: The glucose-induced suppression in glucagon levels is lost after a 50% partial pancreatectomy in humans, most likely as a consequence of reduced insulin levels. This suggest that impaired alpha-cell function in patients with type 2 diabetes may also be secondary to reduced beta-cell mass. Alterations in glucagon regulation should be considered as a potential side effect of partial pancreatectomies.

PS 36 Incretin secretion in humans

567

The postprandial response of the anorexigenic gut hormones PYY and GLP-1 is affected by eating rate

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Background and aims: The rate at which people eat has been suggested to be positively associated with obesity. However, there are no studies examining how eating rate affects appetite and related gut hormones. The present study assessed whether eating the same meal at varying speeds elicits different postprandial orexigenic and anorexigenic gut peptide responses.
Materials and methods: Seventeen healthy adult male volunteers (age: 29.7 ±1.2 years, BMI: 26.1 ±0.9 kg/m2) were consecutively recruited and examined in a crossover design. They consumed a test meal consisting of 300 ml ice-cream (675 kcal, 59% of kcal fat, 33% carbohydrates, 8% protein) at two different rates. In one session, the meal was divided in equal portions and consumed within 5 minutes, while in the other it was consumed in 30 minutes. Blood samples for the measurement of glucose, insulin, plasma lipids, the orexigenic gut hormone ghrelin, and the anorexigenic hormones PYY and GLP-1 were drawn before the meal and at 30 minute intervals after the beginning of meal consumption, until the end of the session, 210 minutes later. Visual analogue scales (VAS) for assessment of the subjective feelings of hunger and fullness were completed during the meal and at 30 minute intervals after meal termination until the end of the study session. The postprandial ghrelin, PYY and GLP-1 responses were assessed and expressed as area under the curve of their plasma concentrations for each session.
Results: PYY levels were higher for the 30 minute meal than for the 5 minute meal 90, 120 and 150 minutes after meal initiation, as were GLP-1 concentrations 60 minutes after the start of the 30 minute meal and until the end of the session. PYY area under the curve (AUC) was higher after the 30 minute meal than after the 5 minute meal (mean ±SEM AUC-5 min meal: 4133 ± 324, AUC-30 min meal: 5250 ± 330 pmol/L*min, p=0.004). The same was true for GLP-1 AUC (mean ±SEM AUC-5 min meal: 6219 ± 256, AUC-30 min meal: 8794 ± 656 pmol/L*min, p=0.001). There was no difference in ghrelin, insulin or glucose response between the sessions, either between specific time points after meal initiation or in terms of AUC. There was a trend for higher VAS fullness ratings immediately after the end of the 30 minute meal compared to immediately after the 5 minute meal (mean ±SEM: 51.7 ±4.9 vs 43.4 ±4.6 mm, p=0.09).
Conclusion: Eating at a physiologically moderate pace leads to a more pronounced anorexigenic gut peptide response than eating very fast, and may thus favor earlier satiety.

568

Differential reduction in incretin hormone response to different macronutrients in obese vs lean healthy subjects

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Background and aims: The incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are released after meal ingestion and potentiate glucose-stimulated insulin secretion. Although it is hypothesized that GLP-1 secretion is reduced in obese subjects after ingestion of a mixed meal, it is not known whether the GLP-1 response to individual macronutrients is similarly reduced. This study therefore examined the incretin hormone responses (total and active forms) to isocaloric glucose, fat and protein challenges in obese (OV) and lean (LV) healthy subjects.
Materials and methods: The study was undertaken in healthy male human volunteers that were either lean (BMI 20-25kg/m^2; n=12;LV) or obese (BMI 30-35kg/m^2; n=12;OV). After an overnight fast, the subjects at three different occasions consumed 2g glucose/kg b wt, 0.88g olive oil/kg b wt or 2.22g protein mix (85% whey protein)/kg b wt. Blood samples were collected before and up to five hours after intake of each macronutrient for measurements of insulin and intact GLP-1 and GIP. Early insulin and incretin hormone response were estimated as suprabasal area under the curve (AUC) for the first 30 minutes (AUC30) whereas total hormon responses were assessed as AUC300 over the entore 300 minutes study period.
Results: GLP-1 and GIP secretion increased after glucose, protein and fat in both lean and obese subjects. OV had a significantly lower AUC30 for total GLP-1 compared to LV (0.4±0.1 vs. 0.7±0.1nM*min, P=0.04). In contrast, there was no difference in total GLP-1 response between OV and LV after protein or fat. No Differences were found for AUC300. By contrast, OV had significant reduction of both AUC30 (0.9±0.2 vs. 1.7±0.3nM*min, P=0.03) and AUC300 (18±3 vs. 10±3nM*min, P=0.046) of total GIP after oral protein, whereas there was no difference in GIP secretion after intake of glucose and fat. No differences were found for the early secretion of the intact forms of the incretins. For insulin there were no difference in the early secretion but significantly higher AUC300 in OV after glucose (80±9 vs. 268±41nM*min, P=0.0005) and protein 37±3 vs. 114±16nM*min, P=0.0007).
Conclusion: Our results showed that obesity is associated with reduced early GLP-1 secretion after oral glucose and reduced early and total GIP secretion after oral protein. Our data suggests that incretin hormone secretion is differentially perturbed in obesity depending on the specific macronutrient ingested.
Supported by: The Swedish Research Council, Region Skåne, Faculty of Medicine, Lund University

569

Deterioration of glucose homeostasis induces impaired incretin effect in healthy normal glucose tolerant subjects

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Background and aims: The incretin effect is severely reduced in patients with type 2 diabetes. It remains unclear whether this deficiency can be induced temporarily in healthy subjects by a short period of glucose homeostatic dysregulation. We aimed to investigate the incretin effect before and after 10 days of prednisolone (a corticosteroid drug) administration, high energy diet and physical inactivity in healthy male subjects
Materials and methods: The incretin effect was measured using 75-g OGTT and isoglycaemic i.v. glucose infusion in 10 healthy Caucasian male subjects without family history of diabetes (age: 24±3 years (mean±SD); BMI: 23±2 kg/m^2; fasting plasma glucose: 5.2±0.4 mM, HbA_1c:5.4±0.1%) before and after dysregulation of glucose homeostasis using high calorie diet, physical inactivity and administration of prednisolone (37.5 mg/day) for 10 days.
Results: No significant change in body weight was observed whereas insulin resistance according to HOMA (1.3±0.1 *vs* 2.9±0.3, (mean±SEM) p=0.0003) and glucose tolerance as assessed by the 120-min plasma glucose-value during the OGTT (4.9±0.4 *vs* 7.8±0.8 mM, p<0.0001) and $AUC_{PG\text{-}OGTT}$ (152±45 *vs* 384±53 mM·4h, p=0.002) deteriorated following the 10-day interventional period. The intervention had a significant negative impact on the incretin effect as assessed by the formula: 100% ($AUC_{insulin,OGTT}$ - $AUC_{insulin,i.v.}$) / $AUC_{insulin,OGTT}$ (72±5 *vs* 43±7%, p=0.002)
Conclusion: These data show that the reduced incretin effect observed in patients with type 2 diabetes is a phenomenon that can be induced in healthy male subjects without risk factors for type 2 diabetes. This finding indicates that the deficient incretin effect in type 2 diabetes is a consequence of a glucose intolerant state rather than a primary event causing type 2 diabetes.
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570

Reduced incretin effect in dexamethasone-induced insulin resistance and glucose intolerance in diabetic offspring

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Background and aims: Insulin resistance and impaired glucose tolerance characterize pre-diabetes. We wanted to evaluate the incretin effect before and after the development of insulin resistance and glucose intolerance. We used a model where we increased insulin resistance, which in some patients also induced glucose intolerance, by short-term dexamethasone treatment.

Materials and methods: 12 healthy, 1st degree relatives to type 2 diabetic patients, with normal glucose tolerance, were examined on 2 occasions before and after a steroid treatment. We performed a 75g OGTT with frequent blood samples to obtain a glucose profile. On day 2 we made an isoglycemic duplication of the glucose curve by an i.v. glucose infusion. The subjects were then treated with 2mg dexamethasone bid for 5 days, and the tests were repeated. During the tests we drew blood to measure insulin and C-peptide. We calculated the incretin effect by relating the incremental insulin response during the OGTT to the incremental insulin response during the i.v. glucose infusion by the following formula: $((iAUC_{insulin,OGTT}-iAUC_{insulin,i.v.})/iAUC_{insulin,OGTT}) \bullet 100\%$. Insulin resistance was evaluated using the $HOMA_{IR}$ index.
Results: The dexamethasone treatment increased the insulin resistance in all the subjects, of which 7 became glucose intolerant. The group that remained glucose tolerant, ((BMI 22±1 kg/m^2) (mean±SEM), 2 men, 3 women), had a change in $HOMA_{IR}$ from 0,88±0,12 to 1,8±0,30 ($p < 0,05$). This group also had a reduction in the incretin effect from 71±5,4% to 61±4,6% ($p < 0,05$). The glucose intolerant group ((BMI 25±0,6 kg/m^2), 5 men, 2 women) had a change in $HOMA_{IR}$ from 0,97±0,16 to 2,5±0,44 ($p < 0,05$). This group had a reduction in the incretin effect from 70±6,2% to 33±11,8% ($p < 0,05$). Comparison between the two groups showed a significantly larger reduction in the incretin effect in the glucose intolerant group as compared with the tolerant group ($p < 0.05$). The increase in $HOMA_{IR}$ did not differ between the groups. A correlation analysis showed no correlation between the increase in insulin resistance and the decrease in the incretin effect ($p = 0,4$).
Conclusion: Our results point to a reduced incretin effect in relatives to patients with type 2 diabetes after an increase in insulin resistance despite a normal glucose tolerance. Nevertheless the reduction in the incretin effect was significantly more pronounced in subjects, who both developed insulin resistance and glucose intolerance. We therefore hypothesize that the diminished incretin effect seen in type 2 diabetes is the end product of a process already beginning with the development of insulin resistance.
Supported by: The Novo Nordisk Foundation

571

The capability increase the incretin effect in response to increasing oral glucose loads is impaired in patients with type 2 diabetes
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Background and aims: The incretin effect (the enhancement of glucose-induced insulin secretion following OGTT compared to isoglycaemic intravenous glucose infusion (IIGI)) is reduced in patients with type 2 diabetes mellitus (T2DM). In healthy subjects the incretin effect increases concurrently with the size of the oral glucose load resulting in similar glucose excursions independently of the size of the glucose load. Whether patients with T2DM are able to regulate their incretin effect in this manner is unknown. We aimed at elucidating this uncertainty.
Materials and methods: The incretin effect was measured over six days by means of three 4-hour OGTTs with increasing glucose loads (25 g, 75 g and 125 g) and three corresponding IIGIs in two groups of volunteers: 1) Eight patients with T2DM (fasting plasma glucose (FPG): 7.7 (7.0-8.9) mmol/l (mean(range)); HbA_{1c}: 7.0 (6.2-8.4)%); and 2) eight healthy control subjects (CTRLs) matched for sex, age and BMI (FPG: 5.3 (4.8-5.7) mmol/l; HbA_{1c}: 5.4 (5.0-5.7)%) were studied.
Results: As opposed to patients with T2DM the CTRLs did not exhibit any difference in glucose excursions in response to the different oral glucose loads. In contrast to the CTRLs, the patients with T2DM only exhibited a tendency to augmentation of their incretin effect ($100\% \cdot (AUC_{insulin,OGTT} - AUC_{insulin,i.v.}) / AUC_{insulin,OGTT}$) in response to increasing oral glucose loads (Table 1). During all three OGTTs the incretin effect was lower in the T2DM group as compared to the CTRLs (Table 1).
Conclusion: Our data suggest that regulation (increase) of the incretin effect in response to increasing oral glucose loads is crucial for controlling postprandial glucose excursions in healthy subjects, and that this regulatory capability is impaired in patients with T2DM.
Supported by: MSD

572

Reduced postprandial GLP-1 response in gestational diabetes mellitus is a fully reversible phenomenon
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Background and aims: Patients with type 2 diabetes exhibit reduced postprandial secretion of glucagon-like peptide-1 (GLP-1). The causality and potential reversibility of this pathophysiological trait are unclear. We aimed to investigate whether women with gestational diabetes mellitus (GDM) exhibit decreased postprandial GLP-1 responses, and if so, whether this deficiency ceases following delivery when normal glucose tolerance is reestablished.
Materials and methods: Eleven women with GDM (plasma glucose concentration at 120 min after 75-g OGTT: 10.0±0.3 mM (mean±SEM); age: 31±2 years; BMI: 31.6±1.9 kg/m2; HbA1c: 5.6±0.2%) and eight pregnant women with normal glucose tolerance (NGT) (age: 27.7±1.0 years; BMI: 29.7±1.7 kg/m2; HbA1c: 5.4±0.1%) were investigated with a 4-hour liquid meal test (100g NAN1: 2170 kJ) during pregnancy (third trimester) and 3-4 months post partum. All patients with GDM reestablished a normal glucose tolerance within 2-3 months following delivery.
Results: Patients with GDM exhibited reduced postprandial GLP-1 responses as compared to their postpartum levels (Table 1) and as compared to healthy non-pregnant women (AUC: 1445±293 vs. 3461±811 pM•4h (mean±SEM), $p<0.0001$). The difference between third trimester and post partum GLP-1 responses among NGT women did not reach statistical significance. However, no differenc-es between the two groups during pregnancy and postpartum, respectively, were observed (Table 1). Pregnancy did not have any impact on postprandial responses of the other incretin hormone, glu-cose-dependent insulinotropic polypeptide, in either of the two groups.
Conclusion: Patients with GDM exhibit reduced postprandial GLP-1 responses and the deficiency is fully reversible alongside the restoration of NGT. GLP-1 responses in healthy pregnant women showed the same tendency, suggesting that reduced postprandial GLP-1 response is a characteristic of type 2 diabetes and most likely occurs as a consequence of an insulin resistant state and worsen alongside the development of overt glucose intolerance.

Table 1. Mean incremental AUC for GLP-1 (pM<font face=

	GDM (n=11)	GDM (n=11)	Difference, unpaired T-test (P-value)
Third trimester	1445±293	1616±870	0.836
Post partum	4520±828	3461±811	0.388
Difference, paired T-test (P-value)	0.002	0.069	

Supported by: The Novo Nordisk Foundation, the Danish Diabetes Foundation, Aase and Ejnar Danielsens Foundation

573

GIP and GLP-1 concentration are prescribed by different factors during oral glucose load in Asian subjects
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Background and aims: It is well known that incretin effect contributes to as much as half of insulin secretory response to oral glucose load and that this effect reduces along with worsening of glucose tolerance. Several studies have shown relations between incretin effect, incretin concentration and other endogeneous factors. These findings are, however, almost based on European subjects, and little is known on Asian subjects who have different genetic background resulting in much lower insulin secretory capacity. Thus, in this study, we aimed to investigate whether Asian subjects show similar incretin effect as previously reported, and to show which factor prescribes incretin secretion.
Materials and methods: Isoglycemic 3-hour oral (75g oral glucose tolerance test (OGTT)) and intraveneous glucose administration was performed in thirteen Japanese subjects (age: 33.8±5.6 years; body mass indx (BMI):

23.2±3.5 kg/m2 (mean±S.D.)) who consists of the NGT and the IGT. In the isoglycemic test, intraveneous glucose infusion (20% wt/vol) was performed aiming to reproduce the plasma glucose profile at OGTT using artificial pancreas system STG-22 (Nikkoso, Tokyo, Japan). Plasma or serum levels of glucose, insulin, C-peptide, glucagon, free fatty acids (FFA), gastric inhibitory polypeptide (GIP) and glucagons-like polypeptide-1 (GLP-1) were determined.
Results: Plasma concentrations of GIP and GLP-1 increased significantly after oral glucose load. The integrated GIP concentration during OGTT (area-under-curve (AUC)-GIP) was not correlated with glucose concentrations but with AUC-FFA and fasting glucagon concentration positively (r= 0.75, P<0.01 and r= 0.62, P<0.05 respectively). There also was a significant strong relationship between GIP levels and BMI (r= 0.79, P<0.005). On the other hand, the AUC-GLP-1 was inversely correlated with fasting glucose level and AUC-glucose significantly (r= -0.68, P<0.05 and r= -0.69, P<0.01 respectively), and was not correlated with FFA, glucogon, or BMI. The AUC-glucose during OGTT was closely reproduced by intravenous glucose infusion. Incretin effect caliculated from the integrated β-cell cell secretory response (of insulin and C-peptide) was comparable to that reported in previously (57.9±4.8 % and 44.7±4.0 % respectively).
Conclusion: In this study, we show for the first time that incretin effect in the Asian subjects is comparable to that in the European subjects. In addition, our findings revealed that GIP and GLP-1 concentrations are prescribed by clearly different factors. Furthermore, glucose metabolism appears to be affected by GLP-1 rather than by GIP even in normal to near-normal glucose tolerant subjects, while GIP appears to be more important in lipid metabolism.

574

Beta cell responsiveness to GLP-1 in nondiabetic lean and obese adults
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Background and aims: The incretin effect describes the enhancement of insulin secretion after ingestion of meals due to the gut factors Glucose-dependent insulinotropic polypeptide (GIP) and Glucagon-like peptide-1 (GLP-1). Recent studies suggest that the incretin effect is not only impaired in patients with T2DM but also in obese subjects without history of diabetes. However it is unclear whether this is due to an impaired beta-cell response to GIP or GLP-1. Therefore we sought to determine a dose-response relationship between GLP-1 and insulin secretion in a cohort of lean and obese subjects.
Materials and methods: For this purpose 8 lean (BMI 23.0±0.8; M/F, 4/4) and 6 obese (36.7±0.8; M/F, 3/3)) healthy subjects with no personal or family history of diabetes were studied. Subjects received a variable glucose infusion to achieve stable blood glucose of 7.3 mmol/l that was clamped for 240 minutes. After 60 min of hyperglycaemia, a step wise infusion of GLP-1 was given at rates of 0, 15, 45, 90, 150, 300, and 400 ng/kg (lean mass)/h for 25 minutes each.
Results: The fasting glucose levels in all subjects were < 5.3 mmol/L and slightly higher in the obese than lean group (4.8±0.1 vs 4.5±0.1, p = 0.05). Obese subjects had a significantly higher fasting insulin than lean subjects consistent with insulin resistance (169±32 vs. 57±3 pM; p<0.001). During hyperglycaemia alone, plasma insulin rose to 688±61 pM in the obese subjects and to 201±25 pM in the lean subjects (p<0.001). At the highest rate of GLP-1 subjects in the obese cohort still secreted significantly more insulin compared to the lean (12579±4218 vs. 6062±579 pM; p<0.05), although the individual responsiveness to GLP-1 was more variable (CV 59 vs. 29%). The relative increment of GLP-1 stimulated insulin secretion over glucose alone was not significantly different between the groups.
Conclusion: While the subjects in the obese cohort were obviously insulin resistant their basal insulin secretion was appropriate with only modestly higher fasting glucose compared to the lean subjects. Both glucose and GLP-1 stimulated insulin secretion was significantly higher in the obese subjects and was proportional compared to the response in leans. In this cohort of obese subjects with insulin resistance but adequate beta-cell function there was no impairment of beta-cell responsiveness to GLP-1.
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PS 37 Insulin secretion in vivo

575

Bromocriptine acutely decreases glucose-stimulated insulin secretion in mice
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Background and aims: Long term treatment with Bromocriptine, a dopamine receptor D2 agonist, improves glucose tolerance and decreases fasting plasma insulin levels in both obese diabetic patients and animal models by a still unknown mechanism. Since dopamine D2 receptors are expressed in pancreatic beta-cells, we hypothesized that Bromocriptine might modulate insulin secretion by a direct action on the pancreas. We therefore investigated the acute effect of therapeutic concentrations of Bromocriptine on insulin release, both *in vivo* and *in vitro*.
Materials and methods: Male C57BL/6 mice were subjected to an intraperitoneal glucose tolerance test (ipGTT) or a hyperglycemic clamp (20 mM) 60 min after they received a single intraperitoneal injection with Bromocriptine (10 or 25 mg/kg BW) or vehicle. Basal and glucose-stimulated insulin secretion (GSIS) was also measured in pancreatic INS-1E beta-cells after acute treatment (60 min) with Bromocriptine (1, 5, 50 and 500 nM) or vehicle.
Results: One hour after a single intraperitoneal injection of 10 or 25 mg/kg BW Bromocriptine the basal glucose levels were dose-dependently increased by 35% and 136% (p<0.01) compared to vehicle, while insulin levels remained unaffected. In addition, Bromocriptine significantly increased the area under the curve of glucose levels by 40% and 46% (p<0.001) and delayed the rise in insulin levels during the ipGTT, indicating glucose intolerance. During the hyperglycemic clamp, Bromocriptine strongly reduced the first-phase (-76% and -74% at 1 min; p<0.01) and second-phase insulin response (-55% and -63%; p<0.01), underlining a clear alteration of GSIS. Additional experiments in INS-1E beta-cells also showed a dose-dependent inhibition of GSIS by Bromocriptine at concentrations above 1 nM (from -14% to -61%; p<0.01), while basal insulin secretion was not modified.
Conclusion: In contrast with the previously reported beneficial effect of long-term Bromocriptine treatment on glucose homeostasis, we report here that acute administration leads to glucose intolerance, provoked by the inhibition of GSIS. Our *in vitro* data suggests that the Bromocriptine-induced decrease in GSIS is the result of a direct effect on pancreatic beta-cells, through activation of dopamine D2 receptors.
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576

Nateglinide stimulated oral glucose tolerance test for functional assessment of residual beta cell capacity in type 2 diabetes
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Background and aims: There is no simple clinical tool for testing beta-cell-capacity in patients with type 2 diabetes. The clamp requires continuous monitoring and is not feasible in an out-patient setting.
Nateglinide is a derivative of the amino acid D-phenylalanine, which acts directly on the pancreatic beta-cells to stimulate insulin secretion particualrly in the the early phase. Our aim was to evaluate a combined nateglinide-/OGT test to assess beta-cell capacity in an out-patient setting.
Materials and methods: We examined in a 79 individuals (30 healthy controls (HC) and 49 type 2 diabetes patients (T2DP) either on dietetic control or treated with OAD (excluding sulfonylurea derivatives) during a modified 3 hour OGTT (50g) combined with 120 mg of nateglinide (Starlix®). We measured glucose, insulin and C-peptide at baseline and after 30, 60, 120 and 180 minutes and calculated HOMA-IR, AUCinsulin, AUCc-peptide and AUCglucose. In order to assess the intraindividual variability 10 HC were tested twice.
Results: Significantly lower serum glucose level were found in HC than in the T2DP at any time points (e.g. max glucose: 6.94±1.44 vs 13.03±3.58mmol/l, AUC glucose: 4.69±0.86 vs 9.45±3.70 mmol*h/l) who reached maximum

level later (35.25±17.61 vs. 63.33±31.76 min). In 18 T2DP we observed normal 2h glucose levels (7 had levels compatible with IGT and 20 had glucose >11.1mmol/l). T2DP can be devided into A leading insulin resistence (HOMA-IR>2,5, n=29) and B secretory deficiency (max. c-peptide < 4ng/mL or increase in c-peptide < 2fold, n=21). Patients with secretory deficiency had higher HbA1c (7.79%±1.80 vs. 6.23%±1,58 p<0.001), AUCglucose (1756.91±766 vs.1612 06±663.21.p.05 n.s) and blood glucose at 30min (12.99mmol/l±2.19 vs. 10.38mmol/l±4.38 n.s.) and 120min (11.14mmol/l±5.05 vs. 9.86mmol/l±4.07 n.s.).
Conclusion: The nateglinide/OGT test is a feasible tool for a routine outpatient clinic to assess the residual beta-cell capacity. Insulin and C-peptide values at 30 and 120 minutes distinguish between normal and deficient insulin secretion. Based on the limited numbers analysed we diagnose relevant insulin deficiency in T2DP if the nateglinide/OGT test shows: - 120 min blood glucose levels > 11.1 mmol/l or - > 7.8 mmol/l < 11.1 mmol/l and maximum c-peptide < 4 ng/mL or stimulated c-peptide is smaller than twofold. We will increase the number of investigated patients in order to confirm these cut-off levels.

577

Stimulation of insulin secretion and enhancement of insulin action, *in vivo*, by a small molecule glucokinase activator
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Glucokinase (GK) is a critical element of glucose metabolism in both the β-cell and hepatocyte, making it an attractive type 2 diabetes drug target. In the β-cell, GK functions as the glucose sensor, determining the threshold for insulin secretion. In the hepatocyte, GK facilitates hepatic glucose uptake during hyperglycemia. The aim of this study was to assess the effects of a GK activator (Cpd C) on *in vivo* insulin secretion and insulin action. Cpd C activates recombinant human GK with an EC_{50} of 50 nM in the presence of 10 mM glucose. Studies were conducted in overnight fasted 10 week old male Sprague-Dawley rats that had underwent arterial and venous catheterization, for sampling and infusion, respectively, 1 week prior to study. In the first set of studies, either vehicle (0.5% methylcellulose, V, n=7) or Cpd C (10 mg/kg, n=5) was dosed orally, followed by an immediate maintenance of euglycemia by an exogenous glucose infusion. Cpd C increased plasma insulin levels (1.8±0.3 vs. 0.8±0.2 ng/mL, p<0.05). As a result, Cpd C also increased the glucose infusion rate (GIR) required to clamp glucose (36±2 vs. 3±1 mg/kg/min, p<0.05). To isolate (insulin-independent) hepatic effects of Cpd C, a second group of rats were dosed orally with either V (n=8) or Cpd C (10 mg/kg, n=8), and then immediately underwent a pancreatic (SRIF+basal glucagon) hyperinsulinemic (2 mU/kg/min) hyperglycemic (190 mg/dL) clamp. Glucagon was clamped at basal levels, and both insulin and glucose were increased similarly (to 1.7 ng/mL and 190 mg/dL, respectively) in both groups. Cpd C significantly increased GIR requirements (30±2 vs. 20±3 mg/kg/min), insulin's stimulation of glucose utilization (29±1 vs. 24±2 mg/kg/min) and suppression of glucose production (-2±4 vs. 4±2 mg/kg/min), and increased liver glycogen (127±13 vs. 71±9 mg/g liver). Thus we have shown that Cpd C stimulates insulin secretion, and independently, also increases hepatic glucose uptake and glycogen synthesis *in vivo*. These data suggest that GK activators hold great potential as novel type 2 diabetes therapeutic agents.

578

Decreased insulin hepatic extraction in women with polycystic ovary syndrome and study of influence of *TCF7L2* gene variant
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Background and aims: The liver is the principal site of insulin degradation. The evaluation of its ability to extract insulin is important to understand pathological states such as impaired glucose tolerance, obesity and diabetes. Polycystic ovary syndrome (PCOS) is considered as a risk factor for diabetes type 2, it is often associated with obesity and beta cell dysfunction as well as insulin resistance. Insulin secretion and hepatic extraction can be calculated from OGTT derived data. Common variants of *TCF7L2* gene are associated with overexpression of this transcription factor and increased insulin gene expression in pancreatic islets, but surprisingly with lower insulin secretion. The aim of study was to assess using functional tests' derived indices the insulin secretion, sensitivity and hepatic extraction and to evaluate the possible influence of *TCF7L2* gene variants in PCOS patients and controls.
Materials and methods: The study involved 109 PCOS patients diagnosed according to ESHRE criteria and 192 control women with different glucose tolerance and body mass index. OGTT was performed in all subjects, in subgroup of 50 PCOS and 23 control women arginin secretion test and euglycemic hyperinsulinemic clamp were performed. The SNPs rs12255372, rs7901695, rs7903146 in the *TCF7L2* gene were assessed by ABI TaqMan SNP Genotyping Assays. Statistics (Kruskal-Wallis one-way ANOVA) was done using NCSS 2004 programme.
Results: PCOS patients vs. controls had higher body mass index (p=0.00), insulin resistance (HOMAR p=0.0004; 1/Matsuda index p=0.00) and insulin secretion (HOMAF p=0.0001; insulinogenic index p=0.00). Insulin hepatic extraction was significantly lower in PCOS patients vs. controls (p=0.00). Independently on study groups, insulin hepatic extraction was higher in lean women (BMI < 25 kg/m^2) compared to overweight women (BMI ≥ 25 kg/m^2). With respect to *TCF7L2* gene variants, PCOS and control women differed only in rs12255372 (G/T) genotypic frequencies (Chi square test, p=0.04). PCOS carriers of the risk T-allele had lower insulin secretion (insulinogenic index p=0.03) and increased insulin hepatic extraction (p=0.02), mainly in lean women. In lean T-allele carriers, acute insulin response (AIR) to arginine during arginine test tended to lower fasting values (AIRf: carriers vs. non-carriers, medians 155 vs. 190 mIU/l, p=0.1) and glucose-potentiated values (AIR_{14}: 768 vs. 965 mIU/l, p=0.2). Euglycemic hyperinsulinemic clamp as well as OGTT derived indices did not detect any difference in insulin sensitivity with respect to genotype.
Conclusion: The study revealed the association of rs12255372 of the *TCF7L2* gene not only with insulin secretion, but also with insulin hepatic extraction in PCOS patients.
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579

Common variant on 1q24.2 (187cM) affects insulin secretion of beta cells and lipid spectrum in French-Canadian and Czech populations
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Background and aims: In order to search for sequence variants conferring risk of type 2 diabetes mellitus (T2DM) we previously conducted a genome-wide linkage study in 108 French-Canadian families from the Saguenay-Lac-St-Jean (SLSJ) region of Quebec (Canada) as we reported on the 43rd EASD Meeting. One of the most significant statistical results was a linkage of insulin resistance index HOMA-IR with locus on chr. 1 (187cM), LOD=2.97. Subsequent FBAT analysis revealed the association of this locus with LDL-cholesterol levels in these families. Our aim was to validate these results by replication in the case/control association study in Czech population.
Materials and methods: The study involved 1465 Czech Caucasian subjects: 347 patients with T2DM (M/F 116/231; age 61.54±7.58/58.37±9.32 years; BMI 29.67±4.63/31.77±5.64 kg/m^2); 327 women with PCOS defined according to ESHRE consensus (age 27.49±6.33 years; BMI 26.96±6.62 kg/m^2); 263 gestational diabetics (age 32.79±4.91 years; BMI 23.82±4.16 kg/m^2); 149 offspring of T2DM (M/F 50/99; age 38.80±10.22/37.24±12.78 years; BMI 26.61±4.52/25.17±4.38 kg/m^2) and 379 controls (M/F 125/254; age 29.39±7.79/29.85±10.77 years; BMI 24.10±2.96/23.27±4.38 kg/m^2) were collected. For better assessment of insulin secretion and sensitivity, the oral glucose tolerance test (oGTT) and insulin tolerance test (ITT) were performed (except of T2DM patients). Moreover, 16 anthropometric parameters, 29 biochemical parameters and hormones, 32 oGTT-derived indices were evaluated. The rs4656671 (G>A) was assessed by TaqMan SNP Genotyping Assay. Statistics was done using nonparametric Mann-Whitney test and χ^2-test (NCSS 2004 software).
Results: The allelic and genotypic distribution was not significantly different among groups and was independent on sex (χ^2-test). The A allele carriers and non-carriers did not differ in age and BMI within these groups. Only results with the highest statistical significances for associations of the A allele (AA + AG vs. GG) with screened parameters in control and offspring of T2DM groups are presented. In spite of the similar oGTT stimulated glucose levels, the carriers of the A allele had significantly lower insulin and C-peptide secretion (Areas Under Curves (AUCs) are presented) and lower levels of total-

and LDL-cholesterol in comparison with non-carriers. Results from T2DM, PCOS and gestational diabetics groups were not significant.
$AUC_{oGTT\ 0\text{-}180min.}$IRI *[mIU/l*180 min.]* Controls: 5377±3567 vs. 5998±3560, *p=0.025*
Offspring of T2DM: 5429±3361 vs. 6495±4233, *p=0.0073*
$AUC_{oGTT\ 0\text{-}180min.}$C-peptide *[nmol/l*180min.]* Controls: 358±120 vs. 384±120, *p=0.024*
Offspring of T2DM: 370±129 vs. 397±132, *p=0.0128*
Total Cholesterol *[mmol/l]* Controls: 4.27±0.79 vs. 4.59±0.90, *p=0.0006*
Offspring of T2DM: 4.42±0.87 vs. 4.62±0.93, *p=0.028*
LDL-Cholesterol *[mmol/l]* Controls: 2.30±0.71 vs. 2.58±0.83, *p=0.001*
Offspring of T2DM: 2.46±0.80 vs. 2.62±0.86, *p=0.033*
Conclusion: In this study on a sample set from the general Czech population we show that SNP rs4656671 that was previously found by linkage study in French-Canadian families, is associated with lower insulin secretion of beta cells and is accompanied with change in lipid spectrum.
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580

C-Peptide correction method to determine exogenous insulin levels in pharmacokinetic studies using Technosphere insulin
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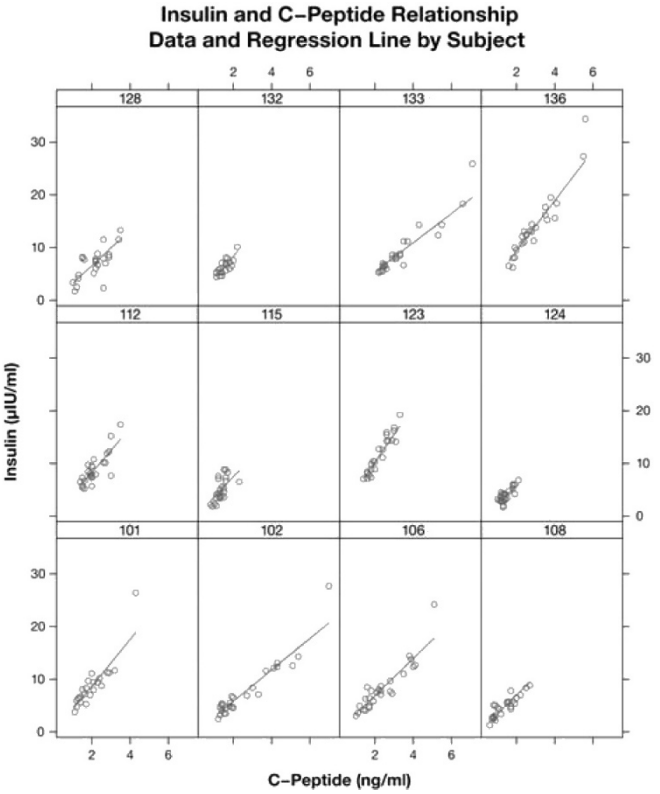
Background and aims: Determining the concentration time profile of exogenous-administered insulin in pharmacokinetic studies usually requires the use of patients with poor or absent β-cell function (type 1 diabetes mellitus), labeled insulin, or insulin clamp studies. These methodologies are difficult and limit the ability to explore the kinetics of novel insulins in broad populations. Although baseline C-peptide corrections have been used, they are not robust or precise.
Materials and methods: We report here the use of repeated intraindividual correlations between insulin and C-peptide to assess exogenous insulin contribution in individuals with intact β-cell function analyzed in several clinical trials (69 subjects from 3 trials). C-peptide and insulin are secreted in a 1:1 molar ratio by the pancreas, but while insulin is removed by the liver, C-peptide is not. The clearance, and ultimate ratio, differs among individuals. Data for the analysis were obtained when exogenous insulin was cleared from the plasma (ie, prior to dosing and 6 h or more after dosing with TI). The relationship was analyzed with a linear mixed effect model where the fixed effect was the population mean for intercept and slope and the random effect was the individual deviation from those means.
Results: As expected, a general linear relationship was found to exist between C-peptide and insulin, but the slope varied by individual.
$Insulin_{i,j} = Intercept_i + Slope_i$ X $C\text{-}Peptide_{i,j}$
C-peptide concentrations allow the estimation of an individual's endogenous insulin concentration from the equation above. Exogenous insulin is then calculated by subtracting the endogenous insulin from the total insulin measured as per the equation below.
$TI\ Insulin_{i,j} = Measured\ Insulin_{i,j} - Insulin_{i,j}$
An example of the C-peptide insulin relationship is displayed below.
Conclusion: We suggest that this novel method may be used to replace determination of individuals' C-peptide elimination experimentally.



PS 38 Incretin secretion - studies in animals

581

Different insulinotropic effecs of GLP-1 and GIP before and during IVGTT with and without systemic DPP4 inhibition in Wistar rats

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Background and aims: Data on insulinotropic potency of GLP-1 and GIP are controversial and systematic investigations are missing. We had therefore performed glucose and insulin measurements in catheter-wearing rats receiving placebo (P), 1, 2, 4 and 8 nmol/kg GIP and 4 and 8 nmol/kg b.w. GLP-1 5 min before an IVGTT. The GLP-1 dose of 4.0 nmol/kg b.w. produced an insulinotropic effect comparable to that of 2.0 nmol/kg b.w. GIP - I-AUC0-25min: 179± 76* vs. 164±63* ng•min•ml-1, insulinogenic Index (iI): 1.11±0.50* vs. 0.97±0.37* ng/mmol. The glucose efflux was less effective after GLP-1 administration - KG: 14.0±1.6 vs. 21.8±5.5* %/min; *p<0.05 vs. placebo). Since the generated data in the tests could be influenced by a) early insulin liberation and b) action of systemic DPP4 activity immediately after incretin injection and before IVGTT, the effects of both these components should be investigated more in detail.
Materials and methods: a) Catheterized Wistar rats were injected with P, 4.0 nmol/kg GLP-1 (GLP-1,(7-36) amide; Neo MPS; Strasbourg, France) and 2.0 nmol/kg GIP (GIP, Probiodrug AG, Halle/Saale, Germany). Incretins were solved in 0.1 % BSA in saline and were injected at 0 min. Arterial blood for blood glucose (BG) and plasma insulin (I) were taken (-5, 0 min and 1, 2, 3, 5, 7, 10, 15 and 20 min). Reactive and absolute G- and I-AUC from 0-20min was calculated.
b) The DPP4 inhibitor P32/98 was injected at -20 min (dose: 30 µmol/kg), the respective incretin at -5 min and the IVGTT (0.4 g glucose/kg) at ± 0 min. Blood sampling was extended to 60 min and AUC from 0-25min were calculated. To compare treatment groups with the control, the two-tailed t-test with Bonferroni-Holm correction was chosen. Within-group changes were tested by t-test, a *p<0.05 was considered significant.
Results: a) Both GLP-1 and GIP induced an insulin increase under basal blood glucose - GLP-1: reactive I-AUC 10.6±6.0*, absolute I-AUC 16.9±4.1* ng•min•mL-1; GIP: reactive I-AUC 8.8±3.4*, absolute I-AUC 16.5±4.7* ng•min•mL-1. 33.9 % and 34.5 % of insulin increase occurred within 3 min in both the tests. Reactive G-AUC was unchanged after 4 nmol/kg GLP-1 vs. P (10.1±6.2 vs. 4.6±4.1 mmol•min•mL-1) but declined after 2 nmol/kg GIP (-1.7±4.2 mmol•min•mL-1*).
b) DPP4 inhibitor declined always plasma DPP4 activity to 25-20 % during the IVGTT. GIP improved more efficient glucose tolerance (G-AUC: GIP, reactive: 24.6±26.1, absolute: 159.0±31.5*; GLP-1, reacive: 64.7±29.9, absolute: 204.2±33.2; P, reacive: 47.7±12.2, absolute: 183.8±17.1 mmol•min•mL-1) and insulin response (I-AUC: GIP, reacive: 76.4±52.9, absolute: 139.9±40.95*; GLP-1, reactive: 61.5±18.6, absolute: 71.4±25.4; P, reactive: 46.7±19.3, absolute: 54.2±22.3 ng•min•mL-1).
Conclusion: Both, 4 nmol/kg GLP-1 and 2 nmol/kg GIP induce an early insulin increase under basal conditions in healthy Wistar rats. Only the incretin GIP exerts a blood glucose decline.
The plasma DPP4 activity on GLP-1 and GIP for a short time may not explain the different insulinotropic potency of GLP-1 and GIP in the IVGTT in rats and especially the great difference in glucose efflux.
We thank Prosidion Ltd. and Probiodrug AG for donation of DPP4 inhibitor and GIP compound

582

Chronic treatment with a glucagon receptor antagonist reduces blood glucose and elevates circulating GLP-1 in diet-induced obese mice

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Glucagon plays a key role in maintaining glucose homeostasis and hyperglucagonemia has been implicated in the pathophysiology of type 2 diabetes. Antagonism of glucagon receptor (GCGR) signaling therefore represents a potential approach for treating diabetes. Cpd 15 is a small molecule GCGR antagonist (GRA) that blocks glucagon binding to the hGCGR with an IC_{50} of 34 nM and antagonizes glucagon-induced cAMP accumulation in CHO. hGCGR cells with IC_{50} of 92 nM. The compound blocks glucagon-induced glucose excursion in hGCGR mice whose endogenous murine GCGR was replaced with the human receptor. Cpd 15 also blocks glucagon induced glycogen breakdown in a real-time NMR based assay to monitor glycogenolysis in perfused liver from hGCGR mice. In a chronic study of 82 days in duration, Cpd 15 dosed daily as admixture to chow resulted in effective and sustained glucose lowering in high fat diet induced obese (DIO) hGCGR mice. Treatment with Cpd 15 also resulted in stable and moderate elevation of both glucagon and GLP-1 that was completely reversible upon washout of the compound. Importantly, there was no hyperglycemic overshoot upon termination of the treatment. Immuohistochemical analysis of pancreatic tissue showed that while GCGR KO mice demonstrated severe α-cell hyperplasia, treatment with Cpd 15 normalized the densities of both insulin positive β-cells and glucagon-positive α-cells in the pancreas of DIO hGCGR mice to the levels observed in lean hGCGR mice without apparent α-cell hyperplasia. Finally, whereas the pancreatic tissue of GCGR knockout mice had 16-fold higher preproglucagon mRNA levels compared to wild type mice, treatment with Cpd 15 caused a 2-fold increase in pancreatic preproglucagon mRNA. Thus, GRA treatment can effectively improve glucose homeostasis and increase total GLP-1 levels without eliciting severe hyperglucagonemia. These results suggest the possibility that combination of a GRA with a DPP-4 inhibitor will lead to improved efficacy in glucose lowering by not only blocking the hepatic action of glucagon but also by providing significantly higher levels of active GLP-1 than could be achieved with either therapy alone.

583

Regulation of proliferation and hormone production of GLP-1 and glucagon-secreting cells

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Background and aims: Glucagon-like peptide-1 (GLP-1) is an incretin hormone, secreted from gut L-cells upon nutrient intake, and forms the basis for novel drugs against type 2 diabetes. Secretion of GLP-1 is impaired in type 2 diabetes. Glucagon, secreted from pancreatic α-cells, acts as the counterpart of insulin. Abnormalities in α-cell function contribute to hyperglycemia and insulin resistance in diabetes. Little is known about the influence of antidiabetic drugs on α- and L-cell function and regeneration. The aim of this study was to investigate the long term impact of anti-diabetic agents on the proliferation and hormone production of GLP-1- and glucagon-secreting cells and mechanisms underlying such effects.
Materials and methods: The GLP-1-secreting cell line GLUTag and glucagon-secreting αTC1-9 cells were cultured in DMEM media. Cell proliferation was evaluated by ^{3}H-thymidine incorporation and MTT assay after a 48 h-incubation with the anti-diabetic agents. GLP-1 and glucagon were measured by specific ELISAs.
Results: In the GLP-1-secreting GLUTag cells, insulin and the stable GLP-1 analogue Exendin-4 stimulated proliferation at a basal (3 mM), but not high (11 mM) concentration of glucose. A similar effect was also observed by applying ATP. Unexpectedly, the proliferation of the cells was suppressed by the cAMP agonist Sp-cAMP. The DPP-IV inhibitor sitagliptin selectively promoted proliferation of GLUTag cells at high glucose, but not at basal concentration of the sugar. In contrast, proliferation of the glucagon-secreting αTC1-9 cells was not affected by the agents under the same conditions. Metformin significantly inhibited proliferation of both GLUTag and αTC1-9 cells, while pioglitazone inhibited proliferation of GLUTag, but not αTC1-9 cells.
Conclusion: The present study shows that insulin, Exendin-4 and sitagliptin stimulate proliferation of GLP-1-secreting cells without effects on glucagon-secreting cells. Metformin suppresses proliferation of both GLP-1- and glucagon-secreting cells. Since serum GLP-1 levels are decreased in type 2 diabetic patients, the mitogenic stimulation of GLP-1-producing cells by insulin, Exendin-4 and sitagliptin noted here suggests a novel beneficial long term effect of these antidiabetic drugs in clinical practice, *i.e.* by augmenting the incretin effect, and understanding the mechanism of this stimulation would be of great importance for the treatment of diabetes. Likewise, the inhibitory influence of metformin on the proliferation of glucagon-secreting cells may benefit diabetic patients treated with this drug, as relative hyperglucagonemia contributes to impaired glycemic control in type 2 diabetes.
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584

GPR119 activation regulates glucose homeostasis through mechanisms requiring incretin receptor activation

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Background and aims: The mechanisms through which nutrients regulate peptide hormone secretion from enteroendocrine cells remains poorly understood. Oleoylethanolamide (OEA) activates incretin secretion via the GPR119 G protein coupled receptor originally detected on islet beta-cells. Although administration of OEA and synthetic GPR119 agonists (GPR119A) reduces glycemic excursion in preclinical studies in association with enhanced incretin and insulin secretion, the importance of GLP-1 and GIP for the glucoregulatory actions transduced through GPR119 remain unclear. We have now examined the importance of GIP and GLP-1 receptor signaling for glucoregulation and satiety mediated by GPR119A through studies of the mechanisms of action of GPR119 agonists in WT and double incretin receptor knockout (DIRKO) mice.
Materials and methods: The actions of a selective GPR119 agonist(GPR119A) were assessed in oral and intra-peritoneal glucose tolerance tests in DIRKO and age and sex-matched C57Bl6 mice. Experimental endpoints included plasma glucose, insulin, GLP-1, GIP and glucagon. Gastric emptying rate was assessed by the acetaminophen assay in mice treated acutely with GPR119A in the presence or absence of the DPP-4 inhibitor Sitagliptin. The effect of GPR119 activation on food intake was assessed in WT C57Bl6, Glp1r-/- and littermate control mice. Statistical analysis was determined using ANOVA.
Results: Oral administration of GPR119A improved glucose homeostasis and augmented plasma levels of both GLP-1 and insulin during an OGTT in WT mice. In contrast, although enteral glucose stimulated GLP-1 and insulin secretion in DIRKO mice, concomitant treatment with GPR119A failed to lower glucose in DIRKO mice. Unexpectedly, oral administration of GPR119A failed to control glucose homeostasis in WT mice during an IPGTT despite an increase in circulating insulin and GLP-1. Acute GPR119 activation significantly reduced the rate of gastric emptying when co-administered with the DPP-4 inhibitor sitagliptin and this effect was independent of GLP-1 action as it remained detectable in Glp1r-/- mice. Moreover, GPR119A significantly but transiently reduced food intake in WT C57Bl6 mice however GPR119A failed to reduce food intake in mice on a metformin-sitagliptin diet.
Conclusion: Our findings suggest that oral administration of nutrients (glucose), together with intact incretin receptor signaling, is required for transduction of the glucoregulatory actions of GPR119A. Hence, under the experimental conditions utilized, direct GPR119A activation of the islet beta-cell is not sufficient to fully control glucose homeostasis in the absence of functional incretin receptors in mice.
Supported by: CIHR

585

Ileal interposition surgery increases circulating GLP-1 and PYY responses and delays diabetes onset in a novel rat model of type 2 diabetes mellitus, the UCD-T2DM rat

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Background and aims: Bariatric surgery often results in resolution of type 2 diabetes mellitus (T2DM). While the mechanisms for diabetes resolution remain undefined, recent studies suggest that endocrine mechanisms may have an important role. Bypass of the proximal intestine increases secretion of glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), gut hormones that influence energy and glucose homeostasis, in part by increasing the delivery of less completely digested nutrients to the distal intestine. Ileal interposition (IT) surgery involves transposing a 10 cm segment of ileum into the proximal intestine and provides a model whereby the effects of bariatric surgery on gut hormones can be isolated. We hypothesized that IT surgery would delay the onset T2DM in UCD-T2DM rats, a model of polygenetic obese T2DM more similar to T2DM in humans than other existing rodent models, in part by increasing nutrient-stimulated GLP-1 and PYY release.
Materials and methods: IT (n=18) or sham surgery (n=19) was performed on 4 month old UCD-T2DM rats weighing ~580 g. All animals underwent an oral glucose tolerance test (OGTT) 1 month after surgery. 10 animals from each group were euthanized 2 months after surgery for tissue analyses. The rest were followed until onset and underwent insulin tolerance (ITT) and oral fat tolerance tests (OFTT) at 2.5 and 3.5 months after surgery, respectively. Diabetes onset was determined by weekly non-fasted blood glucose (<200 mg/dl).
Results: At this point in the study, sham animals have higher diabetes incidence (78%) and earlier age of onset (262 ± 12 days) compared with IT (63% and 349 ± 22 days; P<0.01). Body weight and food intake do not differ between groups. During OGTT, IT animals exhibited lower glucose (AUC: Sham = 15141 ± 1317, IT = 10972 ± 790 mg/dl x 120 min; P<0.01) and insulin excursions (AUC: Sham = 142 ± 17, IT = 115 ± 11 ng/ml x 120 min; P<0.01) and greater active GLP-1 secretion (AUC: Sham = 53 ± 14, IT = 211 ± 23 pM x 60 min; P<0.01). Since the position of the duodenum remains unchanged after both surgeries, as expected, GIP excursions were not different between groups (AUC: Sham = 9863 ± 537, IT = 10121 ± 447 pg/ml x 60 min). IT animals also exhibit improved insulin sensitivity. Plasma glucose levels fall by 19 ± 3% in IT animals compared with 8±4% in Sham animals 45 min after insulin administration (P<0.05). Total PYY excursions during OFTT were 4-fold greater in IT animals (AUC: Sham = 4704 ± 1175, IT = 19670 ± 3367 pg/ml x 180 min; P<0.01). While body weight only tended to be lower in IT animals, the retroperitoneal (Sham: 12 ± 1 g, IT: 9 ± 1 g; P<0.05) and epididymal (Sham: 9 ± 1 g, IT: 7 ± 1 g, P<0.05) fat pads weighed significantly less in IT animals. In addition, peak mesenteric adipocyte volume was almost two-fold smaller in IT animals (Sham: 409 ± 31, IT: 268 ± 27 pl; P<0.01), whereas subcutaneous adipocyte size did not differ between groups.
Conclusion: Thus, IT surgery improves glucose tolerance and insulin sensitivity and delays the onset of T2DM in UCD-T2DM rats, which may in part be due to increased nutrient-stimulated release of GLP-1 and PYY, and decreased mesenteric adipocyte size.
Supported by: NIH

PS 39 Hypoglycaemia in type 1 diabetes

586

Do patients with greater glycaemic variability on continuous glucose monitoring have a greater risk of subsequent hypoglycaemia?

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Background and aims: Measures of glycaemic variability derived from 1 month of home capillary glucose testing are reported to predict risk of subsequent hypoglycaemia. It is not known if much shorter (48 hours) exposure to much more detailed glucose information available with continuous glucose monitoring (CGM) will likewise predict risk of hypoglycaemia. . There is also no consensus on which measure of variability provides the best indicator of an individual's subsequent hypoglycaemia risk. Aims: To determine if 3 days of CGM (a single sensor) can identify patients at an increased risk of hypoglycaemia in the following year.
Materials and methods: 75 patients with type 1 diabetes from the UK Hypoglycaemia study [HbA1c 7.5 (0.9%) diabetes duration either <5 years or >15 years] had a session of CGM and prospective documentation of severe and symptomatic hypoglycaemia over the subsequent 9 months From the first complete 48 hours of CGM from each subject, measures of variability (Low Blood Glucose Risk Index (LBGI); High Blood Glucose Risk index (HBGI); Blood glucose Risk Index (BGRI) and Continuous Overall Net Glycaemic action (CONGA)) were calculated. Correlations between different measures of variability, HbA1c and subsequent hypoglycaemia risk were calculated.
Results: Glycaemic variability was greater in the longer duration group and was significantly correlated with HbA1c (CONGA1 r= 0.35; p=0.003). CONGA and BGRI showed strong correlation with each other (r= 0.691; p<0.001) but there was poor agreement between measures of variability in identifying those with the highest degree of variability (kappa 0.3 between BGRI and CONGA), with both measures only agreeing in 9/17 of those in the highest quartile of variability.. Only glycaemic variability measured by CONGA was significantly correlated with risk of severe (r = 0.3; p= 0.016) and symptomatic (r = 0.41; p< 0.001) hypoglycaemia CONGA1 < 30 was associated with minimal risk of severe hypoglycaemia over the following year.
Conclusion: Some measures of variability taken from 48 hours of CGM can identify patients at high risk of severe hypoglycaemia over the next 9 months and may identify patients needing treatment to be focussed towards avoidance of hypoglycaemia and reduction of hypoglycaemia risk.

587

Improvement in hypoglycaemia awareness and amelioration if glycaemic profile using CSII in type 1 diabetic subjects with repeated severe hypoglycaemia

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Intensive insulin therapy significantly reduces the risk of complications in subjects with type 1 Diabetes (T1D) and represents the standard treatment from the onset of the disease. However, it is associated with a higher risk of non-severe (NS) and severe hypoglycaemic episodes (SH).
Aim: To evaluate the long-term effect of CSII on hypoglycaemia awareness and on glucose profile in T1D subjects with repeated NS/SH.
Methods: Subjects with: Age ≥18-years, T1D duration >5 years, on multiple doses of insulin, with an absence of micro/macrovascular complications and >4 NS/week (last 8-weeks) and >2 SH (last 2-years) were included. The number of NS/SH episodes were registered. Hypoglycaemia awareness was also evaluated (Clarke's test). A 72h continuous glucose monitoring (CGM) was performed before starting CSII. Quality of life (QoL) assessment was also performed. After 6, 12 and 18-months, all the subjects were reevaluated.
Results: 29 subjects were included. Twenty started CSII (aged 34.4±7.5 years; 13 women, A_{1c} 6.7±1.1%) and 9 declined. At baseline 19/20 subjects displayed hypoglycaemia unawareness which diminished significantly during the follow-up (3/20). NS episodes/week diminished from 5.40±2.09 at baseline to 3.80±2.23 at the end of the study (p<0.02). SH episodes fell from 1.30±0.47 per-subject-year at baseline to 0.05±0.22 after 18-months (p<0.001). HbA_{1c} remained unaltered. Considering CGM, the percentage of values within 70-180mg/dl significantly increased (53.1±10.9 to 65.9±17.6; p<0.01), the percentage below 70mg/dl significantly decreased (13.7±9.4 to 9.5±5.5; p<0.05) and the area <70mg/dl significantly decreased (2.45±1.90 to 1.36±1.61; p<0.05) after 18-months. In addition, considering SD from CGMS it also decreased at the end of follow-up (63.7±14.5 to 52.3±14.5; p<0.05). An improvement in nearly all the aspects of QoL was observed. In the group of 9 subjects who declined CSII we did not observe any change neither in the number of NS/SH, nor in hypoglycemia awareness.
Conclusion: CSII prevents hypoglycaemic episodes, improves hypoglycaemia awareness and ameliorates glycaemic profile for a lasting period in T1D subjects with repeated NS/SH. Likewise; its use is associated with an improvement in diabetes QoL.
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588

ACTH stimulation test in patients with type 1 diabetes and recurrent severe hypoglycaemia

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Background and aims: Adrenal insufficiency increases insulin sensitivity. This may increase the risk of hypoglycaemia in patients with type 1 diabetes. However, data on the prevalence of adrenal insufficiency are very limited. The primary aim of the study was to determine the prevalence of undiagnosed adrenal insufficiency in patients with type 1 diabetes and recurrent severe hypoglycaemia.
Materials and methods: Cross-sectional data from a clinical trial investigating the effect of human insulin vs. insulin analogues on the frequency of severe hypoglycaemia. Patients with type 1 diabetes for minimum five years and at least two episodes of severe hypoglycaemia (defined as the need of assistance from another person to normalise blood glucose) in the previous year were enrolled in the study. At entry, an ACTH stimulation test (Synacthen®, 0,25 mg i.v.) was performed. Basal and stimulated (after 30 minutes) serum cortisol was measured in all patients.
Results: 128 patients were included (age 53 (13) years (mean (SD)), age at diagnosis of diabetes 23 (13) years, duration of diabetes 30 (13) years, 60% men). Mean (SD, range) basal serum cortisol concentration was 452 nM (120; 119-894). Mean stimulated serum cortisol concentration was 731 nM (125; 515-1134). Delta cortisol was 279 nM (95; 74-614). None of the patients had adrenal insufficiency (defined as stimulated serum cortisol < 500 nM). Stimulated but not basal serum cortisol was significantly higher in women than in men (2 women taking oral contraceptives excluded), p=0.001. Basal and stimulated serum cortisol were neither associated with age (r^2=0.02, p=0.1 and r^2=0.028, p=0.06, respectively) nor with duration of diabetes (r^2=0.002, p=0.59 and r^2=0.0002, p=0.87, respectively). In an analysis adjusted for sex, age and duration of diabetes as explanatory variables basal serum cortisol was significantly positively associated with age (1.97 nM/year, p=0.039), while stimulated serum cortisol was significantly associated with age (1.94 nM/year, p=0.038) and sex (75 nM/female, p=0.001).
Conclusion: Adrenal insufficiency as a contributory factor in development of recurrent severe hypoglycaemia is rare. ACTH stimulation test seems justified only when other signs of adrenal insufficiency are present (e.g. increased insulin sensitivity, common symptoms and signs, etc).
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589

Sustained reduction of biochemical, clinical and severe hypoglycaemia with extended CGM use: results of JDRF CGM six month extension study

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Background and aims: A potential value of continuous glucose monitoring (CGM) is to reduce biochemical hypoglycemia, which is commonly observed in patients with well-controlled type 1 diabetes.
Materials and methods: 83 of 86 subjects ≥25 years old randomized to CGM use in the JDRF CGM randomized clinical trial were followed for 12 months. After 12 months, all but one subject was still using CGM, averaging 6.0±1.6 days/week. HbA1c levels (7.1% at baseline) were reduced to 6.8% at 6 and 6.9% at 12 months (P<0.001 at each time point compared with baseline).
Results: The mean amount of time (minutes) per day in the hypoglycemic range decreased from baseline to 6 months and remained decreased at 12 months (see table below).

	Baseline	6 months	12 months	p-value*
CGM Glucose (min/day)				
≤70 mg/dL	81	69	65	0.06
≤60 mg/dL	44	30	28	0.003
≤50 mg/dL	23	12	11	0.002

*comparing change from baseline to 12 months

A similar trend was seen both in subjects whose baseline HbA1c was ≥7.0% and in those whose baseline HbA1c was <7.0%. A decrease in time in the hypoglycemic range occurred both for daytime and nighttime. The decrease in the frequency of biochemical hypoglycemia was accompanied by a reduction in the rate of severe hypoglycemic events. The rate of severe hypoglycemia was 21.8 events per 100 person-years in the first 6 months and 7.1 events per 100 person-years in the last six months. Indeed, in subjects with baseline HbA1c <7.0 % who had 23.6 events per 100 person-years during the first 6 months, there were no episodes of severe hypoglycemia during the last 6 months even though mean HbA1c levels remained unchanged at 6.4%.
Conclusions: CGM provides an effective means to achieve and maintain target HbA1c levels for up to 12 months in association with a substantial reduction in the frequency of biochemical hypoglycemia in intensively treated adults with type 1 diabetes. More experience with the devices and reduced exposure to biochemical hypoglycemia with associated improvements in counter-regulatory response may have contributed, in turn, to the extraordinarily low rates of severe hypoglycemic events that were observed during the second 6 months of sensor use.
Supported by: JDRF

590

Insulin-like growth factor-1 as a risk marker for severe hypoglycaemia in type 1 diabetes

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Background and aims: Low levels of circulating insulin-like growth factor-1 (IGF-1) are associated with increased risk of severe hypoglycaemia (SH) in pregnant women with type 1 diabetes, especially in early pregnancy. We studied whether a similar relationship between IGF-1 and SH exists in non-pregnant people with type 1 diabetes.
Materials and methods: A total of 267 patients with type 1 diabetes were recruited from our outpatient clinic. Information about demography and diabetes was collected at baseline along with blood samples. The participants were followed for one year during usual diabetes treatment. Participants were instructed to phone a trained diabetes nurse within 24h in case of severe hypoglycaemia. The nurse performed a detailed interview with the participant to validate if the episode was actually caused by severe hypoglycaemia. Also, participants were interviewed about mild hypoglycaemia and hypoglycaemia awareness. Biochemical hypoglycaemia (blood glucose < 3.0 mmol/l) was assessed by monthly self-monitored 5-point blood glucose profiles.
Results: During the study 257 episodes of severe hypoglycaemia (1.0 episode per patient-year) were reported by 89 patients (33%). Serum IGF-1 was 112 (36-479) ng/ml (median (range)). IGF-1 was not associated with occurrence of severe hypoglycaemia in the entire cohort (r^2=0.002, p=0.52) or in any of the two sexes when analysed separately. Median serum IGF-1 levels were 112, 101, and 119 ng/ml in patients without SH (n=177), with 1 SH (n=43), and with ≥2 SH (n=47), respectively. Levels of IGF-1 were positively, but weakly, associated with frequency of biochemical hypoglycaemia (p=0.045), but not when corrected for HbA1c level. IGF-1 levels were neither associated with frequency of mild hypoglycaemia (p=0.36) nor hypoglycaemia awareness (p=0.37).
Conclusion: Although low levels of IGF-1 are associated with increased risk of SH in pregnant women with type 1 diabetes, IGF-1 levels are not associated with a hypoglycaemic phenotype in non-pregnant people with type 1 diabetes.
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591

Hypoglycaemia unawareness in patients with diabetes - could galvanic skin response (GSR) measurements provide the answer?

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Background and aims: Hypoglycaemia Unawareness (HU), which occurs in approximately 25% of patients with type 1 Diabetes (T1DM), is the main limiting factor in obtaining tight blood sugar control and therefore, preventing or delaying onset of complications related to diabetes in these patients. Reduced autonomic nervous system (ANS) responses, presumed to be secondary to recurrent exposure to cortisol and / Corticotrophin releasing hormone are two of the several theories implicated in the pathogenesis of HU. Measurements of GSR can be used to represent the ANS function, especially the peripheral sympathetic system. The aim of this study was to measure differences in GSR and therefore, the differences in ANS responses to recurrent hypoglycaemia, in healthy subjects and those with disorders of the hypothalamic-pituitary-adrenal (HPA) axis.
Materials and methods: We used a 2 day model of recurrent hypoglycaemia using the hyperinsulinaemic hypoglycaemic clamp technique in two groups of subjects. Group 1 included 11 healthy subjects (age - 41.77 ± 13.72 years, BMI - 24.08 ± 2.86). Group 2 included 7 patients with abnormalities of the HPA axis (age - 46.77 ± 10.87, BMI 26.18 ±2.27). Group 2 included 1 patient with secondary adrenal failure and 6 patients with primary adrenal failure. All patients were on appropriate replacement therapy and took double their normal hydrocortisone dose on both the days of the study. All subjects provided written consent for the study, which was approved by the regional and local research ethics committee. GSR was measured using GSR100C module of the MP150 BIOPAC system. Two Ag-AgCl skin conductance electrodes (TSD203) were attached to the pulps of second and third fingers and GSR recorded using a portable system. Biopac isotonic gel (GEL 101) was used to ensure good contact and the subjects wore the electrodes for minimum of 5 minutes before registration was commenced. The data thus sampled was stored and analyzed digitally using AcqKnowledge software. All subjects underwent clamp studies lasting for 4 hours; this included 30 minutes of euglycaemia (Blood sugar ≈ 4.5 mmol/l), 120 minutes of hypoglycaemia (Blood sugar ≈ 2.5 mmol/l) and the final 60 minutes of euglycaemia. Both the initial shift from euglycaemia to hypoglycaemia and the recovery phase from hypoglycaemia to euglycaemia lasted 15 minutes.
GSR was recorded on 3 occasions during each study - 1) at baseline prior to starting the study 2) during the phase of hypoglycaemia and 3) during the second phase of euglycaemia. Peak GSR responses refers to the maximum GSR responses recorded for any subject during each day of the study.
Results: The peak GSR responses between day 1 and day 2 were significantly different in healthy subjects (p = 0.04) compared to patients (p = 0.18) with the responses being higher on day 2 than day 1 (10.58 ± 5.19 μmho vs 8.59 ± 4.94 μmho). In the patients, the peak GSR on day 1 was 6.33 ± 2.42 μmho and on day 2 was 5.11 ± 2.81 μmho.
Conclusion: The reduced GSR responses to hypoglycaemia in patients with disorders of the HPA axis following exposure to recurrent hypoglycaemia, suggest that their altered HPA axis may be responsible for their reduced ANS responses. This supports the theory that HPA axis dysregulation could account for HU in those with recurrent hypoglycaemia. GSR measurements could therefore be potentially used to study altered ANS responses in patients with diabetes.
Supported by: JDRF

PS 40 Hypoglycaemia

592

Severity of self-reported hypoglycaemia and quality of life in patients with type 2 diabetes mellitus treated with oral anti-hyperglycaemic agents

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Background and aims: Oral anti-hyperglycemic agents (OAHAs), especially sulfonylureas (SU), may increase risk of hypoglycemia and thereby reduce patient quality of life. Our objective was to assess the impact of self-reported hypoglycemia on health-related quality of life (HRQoL) among patients with type 2 diabetes mellitus (T2DM) treated with OAHAs.
Materials and methods: A follow-up survey was conducted among 2008 participants with self-reported T2DM treated with OAHAs only from the US National Health and Wellness Survey 2007.Data were collected on the severity and frequency of hypoglycemic episodes with severity defined as mild (no interruption of activities), moderate (some interruption of activities), severe (needed assistance of others), or very severe (needed medical attention). HRQoL was assessed using the EQ-5D US weighted summary score (utility) and Worry subscale of the Hypoglycemia Fear Survey (HFS).
Results: Mean age was 58 (± 11) years, 57% were male, and 72.2% reported HbA_{1C} goal attainment (<7%). The proportions of patients on SU monotherapy, SU combination therapy, and other treatment regimens were 10.7%, 39.7%, and 49.6%, respectively. Hypoglycemic episodes, in the 6 months prior to the survey, were reported by 61.6% of patients (45.6% mild, 37.4% moderate, 13.2% severe and 3.8% very severe). For patients reporting hypoglycemia, mean utility score was 0.08 lower (0.78 versus 0.86, p<.0001), and mean HFS score was 11.3 higher (17.5 versus 6.2, p<.0001), compared to patients with no symptoms. Differences in mean scores between those with and without hypoglycemia increased with severity for utility (0.03, 0.09, 0.18, 0.23) and HFS (6.1, 13.9, 20.1, 25.6). After adjusting for age, gender, weight gain, microvascular and macrovascular complications, the utility decrement was 0.045 (by severity: 0.008, 0.055, 0.131, 0.209), and the HFS increase was 9.6 (by severity: 5.3, 12.4, 17.6, 23.2). HRQoL further decreased with higher frequency of hypoglycemic episodes.
Conclusion: Self-reported hypoglycemia is independently associated with lower HRQoL, and the magnitude of this reduction increases with both severity and frequency of episodes.
Supported by: Merck & Co., Inc.

593

^{13}C magnetic resonance spectroscopy of cerebral glucose metabolism during acute mild hypoglycaemia in humans

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Background and aims: Normal brain function requires constant supply of glucose and may thus be jeopardized by insulin-induced hypoglycemia. Hypoglycemia causes profound effects on brain physiology, however its effect on metabolism of glucose in the brain is largely unknown. The aim of this study was to investigate the effect of acute hypoglycemia on cerebral glucose metabolism in humans in vivo using 13C magnetic resonance spectroscopy (MRS).
Materials and methods: Eight healthy volunteers underwent ^{13}C MRS measurements during 120-minute hyperinsulinemic (60 mU/min/m^2) euglycemic and hypoglycemic glucose clamps, performed in random order on separate days. [1-^{13}C]glucose was infused during the clamps to increase plasma ^{13}C isotopic enrichment, required to enable ^{13}C MRS. Measurements were performed at a magnetic field strength of 3T in a 125 ml voxel in occipital brain tissue, with a TR of 2s and a duration of 2.5 minutes per scan. Arterial blood was sampled every 5 minutes for the measurement of plasma glucose and plasma ^{13}C enrichment, and every 30 minutes for the measurement of counterregulatory hormone levels. MR spectra were analyzed to assess cerebral concentrations of the glucose metabolite isotopomers glutamate, glutamine, aspartate and lactate. All metabolite concentrations were corrected for plasma ^{13}C enrichment.
Results: Plasma glucose levels were 5.2±0.1 mmol/l during euglycemia and 3.0±0.2 mmol/l during hypoglycemia. Arterial plasma levels of glucagon (77.0±41.9 vs. 27.6±5.1 pmol/L), adrenaline (4.84±2.51 vs. 0.41±0.12 nmol/L), noradrenaline (1.96±0.64 vs. 1.14±0.31 nmol/L) and cortisol (0.89±0.21 vs. 0.42±0.13 µmol/L) were all significantly higher under hypoglycemic than under euglycemic conditions (all P<0.01). Plasma ^{13}C isotopic enrichment remained stable at 35±1% during euglycemia and at 30±1% during hypoglycemia. The quality of the ^{13}C-MR spectra was good under both conditions. After correction for plasma isotopic enrichment, label incorporation into the first glucose-derived metabolites Glutamate C4 and Glutamine C4 did not differ between euglycemia and hypoglycemia (figure). There were also no differences for glutamate C_3 and C_2, glutamine C_3 and C_2, aspartate and lactate.
Conclusion: Despite significant enhancement of counterregulatory hormone responses, overall cerebral glucose metabolism appears unaltered during insulin-induced hypoglycemia in vivo in healthy humans. These data indicate that the human brain is able to maintain normal overall glucose metabolism despite a considerable fall in glucose delivery.

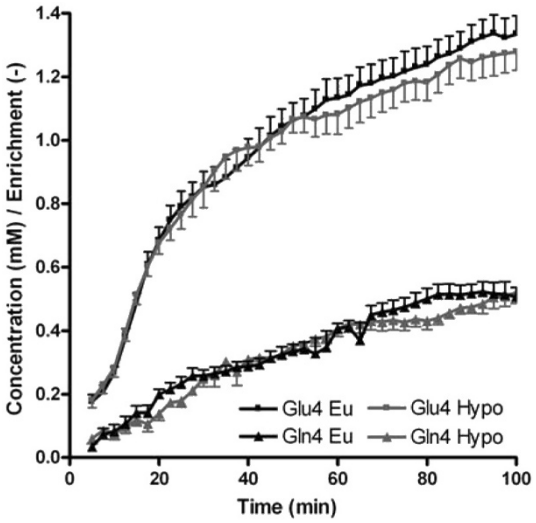

Figure: Incorporation of the 13C label inglutamate C4 (diamonds) and glutamine C4 (triangle) for a euglycemic (black) and hypoglycemic (grey) clamp, corrected for 13C enrichment of plasma glucose. Mean ±SEM.

Supported by: Dutch Diabetes Research Foundation and NIH

594

Somatostatin type 2 receptor antagonism improves glucagon and corticosterone counterregulation to hypoglycaemia in STZ-diabetic rats

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Background and aims: The pancreatic α-cell glucagon response to a variety of stimuli including exercise, arginine, and neurogenic stress is normal, or even excessive, in diabetic subjects. However, we suggest that the markedly diminished glucagon responsiveness to *hypoglycemia* is mainly due to excessive insulin delivery since insulin suppresses glucagon release. The lack of glucagon response may be due to a lack of decrement of intraislet insulin, increased α-cell sensitivity to exogenous insulin, or chronic hyperglycemia. The impaired α-cell response to hypoglycemia may also be due, in part, to augmented circulating and especially pancreatic somatostatin (SST) in diabetic humans and animals. Presently, we hypothesize that since glucagon release is inhibited in part due to the inhibitory effect of excessive SST, a SST receptor type 2 antagonist (SSTR2a), BIM23458, will normalize the glucagon response to hypoglycemia. SST inhibits glucagon secretion via SSTR2 on α-cells.
Materials and methods: Non-diabetic (N) and STZ-diabetic (D) Sprague-Dawley rats underwent 3h of insulin-induced hypoglycemia (10 U/kg, sc) clamped at 2.5±0.5 mM.
Results: Expectedly, D rats had a greatly attenuated glucagon response to hypoglycemia (P<0.007). To evaluate the inhibition of SST suppression on glucagon release during hypoglycemia, diabetic rats were given iv SSTR2a (D+SSTR2a) at both 1.5 or 3.0 mmol/kg/h for 1h before and 3h after insulin

injection. These groups showed a dose-dependent increase of glucagon such that the latter dose fully restored the glucagon response (N: 325±63, n=9; D: 98±19, n=9; D+SSTR2a: 384±67, n=18; AUC responses, $P<0.002$). Furthermore, the attenuated corticosterone response in D rats ($P<0.002$) was also enhanced by SSTR2a (N: 59±5; D: 30±3; D+SSTR2a: 46±9; AUC responses, $P<0.05$), presumably because SST inhibits the secretions of hormones of the hypothalamo-pituitary-adrenal axis, CRH and ACTH. Thus, antagonism of SSTR2 potentiates not only glucagon but also corticosterone counterregulation. To evaluate the effect of 4h SSTR2a infusion *per se*, plasma glucagon, corticosterone, and glycemia were measured in N, D, and D+SSTR2a (n=6/group) without hypoglycemia. Glucagon, corticosterone, and glycemia were unaltered in these groups. It is known that SSTR2 in the α-cell is coupled to the *L*-type high threshold calcium channel, which is responsible for stimulated secretion of glucagon, but not the *N*-type calcium channel, which mediates basal glucagon secretion, and we presently demonstrate that *antagonism of SSTR2 enhances hypoglycemia-stimulated, but not basal, glucagon or corticosterone release in diabetic rats and that SSTR2a does not affect glycemia in the absence of hypoglycemia*. D exhibit 60-70% more plasma and pancreatic SST than N, regardless of hypoglycemia. SSTR2a did not significantly affect plasma or pancreatic content of SST.

Conclusion: This data supports our hypothesis that *increased SST may contribute to impaired glucagon and corticosterone responsiveness to hypoglycemia and that its selective inhibition normalizes glucagon, and improves corticosterone, counterregulation*. Intervention to improve counterregulation will decrease the threat in those at risk of hypoglycemia.

Supported by: Canadian Diabetes Association and NSERC grants (MV); CIHR Doctoral Research Award (JTYY)

595

The cingulate cortex is activated by acute but not recurrent hypoglycaemia in rats - evidence for a role of limbic system in impaired responses to hypoglycaemia?

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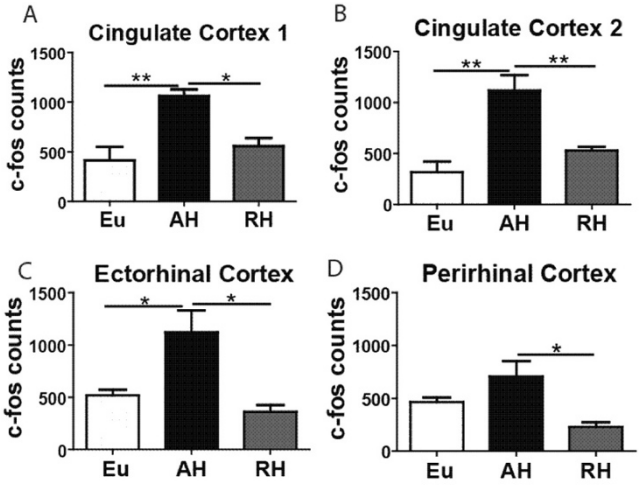
Background and aims: Some patients, particularly those with exposure to recurrent hypoglycaemia (RH) develop a debilitating loss of counterregulatory (CR) neurohumoral defences to low glucose associated with symptomatic unawareness of hypoglycaemia (HU). Although hypoglycaemia sensing probably occurs predominantly in hypothalamus, it is unclear whether impaired CR/ HU is a consequence simply of altered hypothalamic glucose sensing or whether other brain areas, for example the limbic system contribute. We hypothesized that the cingulate cortex (CC), a cortical/limbic area involved in monitoring the internal milieu, would be activated by acute hypoglycaemia (AH) and, if so, that this activation would be abrogated with RH.

Materials and methods: 3 groups (n=4-5 for each) of male Sprague Dawley rats (250-300g) were injected s.c. on three consecutive days as follows; (i) control (CON) had day 1-3 saline (ii) AH had day 1-2 saline and insulin (humulin S, 10 U/kg) on day 3 (iii) RH had day 1-3 insulin (humulin S, 10-6 U/kg). The RH protocol was selected from our previous studies showing that this results in impaired CR. 120 minutes after injection on day 3, rats were perfused transcardially with fixative and brains were extracted and processed immunohistochemically for c-fos, a marker of neuronal activation, using a monoclonal antibody against rabbit c-Fos (1:10,000, Calbiochem) with one-way ANOVA analysis and post hoc Tukey's for multiple comparisons. The CC was subdivided into 2 anatomically separated areas (CC1 and CC2).

Results: As shown in the figure, AH induced an increase in c-fos staining within both the CC1 and CC2 compared with CON. In keeping with our hypothesis, this activation was markedly attenuated following RH. Looking for a similar pattern in other non-hypothalamic areas, we found a significant increase in c-fos activation with AH in the ectorhinal cortex, an area with projections to the CC, which was attenuated with RH. The adjacent perirhinal cortex also demonstrated decreased c-fos activation with RH compared with AH.

Conclusion: In summary, our data show that the CC and connected brain areas are activated by hypoglycaemia with these responses being lost with RH, a pattern mimicking that of the development of impaired CR. These areas are not known to be direct glucose sensing areas but collectively are involved in memory and perception and sensing of physiological danger. We speculate that altered function in limbic areas may either contribute to the development of impaired CR and/ or contribute to the associated hypoglycemia unawareness.



AH induces c-fos expression whilst RH attenuates such a response. (A-D) Quantitative comparison of c-fos cell counts following AH and RH in the CC1 (A), CC2 (B), Ectorhinal (C) and Perirhinal (D) cortices. Data are mean ± SEM, * P<0.05, ** P<0.01.

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596

Hypoglycaemia is related to mortality in the ICU

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Background and aims: The world wide implementation of intensive insulin therapy in the intensive care unit (ICU) is accompanied by increased risk of hypoglycaemia. However, evidence about the impact of hypoglycaemia on ICU mortality is scarce and issue of ongoing debate.

Materials and methods: All 291 first episodes of hypoglycaemia (Glucose ≤ 2.5 mmol/l) were derived from 155,195 glucose values in 5,983 patients admitted to an 18-bed medical/surgical ICU from 2004 to 2007. Readmissions and patients with a withholding care policy were excluded. Patients were treated with a computerized insulin algorithm (target glucose range 4-7 mmol/l). Using Poisson regression, the incidence rates (IR) for ICU death and incidence rate ratio (IRR) comparing exposure and non-exposure to hypoglycaemia, were calculated. To investigate whether hypoglycaemia might have a causal role in worsening patient outcome or might rather be a marker of severe disease, we corrected for severity of disease using daily Sequential Organ Failure Assessment (SOFA) score. Furthermore, age, sex and diabetes mellitus were included as possible confounders.

Results: Mean age was 65 ± 14 years, 35% was female and 6.7% of patients died in the ICU. The overall IR of ICU death was 18.8/1000 person days (PD). The IR of death in patients exposed to hypoglycaemia was 35.6/1000 PD, as compared to 16.9/1000 PD in patients without hypoglycaemia exposure. The corrected IRR was 1.6 (95% CI 1.2.-2.1), $P< 0.001$.

Conclusion: Hypoglycaemia is a strong predictor of ICU death, independent of severity of disease. This suggests a causal role for hypoglycaemia in ICU mortality. Hypoglycaemia is the prime side effect of intensive insulin therapy in the ICU.

597

Mechanisms of insulin resistance following hypoglycaemia in humans: the role of lipolysis induced by hypoglycaemia counterregulation

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Background and aims: Changes in glucose metabolism that occur during counterregulation are, in part, mediated by increased plasma FFA as result of

hypoglycemia-activated lipolysis. However, it is not known whether FFA play a role in the development of post-hypoglycemic insulin resistance as well. To test the hypothesis that hypoglycemia-induced lipolysis plays a role in the pathogenesis of post-hypoglycemic insulin resistance, we conducted a series of studies in 8 healthy volunteers using acipimox, an inhibitor of lipolysis, in order to determine the metabolic effects on insulin action of prevention of increase in FFA during hypoglycemia in normal volunteers. Insulin action was measured during 2 h hyperinsulinemic-euglycemic clamp (plasma glucose, PG, 5.1 mmo/l) from 5 to 7 pm, after volunteers had undergone a 3 h morning hyperinsulinemic glucose clamp (from 10 am to 1 pm) either euglycemic (*S1*) or hypoglycemic (PG 3.2 mmol/l, *S2, S3, and S4*) during which FFA levels were either allowed to increase (*S2*) or suppressed by acipimox (*S3*) or replaced by infusing lipids (*S4*). [6,6-2H2]glucose was infused to measure PG fluxes in all studies.

Results: Plasma adrenaline, noradrenaline, GH and cortisol levels were not different (P>0.2). Glucose infusion rates (GIR) during the euglycemic clamp was reduced by morning hypoglycemia in *S2* vs *S1* (16.8±2.3 vs 34.1±2.2 μmol.Kg^{-1}.min^{-1}, respectively, p<0.001). The effect was largely removed by blockade of lipolysis during hypoglycemia in *S3* (28.9±2.6 μmol.Kg^{-1}.min^{-1}, p>0.2 vs *SI*), and substantially reproduced by replacement of FFA in *S4* (22.3±2.8 μmol.Kg^{-1}.min^{-1}, p<0.03 vs *SI*). Compared to *S2*, blockade of lipolysis in *S3* decreased endogenous glucose production (2±0.3 vs 0.85±0.1 μmol.Kg^{-1}.min^{-1}, p<0.05) and increased glucose utilization (16.9±1.85 vs 28.5±2.7 μmol.Kg^{-1}.min^{-1}, p<0.05). In *S4* GIR fell by ~ 23% (22.3±2.8 μmol.Kg^{-1}.min^{-1}, vs *S3*, p=0.058) indicating a role of acipimox *per se* on insulin action.

Conclusion: In conclusion, lipolysis induced by hypoglycemia counterregulation largely mediates post-hypoglycemic insulin resistance in healthy subjects, with an estimate overall contribution of ~ 50%.

PS 41 Glucose metabolism - experimental

598

Visualisation of protein-protein interactions of glucokinase in living cells

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Background and aims: Several binding proteins of glucokinase (GK) have been identified from independent studies. The liver-specific regulatory protein GKRP sequesters GK in the nucleus at basal glucose concentrations, whilst PFK2/FBPase2 is thought to interact with GK in the cytoplasm in both liver and pancreatic beta cells. However, little is known regarding the regulation of the GK-PFK2/FBPase2 interaction at the cellular level.

Materials and methods: The interaction between GK and PFK2/FBPase2 was investigated using Bimolecular Fluorescence Complementation (BiFC). GK, FBPase2 and PFK2/FBPase2 were tagged with either N- (YN155) or C-terminal (YC155) YFP halves at the C-terminus and transiently co-expressed in Cos1, WRL68 and MIN6 cells. Interactions between GK and PFK2/FBPase2 were also investigated in primary hepatocytes and MIN6 using a proximity ligation assay.

Results: Using the BiFC assay a direct interaction between GK and PFK2/FBPase was visualised as a clear fluorescent signal in the cytoplasm of Cos1, WRL68 and MIN6 cell lines. Removal of the PFK2 domain did not alter the interaction, consistent with binding of GK to the FBPase2 domain. Deletion of the GK interaction interface on FBPase2 (SLKVWT) eliminated the cytoplasmic signal confirming specificity. Two MODY-2 GK mutants which show decreased cellular stability when expressed in a beta-cell system, showed lower levels of interaction with PFK2/FBPase2, quantified as <50% of wild-type GK with respect to YFP intensity and frequency of signal. Studies of the adaptive changes in binding of GK to PFK2/FBPase2 in hepatocytes and MIN6 showed that in primary hepatocytes expressing endogenous GKRP, binding of GK to PFK2/FBPase2 was enhanced by high glucose and pharmacological GK activators and counteracted by resorcinol, which counteracts translocation of GK from the nucleus, and by dibutyryl-cAMP, which lowers fructose 2,6-bisphosphate. The interaction between GK and PFK2/FBPase2 in MIN6 beta cells was also enhanced by high glucose and counteracted by dibutyryl-cAMP, indicating adaptive changes in binding of GK to PFK2 independently of GKRP.

Conclusion: This study shows a direct interaction between GK and PFK2/FBPase2 in mammalian cells. The adaptive nature of this interaction, which is responsive to glucose and GK activators, and the finding that MODY-2 GK mutants show decreased interaction with PFK2/FBPase2, support a role for the GK-PFK2/FBPase2 interaction in the glucose-mediated control of GK activity.

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599

Indirect pathway contribution to hepatic glycogen synthesis is underestimated by enrichment of glucuronide position 3 from deuterated water

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Background and aims: Analysis of urinary glucuronide enrichment from deuterated water (^2H_2O) is a simple method for assaying direct and indirect pathway contributions to hepatic glycogen synthesis (Fig 1). The method assumes that glucuronide derived via indirect pathway is enriched in position 5 (H5) while that derived via direct pathway is not. With transaldolase-mediated exchange (TA), direct pathway precursors are enriched in H5. Thus when TA is active, H5 overestimates the indirect pathway contribution. Since TA does not alter position 3 enrichment (H3), we reasoned that H3 represents the real indirect pathway contribution and that TA accounts for the increased

H5 enrichment relative to H3, (i.e. H5/H3 > 1.0). To test this hypothesis, TA was measured using [U-d_7]glucose and theoretical H5/H3 ratios from ^2H_2O enrichment were calculated. These were compared to real H5/H3 ratios obtained from subjects administered with ^2H_2O.
Methods: 14 Overnight-fasted healthy subjects took breakfast (540 Kcal, 60% CHO/20% fat/20% protein) at 08:00. The CHO portion included 10 grams of glucose. Peppermint oil (200 mg) was taken at 04:00 and 08:00 and urine was collected from 10:00-12:00. TA exchange was measured in 6 of these subjects by enriching the meal glucose with 30% [U-d_7]glucose and quantifying H5 and H3 from urinary menthol glucuronide. H5 and H3 from ^2H_2O was measured in the remaining 8 subjects by providing ^2H_2O (0.3% of body water) 8 hours before the breakfast meal. In all cases menthol glucuronide was isolated by solid phase extraction-preparative HPLC and directly analyzed by ^2H NMR.
Results and discussion: ^2H NMR analysis of glucuronide enrichment from [U-d_7]glucose revealed that 23 ± 5 % of direct pathway flux underwent TA exchange. From this TA activity, a theoretical H5/H3 ratio of 1.31 ± 0.05 was obtained. Real H5/H3 ratios from ^2H_2O was significantly higher than the theoretical value (2.12 ± 0.30, $p < 0.05$) indicating that the H3 and H5 enrichment differences from ^2H_2O were not all accounted for by TA. The lower than expected H3 is likely due to a primary kinetic isotope effect that discriminates against ^2H-incorporation into hexose position 3 at the level of triose phosphate isomerase. Consequently, H3 underestimates the indirect pathway contribution to hepatic glycogen synthesis.
Conclusion: The indirect pathway contribution to hepatic glycogen synthesis can be noninvasively quantified by ^2H NMR analysis of urinary menthol glucuronide ^2H-enrichment following ingestion of ^2H_2O and Peppermint oil. Position 5 enrichment over-estimates the indirect pathway contribution because of transaldolase exchange activity. Glucuronide H3 underestimates the indirect pathway contribution possibly because of isotopic discrimination at the level of triose phosphate isomerase.

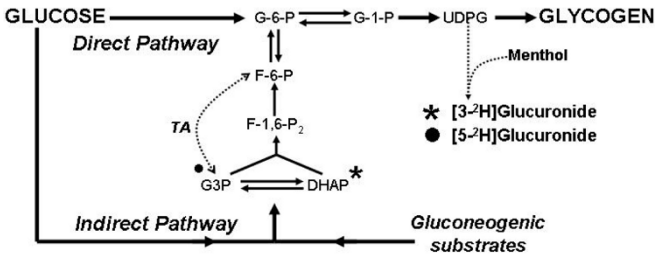

Figure 1: Labeling of menthol glucuronide from ^2H_2O via the indirect pathway. At the triose-P level, glyceraldehyde-3-phosphate (G3P) is labeled with ^2H such that it subsequently forms [5-^{2}H]glucuronide (•) and dihydroxyacetone phosphate (DHAP) is labeled with ^2H such that it forms [3-^{2}H]glucuronide (*). Transaldolase exchange activity (TA) increases the amount of [5-^{2}H]glucuronide independently of indirect pathway flux but has no effect on the production of [3-^{2}H]glucuronide.

Supported by: JDRFI

600

Metabolic profiling of hepatic glycogen and lipid synthesis in spontaneously feeding rats: effects of switching from solid to liquid diet
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Background and aims: The liver has a central role in glucose and lipid homeostasis and disarrangements of hepatic glucose and lipid fluxes are early defining events in the development of insulin resistance and Type II diabetes (T2D). Under normal conditions, hepatic carbohydrate and lipid metabolic fluxes are highly coordinated with respect to each other and to overall nutritional state. To better understand how this is altered in T2D requires an integrated analysis of hepatic glucose and lipid metabolic fluxes. The analysis cannot perturb feeding habits since such changes can also acutely modify hepatic glucose and lipid fluxes. This is difficult to achieve in rodent models since metabolic flux assays usually involve invasive catheterization procedures whose effects *per se* on the animal´s nutritional status may be significant. We are developing methods for integrating hepatic glucose and lipid fluxes in spontaneously feeding animals. Tracers are delivered via diet and as a single injection of deuterated water (^2H_2O). As an important first step towards an integrated metabolic profile of hepatic glucose and lipid fluxes, of we present simultaneous measurements of hepatic glycogen synthesis and *de novo* lipogenesis (DNL) in spontaneously feeding animals.
Materials and methods: Male Wistar rats raised on standard chow solid diet (SD) were randomly divided in two groups of 7. One group was placed for 7 days on a liquid diet (LD) that was isocaloric with SD. The control group was maintained on SD for this period. Animals were maintained with a constant light/dark cycle of 12/12 h. At 20:00 of day 6 rats were injected with 2.1 ml/100 g body wt of 99% ^2H_2O. At 08:00 on day 7, rats were sacrificed and the liver was excised, frozen and lyophilized. Glycogen was extracted by alkali method and hydrolysed to glucose by α-amyloglucosidase. Extracts were dried and glucose was derivatized to monoacetone glucose (MAG) for NMR analysis of ^2H-enrichment and glycogen quantification. Lipids were extracted by Folch method and triglyceride ^2H-enrichment was analyzed by ^2H NMR. Body water ^2H-enrichment was directly determined from plasma by ^2H NMR. From a single ^2H NMR spectrum of MAG, total hepatic glycogen was determined by an internal standard. The fraction of total glycogen synthesized overnight was determined from enrichment of glycogen position 2 relative to body water (H2/BW). The contribution of direct and indirect pathways to overnight glycogen synthesis was determined from enrichment in position 5 relative to position 2 (H5/H2). From the triglyceride ^1H and ^2H NMR spectra, total hepatic triglyceride content and the contribution of DNL to hepatic triglycerides during overnight feeding was determined.
Results: Total glycogen synthesized was significantly lower in LD group (3.1 ± 0.7 mg) than in SD group (17.8 ± 4.3 mg), $p<0.01$. Indirect pathway accounted for 5.7 ± 2.2 and 1.1 ± 0.4 ($p<0.01$), of the total glycogen synthesised, in SD and LD groups respectively. In contrast, DNL was not affected by the diet formulation: SD group synthesized 85.1 ± 11.5 μmol/g*dw*, whereas LD group synthesized 85.7 ± 15.2 μmol/g*dw*. Total hepatic triglycerides levels were also similar for both groups (255.4 ± 30.7 μmol/g*dw* for SD and 286.5 ± 22.4 μmol/g*dw* for LD).
Conclusion: Seven days weaning from solid to liquid diet induced significant alterations in overnight hepatic glycogen synthesis but not for triglyceride synthesis.
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601

Effects of resistin on glucose metabolism in C2C12 myocytes
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Background and aims: Resistin is an adipokine highly related to insulin resistance (IR). Our study is to investigate how resistin influences skeletal glucose metabolism and explore its mechanisms.
Materials and methods: The recombinant plasmids PcDNA3.1 expressing resistin were constructed and then transfected into C2C12 myocytes. The expression of resistin in C2C12myocytes was detected by Western blotting. Glucose uptake was measured by ^3H labeled glucose; glucose oxidation and glycogen synthesis were detected with ^{14}C-labeled glucose. GLUT-4 mRNA was measured by reverse transcript polymerase chain reactions (RT-PCR).
Results: Resistin was expressed in transfected myocytes. Resistin decreased insulin stimulated glucose uptake rate by 28%~31% and inhibited the expression of GLUT4 mRNA. However, there were no significant differences in basal glucose uptake, and glucose oxidation and glycogen synthesis remained unchanged in all groups.
Conclusion: Resistin inhibited insulin stimulated glucose uptake in myocytes by downregulating the expression of GLUT-4 and it had no effects on glucose oxidation and glycogensynthesis. Our findings may provide a clue to understand the roles of resistin in the pathogenesis of skeletal IR.
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602

Regulation of hepatic glucose homeostasis by glucokinase localization
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Background and aims: Glucokinase (GK) acts as a glucose sensor for coupling millimolar glucose concentrations to metabolism. A specific glucokinase regulatory protein (GRP) inhibits GK activity in liver and mediates the translocation of GK between nucleus and cytoplasm in dependence on glucose and fructose metabolites. While the GK nuclear import is clearly mediated by the GRP, the mechanism of GK nuclear export remains open. It has

been shown, that GK activators recently described as a potential new class of drugs for the treatment of type 2 diabetes modulate GK localization in liver. Thus, the elucidation of the underlying molecular mechanisms of GK shuttling is also important for an appropriate pharmacotherapeutic intervention. Therefore the aim of this study was to analyze the GK-GRP interaction and the GK nuclear export mechanism with respect to hepatic glucose homeostasis.
Materials and methods: Interaction between GK and GRP was analyzed in COS cells using a fluorescence based mammalian two-hybrid system (MMTHS) and in primary rat hepatocytes via fluorescence resonance energy transfer (FRET). The glucose concentration in the cytoplasm and nucleus was determined by a recently established FRET based glucose specific nanosensor (FLIPglu). Primary rat hepatocytes were transduced with a recombinant adenovirus expressing the FLIPglu sensor and a nucleus localized FLIPglu (FLIPglu-nuc). COS cells were transiently transfected with FLIPglu and FLIPglu-nuc constructs.
Results: The MMTHS allowed monitoring of the effects of environmental changes on the GK-GRP interaction. In medium supplemented with 5 mmol/l glucose an interaction between GK and GRP was measured. Increasing the glucose concentration in the cell culture medium significantly decreased the interaction between the proteins. Using an antibody based acceptor photobleaching FRET technique in primary rat hepatocytes differences in the GK-GRP association strength were detectable. Interaction of endogenous GK and GRP in the nucleus of hepatocytes was tenfold higher after incubation at 5 mmol/l glucose than at 20 mmol/l glucose. Addition of fructose-6-phosphate further induced the interaction. The cytoplasmic and nuclear glucose concentration upon changes in the extracellular glucose concentration could be determined expressing the FLIPglu sensor and calculating the EYFP/ECFP ratio upon ECFP excitation. After addition of glucose containing Krebs Ringer medium the intracellular glucose concentration significantly increased. In both primary rat hepatocytes and COS cells the cytoplasmic glucose rise was accompanied by a comparable increase in the nucleus.
Conclusion: Subcellular alterations in glucose homeostasis in primary cells could be monitored using the FLIPglu sensor. Glucose plays a major role in the GK compartmentation by dissolving the GK-GRP complex. As there is a simultaneous rise of the glucose concentration in the nucleus, paralleling this in the cytosolic compartment, it is likely that GK leaves the nucleus at least partially in a GRP independent manner. This newly established cellular system will allow the elucidation of the effect of GK activators on this process in future studies.

603

Measuring glucokinase in fresh and frozen liver
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Background and aims: Glucokinase (GK) is a key enzyme of glucose homeostasis influencing both insulin secretion and control of hepatic glucose balance, especially when post-prandial glycemia increases. The absolute activity of GK is key to both processes as illustrated by numerous studies on man and in animal models. Mutation of the gene for this enzyme can result in GK protein with increased or decreased activity, contributing to chronic hyperglycemia (MODY2) or familial hypoglycemia respectively. Activity of the liver enzyme can be readily measured in fresh liver samples. However, we are unaware of data derived from frozen liver samples: there is no reason in principle why activity of the enzyme should be impaired by freezing. Our initial attempts at preparing a 10% liver homogenate from frozen rat liver gave activities of glucokinase significantly higher than literature values reported both by ourselves and others. Because of the potentially important physiological implications of this finding with respect to understanding of the mechanisms of normal glucose homeostasis, and especially that of liver glycogen synthesis we began a systematic review of the methodology used to determine activity of this enzyme, and a preliminary report of key findings to date is reported here.
Materials and methods: GK and HK activity were determined as previously described: briefly, samples of both fresh and thawed frozen liver were homogenized by hand using a glass homogenizer and samples ultra-centrifuged at 100,000g to obtain a clear supernatant: the fatty layer was removed and supernatant assayed for GK and HK by the G6PDH NAD-linked assay.
Results: Activity of GK was significantly higher in frozen liver sections compared with fresh liver, and hexokinase activity significantly decreased. The enzymes were separated by gel filtration to investigate their kinetic parameters independently: GK demonstrated unchanged sigmoidal and HK, Michaelis-Menten, kinetics with respect to glucose. The Vmax for GK was obtained at about half the glucose concentration used by previous workers in both fresh and frozen liver tissue, and S0.5 was unchanged (approximately 8mM).
Conclusion: Since the increased activity of GK in frozen liver was not the result of altered kinetics of the enzyme we postulate that freezing the tissue from ad libitum fed rats results in more efficient liberation of the enzyme from a nuclear pool sequestered by the glucokinase regulatory protein (GKRP). In fresh liver tissue it is possible that some of this pool escapes mechanical release during homogenization and ultra-centrifugation and is then pelleted and lost to the supernatant. This would indicate that the nutritional conditions under which animals are killed for study of glucokinase activity using fresh liver samples could influence measures of GK activity, and hence the conclusions drawn. Such data might also explain why measured activity of GK appears to change remarkably quickly under differing nutritional circumstances.
Supported by: the Wellcome Trust

604

Short term and long term disease mathematical modelling of diabetes in Zucker and ZDF rats
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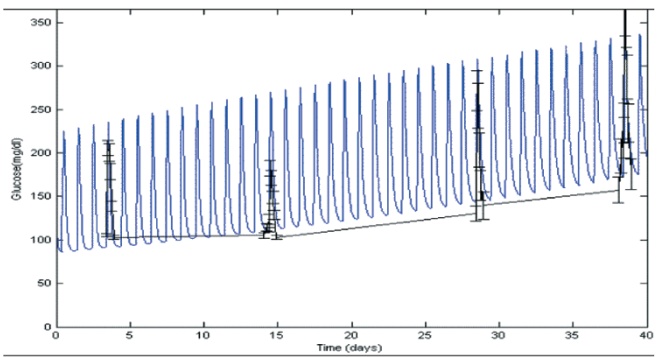
Background and aims: The aim is to create a universal mathematical model for glucose and insulin dynamics in rats both in the short term, for example to model meal feeding, and also in the long term, to model disease progression.There have been several attempts to model the glucose and insulin system before, but very few of these have tried to combine both short- and long-term dynamics. This new model takes elements from previous mathematical models in an attempt to create a universally applicable model.
Materials and methods: The model was created using a combination of features from existing models, such as beta cell mass from the beta cell mass model, and newly-designed elements to characterise both short- and long-term dynamics of the glucose and insulin system. The model incorporates elements that allow the simulation of short-term aspects which include feedback between insulin and glucose dynamics. Long-term dynamics display elevated levels of glucose, stimulating an increase in insulin secretion capacity, but very high levels of glucose can cause damage and reduce insulin secretion capacity. The model additionally allows simulation of gut delay of glucose absorption, as well as incorporating increased glucose as a result of feeding. The model can be numerically simulated within MATLAB, using a stiff equation solver, and an interface was created to enable easy use of the model by researchers and other interested parties.
Results: Simulations show that the long-term progression into diabetes can be modelled by decreasing both insulin sensitivity and the first phase of insulin secretion. The wider applicability of the model is demonstrated by its ability to simulate the adaptation of the system to the change in insulin secretion to cope with a decay in insulin sensitivity. The data used to perform model fitting and validation were from Zucker and Zucker Diabetic Fatty (ZDF) rats which were meal fed for 4 hours per 24 hour period over 40 days, with glucose and insulin profile measurements taken on days 3, 14, 28 and 38 of the study. The figure shows a model fit for glucose data, with black denoting the real data, showing the model responses for each day.
Conclusion: A mathematical model has been developed that can characterise the disease status of the glucose and insulin system in rats, incorporating both short- and long-term aspects. The model can produce dynamic responses that compare well with real data.



Supported by: AstraZeneca

PS 42 Metabolic effects of various hormones

605

Islet-cell secretion of pancreatic polypeptide in response to ghrelin and obestatin acting on distinct receptors

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Background and aims: Des-acyl ghrelin (28 a.a. residues) and obestatin (23 a.a.) are hormonal cleavage products of proghrelin, which is synthesized in endocrine A-like cells in the gastric mucosa.
During processing, a fraction of des-acyl ghrelin is octanoylated in position 3 (serine) to form acyl ghrelin. Being cleavage products of proghrelin, obestatin and the two ghrelin peptides are likely to be released together from the A-like cells, conceivably affecting their targets in concert. In the present study we used isolated pancreatic islets from the mouse to study whether acylghrelin and obestatin act on the same or on distinct receptors to inhibit the secretion of pancreatic polypeptide (PP).
Materials and methods: Isolated mouse pancreatic islets were used to study the dose dependent response of acylghrelin & obestatin in absence and presence of des-acylghrelin on islets hormone secretion.
Results: Both acyl ghrelin and obestatin abolished PP release by dose-dependent manner with marked effect at concentrations of 10^{-10} M. Des-acyl ghrelin was without effect.The inhibitory effect of 10^{-10} M acyl ghrelin was counteracted by des-acyl ghrelin in a concentration-dependent manner, while the inhibitory effect of 10^{-10} M obestatin was not.. Under these circumstances the IC_{50} value for des-acyl ghrelin was $4.1\pm 1 \times 10^{-8}$ M. This means that quite high concentrations of des-acyl ghrelin were needed to impair the inhibition of PP release induced by 10^{-10} M acyl ghrelin. The IC_{50} value for acyl ghrelin was calculated to be $4.1 \pm 1 \times 10^{-12}$ M (n=6), while the IC_{50} value for obestatin was $3.2 \pm 1 \times 10^{-12}$ M (n=8). The concentration-response curve for acyl ghrelin was greatly affected by different concentrations of des-acyl ghrelin, shifting the curve to the right with increasing concentrations of des-acyl ghrelin, in a manner suggestive of competitive inhibition and the effect of obestatin was unaffected by the presence of des-acyl ghrelin.
Conclusion: Our data suggest that des-acylghrelin per se was found no effect on PP cells while the inhibitory effect of acylghrelin on PP secretion was reversed by des-acylghrelin, the suppressive effect of obestatin on PP cells was unchanged. This observation indicates that acylghrelin and obestatin act on PP cells through distinct receptors. Understanding the mechanisms that control PP release might help to clarify the physiological significance of PP cells.
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606

Vitamin D and glucose metabolism in polycystic ovary syndrome

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Background and aims: Several studies suggest an association of low 25-OH vitamin D [25(OH)D] levels with impaired β cell function and type 2 diabetes. Women affected by polycystic ovary syndrome (PCOS) frequently present with insulin resistance and are at an increased risk for the development of type 2 diabetes. The aim of this study was to investigate the relationship between 25(OH)D levels and parameters of glucose metabolism in PCOS women.
Materials and methods: 25(OH)D levels were measured by means of ELISA in 205 women affected by PCOS. Metabolic, endocrine, and anthropometric measurements and oral glucose tolerance tests were performed. Metabolic syndrome was defined in accordance with National Cholesterol Education Program/Adult treatment panel III criteria.
Results: PCOS women with hypovitaminosis D (<29.9 ng/ml) had significantly higher BMI, waist circumference, HOMA-IR, HOMA-β, AUCgluc, 1h glucose, fasting insulin, and triglycerides and lower levels of QUICKI and HDL (p<0.05 for all). In turn, PCOS women with impaired glucose tolerance had significantly lower 25(OH)D levels than PCOS women with normal glucose tolerance (p=0.006). In multivariate regression analysis, 25(OH)D and BMI were independent predictors of HOMA-IR and QUICKI (p<0.05 for all). PCOS women with impaired glucose tolerance and metabolic syndrome (MS) had lower 25(OH)D levels than PCOS women without these features (p<0.05 for all). In logistic regression analysis, 25(OH)D (OR 0.86, p=0.021) and BMI (OR 1.3, p<0.001) were independent predictors of MS in PCOS women.
Conclusion: We present evidence that low 25(OH)D levels are associated with obesity, IR, impaired β cell function, impaired glucose tolerance, and MS in PCOS women. Large intervention trials are warranted to evaluate the possible beneficial role of vitamin D supplementation on disturbances in glucose metabolism in PCOS women.

607

Altered plasma leptin level is associated with low stearoyl-CoA desaturase-1 activity in type 2 diabetes

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Background and aims: Leptin is a multifunctional hormone which may be involved in the pathogenesis of type 2 diabetes mellitus (T2DM) by influencing insulin production or sensitivity. The exact mechanism of insulin secretory dysfunction associated with altered plasma leptin level in T2DM is still under investigation. One hypothesis is that the hypoleptinemia induced insulin secretory defect is caused by steatosis of β-cells of pancreas resulting from altered fatty acid metabolism. The present study was designed to determine any change in fatty acid metabolism associated with altered leptin level in type 2 diabetes.
Materials and methods: A total of 119 subjects were included. In Group I (Case), 59 newly diagnosed T2DM and in Group II (Comparison), 60 age (year, 36±4 vs. 35±4, M±SD) and Body Mass Index (BMI) matched (kg/m^2, 24.0±3.1 in Group I vs. 23.6±2.0 in Group II) healthy control subjects were included in this cross sectional analytical study. Plasma insulin (fasting and 30 min post glucose) and leptin were estimated by Enzyme Immunoassay. Insulin secretory capacity (HOMA-B%) and insulin sensitivity (HOMA-S%) were calculated by Homeostasis Model Assessment using HOMA-CIGMA software. Fasting serum non-esterified fatty acid (NEFA) was measured by enzymatic colorimetric method. Plasma fatty acid composition (%) was analyzed by gas liquid chromatography. Stearoyl-CoA desaturase-1 (SCD-1) activity was calculated by plasma palmitoleic/palmitic (C16:1/C16:0) ratio.
Results: The diabetic subjects showed highly significant β-cell dysfunction and also insulin resistance as evident from HOMA B% [20.2(4.2-89.6) in diabetic vs. 78.4(35.5-365.7) in control, p<0.001] and HOMA S% [84.6(39.1-226.4) vs. 118.8(22.0-3573.0), p<0.004]. Serum fat normalized leptin level was found significantly lower in diabetic subjects [ng/ml, 5.44 (0.65-34.7)] compared to controls [8.35 (1.36-55), p=0.012]. The serum leptin level inversely correlated with insulin secretory dysfunction. The total NEFA level in the diabetic subjects was higher than control (mmol/l, 0.652±0.196 vs. 0.42±0.15, p<0.001). NEFA was inversely correlated with HOMA S% (r=-0.369, p<0.01). HOMA S% was positively associated with eicosapentaenoic (20:5) acid (r=0.354, p=0.001). HOMA B% was inversely associated with palmitic (16:0) acid (r=-0.577, p=<0.01). Leptin level was positively correlated with stearoyl-CoA desaturase-1 activity (r=0.185, p=0.04), erusic (22:1) acid, (r=0.561, p=0.046), as well as the ratio of linoleic/linolenic (C18:2/C18:3) acid (r=0.165, p=0.05), and inversely correlated with behenic acid (r=-0.319, p=0.039).
Conclusion: The data suggest that a) Serum fat normalized leptin level in normal to mildly overweight Bangladeshi type 2 diabetic subjects is lower compared to age and BMI-matched healthy population; b) Lower plasma leptin in type 2 diabetes mellitus subjects is associated with insulin secretory dysfunction; c) Lowered plasma leptin level in Type 2 diabetes is associated with lower levels of stearoyl-CoA desaturase-1 (SCD-1) activity, linoleic/linolenic (C18:2/C18:3) ratio and plasma erusic acid level, and higher level of plasma behenic acid.
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608

Plasma thyroid hormone levels are positively associated with insulin resistance at all stages of type 2 diabetes

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Background and aims: Thyroid hormone levels have generally been found normal in diabetic patients. Whether variation in those levels within the euthyroid range influence insulin sensitivity at the various stages of type 2 diabetes remains to be established.
Materials and methods: A meal was given to four groups of subjects (age 34+2.6, 37+2.7, 46+4.2, 51+2.2 yrs, respectively, and BMI 23.1+0.63, 23.9+0.53, 24.9+0.8, 24.9+0.56 kg/m^2, respectively) whose baseline characteristics are shown in Table 1. Blood was withdrawn for 360 min from the radial artery for measurements of glucose and insulin. Fasting plasma free T4 (FT4) and free T3 (FT3) levels were also measured. Fasting and postprandial insulin resistance was assessed by HOMA and ISI indices respectively.
Results: FT4 levels were lower in controls versus relatives, IGT and DM (p=0.048). FT3 levels were also lower in controls versus relatives, IGT and DM subjects (p=0.005). HOMA was positively associated with FT4 and FT3 levels (β-co-efficient=0.110±0.33, p=0.001 and 0.521±0.160, p=0.002 respectively). ISI was negatively associated with FT4 and FT3 levels (β-co-efficient=-6.597±1.449, p=0.001 and -21.036±6.152, p=0.001, respectively).
Conclusion: In type 2 diabetes, increases of thyroid hormone levels within the normal range associate positively with insulin resistance. These data suggest that thyroid hormones may be part of the pathogenetic mechanism to explain metabolic derangement at all stages of type 2 diabetes.
RELATIVES: 1st degree relatives of diabetic patients with a normal glucose tolerance test, IGT: subjects with impaired glucose tolerance, DM: subjects with type 2 diabetes.

Table 1: Baseline subjects' characteristics

	CONTROLS	RELATIVES	IGT	DM	P overall
Table of Contents				2	
• No/Subjects	17	22	9	4	
Free T3 (pmol/l)	4.12±0.1	4.3±0.12	4.51±0.2	4.7±0.11	0.005
Free T4 (pmol/l)	14.9±0.26	15.32±0.12	16.1±0.7	17±0.6	0.048
Plasma Glucose (mmol/l)					
-fasting	4.46±0.1	4.81±0.09	5.8±0.22	7.5±0.27	<0.0001
-120min	5.6±0.2	6.85±0.35	9.26±0.25	14.35±0.7	<0.0001
Plasma Insulin (mU/l)					
-fasting	4.65±0.5	7.03±1.2	7.13±0.6	5.92±0.5	NS
-120min	34,4±3.2	51.5±4.7	81.8±8.7	51.07±7.1	0.0007
HOMA Index	0.91±0.11	1.3±0.11	1.84±0.18	2±0.17	<0.001
ISI Index (mg*l^2/ mmol*mU*min)	101.2±7.8	76.5±6.4	46.1±2	35.5±3.16	<0.001

Data are expressed as means±SEM, and P values represent overall comparison (repeated-measures ANOVA) between control and patient groups after adjustment for possible covariates (age, sex). BMI, body mass index. Free T3 and Free T4 concentrations are given in International Units.

609

Growth hormone deficiency and rhGH-therapy to individuals with low IGF-1 levels contribute to the regulation of STAMP2 gene in subcutaneous adipose tissue

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Introduction: Growth hormone deficiency (GHD) is known to be associated with metabolic dysregulation, increased inflammation and compromised growth and differentiation of adipose tissue. We investigated the effect of GHD as well as GH supplementation in patients with low IGF-1 levels on the expression of six-transmembrane protein of prostate 2 (STAMP2), the potential coordinator of nutrient and inflammatory response in human subcutaneous adipose tissue (SAT).
Methods: The first cohort consisted of 20 GHD patients (age 30.7±7 yrs, M/F 12/8, BMI 21.7-36.8 kg.m^{-2}) and 19 healthy age, gender and BMI-matched controls. Insulin sensitivity (IS) was measured by euglycemic hyperinsulinemic clamp (EHC). Abdominal fat mass and distribution was determined using MRI. Samples of SAT were taken by needle biopsy and fat cell size (FCS) was assessed by light microscopy. Gene and protein expressions were measured by qRT-PCR and immunoblotting, respectively. The second cohort consisted of ten 40-70yrs old sedentary male subjects with waist circumference >102 cm, BMI 27 - 35 kg.m^{-2}, and blood levels of IGF-1 bellow 241 ng/ml who were for a period of 24 wks treated by pharmacological levels (0.95 mg/day) of rhGH (Genentech, CA, USA).
Results: GHD patients displayed lower IS, increased FCS and visceral obesity. GHD was associated with a significant decrease in STAMP2 gene and protein expressions, the change in mRNA being more pronounced during the EHC (2.2-fold, p<0.0001) compared to the fasting state (1.4-fold, p<0.05). STAMP2 was positively correlated to the protein expression of adipokines regulating growth and differentiation of adipose tissue (PDGF, SDF1, GRO; r=0.59, r=0.41, r=0.62; p<0.05; n=24). A strong positive association was found between STAMP2 and adiponectin gene expression during fasting state and the euglycemic hyperinsulinemia (r=0.52, r=0.67; p<0.01; n=34). Furthermore, patients receiving GH-supplementation displayed 1.4-fold increase (p=0.02) in the expression of STAMP2 gene in SAT.
Conclusion: Our results demonstrate that STAMP2 is decreased in GHD associated with whole-body metabolic derangements and upregulated by rhGH therapy of obese individuals with low IGF-1 levels. The relationships of STAMP2 to adipokines regulating adipose tissue growth, differentiation and inflammation support a positive role this protein might play in human metabolic health.
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610

Type 2 diabetes mellitus and primary hypothyroidism. TSH test in all diabetic patients?

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Background and aims: Type 2 diabetes mellitus (DM) and primary hypothyroidism are recognized risk factors for atherosclerotic cardiovascular disease. This study is an effort to identify the high prevalence of association between these two diseases in our population.
Materials and methods: A cross-sectional study from an outpatient clinic in Monterrey, Nuevo Leon, México. 1848 adult patients with DM were included in the study group and were compared with 33732 matched controls without a diagnosis of type 2 DM. Primary hypothyroidism was defined in all patients using thyroid medication. Subjects with thyroid neoplasm, surgery complications, or other pathologies were excluded.
Results: The two groups were similar respect to demographic characteristics. Of the 1743 patients of the study group, 105 had hypothyroidism, and the

control group 33,131 patients, 601 had hypothyroidism, OR 3.32 (95% IC 2.64-4.10). Cholesterol, triglycerides, systolic and diastolic pressure values were significantly higher in the study group.
Conclusion: We demonstrated an elevated association between DM and hypothyroidism. These findings are consistent with increased cardiovascular risk. Thyroid profile measurement should be a diagnostic test in all patients with DM, similar as type 1 diabetes mellitus.

PS 43 Sex hormones and metabolism

611

Menopausal hormone therapy and new-onset diabetes in the French E3N cohort

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Background and aims: Two US randomized trials found a lower incidence of type 2 diabetes among women treated by menopausal hormone therapy (MHT) with oral conjugated equine estrogen combined with medroxyprogesterone acetate. The purpose of this study was to evaluate the influence of various MHTs, according to their formulation and route of administration, on new-onset diabetes in a cohort of postmenopausal women.
Materials and methods: E3N is a cohort study of 98,995 French women born in 1925-1950 and followed by questionnaire every 2 years. We included only the women who responded to a dietary history questionnaire sent in 1993, and who were postmenopausal when they replied to the 8th questionnaire sent in 2005. We excluded women with non validated diabetes, prevalent diabetes, no follow-up or lack of information on MHT use, leaving 63,624 postmenopausal women for analysis. Cases of diabetes were identified through self-report or drug reimbursement record linkage, and further validated. The association between MHT use and new-onset diabetes was investigated by Cox regression analysis.
Results: A total of 1220 new-onset diabetes were validated. We observed a lower risk of diabetes among women having ever used MHT (Hazard Ratio: HR=0.82 [0.72 - 0.93]), compared to MHT never users. Adjustment for BMI during follow-up did not substantially modify this association. An oral route of estrogen administration was associated with higher decrease in diabetes risk than cutaneous route (HR=0.68 [0.55-0.85] vs. 0.87 [0.75-1.00], p for homogeneity=0.028). When further taking into account the type of progestagen used in combined MHT, there was no statistically significant heterogeneity between progestagens, neither between route of estrogen administration, with regard to diabetes risk.
Conclusion: MHT might decrease the risk of diabetes, independent of BMI. Further studies are needed to confirm the stronger effect of oral administration of estrogen compared to cutaneous administration.
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612

Hyperandrogenism is associated with an increased risk for type 2 diabetes and metabolic disturbances in a large cohort of postmenopausal women

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Background and aims: Women with polycystic ovary syndrome present with hyperandrogenism and are frequently affected by insulin resistance and impaired glucose tolerance. However, the association of hyperandrogenism with type 2 diabetes is incompletely described in postmenopausal women. The aim of this study was to evaluate the relation between testosterone and type 2 diabetes and metabolic disturbances in postmenopausal women.
Materials and methods: We measured testosterone [µg/l], estradiol [ng/l], and metabolic parameters in 728 postmenopausal women not using hormone therapy enrolled in the Ludwigshafen Risk and Cardiovascular Health (LURIC) study.
Results: Median testosterone levels were significantly higher in postmenopausal women with type 2 diabetes (0.8 [0.5-1.0]) than in women without type 2 diabetes (0.7 [0.5-1.0]) (p=0.019) whereas median estradiol levels were

similar (27 [21-36.5] vs 26 [20-44). Postmenopausal women were stratified into 2 groups (higher or lower than the median testosterone level of the study sample). Postmenopausal women with higher testosterone levels had a 37.5 % higher risk of type 2 diabetes (RR 1.38; 95% CI [1.02-1.86]; p=0.039] than women with lower testosterone levels. Postmenopausal women with testosterone levels >0.7 µg/l had higher weight, waist circumference, fasting glucose, fasting insulin, fasting proinsulin, HOMA-IR, LDL, and CRP and lower HDL levels than women with lower testosterone levels (all p<0.05).
Conclusion: These data suggest that endogenous testosterone levels are associated with type 2 diabetes and metabolic disturbances in postmenopausal women. Identification of postmenopausal women with hyperandrogenism may be important for prevention and treatment of type 2 diabetes.

613

Progesterone directly affects mitochondrial function - a mechanism relevant to gestational adaptations of glucose metabolism?
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Background and aims: Late pregnancy is associated with adaptations of glucose metabolism, which have been attributed, at least in part, to high concentrations of circulating progesterone. In preceding studies, we found that progesterone directly and immediately modulates glucose handling by isolated specimens of rat skeletal muscle via a non-genomic mechanism of action. The results revealed not only inhibitory action on glucose transport, but also that progesterone impairs cell respiration. 1 µmol/l progesterone, a concentration that can be reached in plasma of pregnant humans and rats, reduced the rate of pyruvate oxidation by specimens of skeletal muscle by 28±2% (p<0.0001). Based on this finding, the present study investigated, if progesterone can affect respiratory function of mitochondria directly.
Materials and methods: Mitochondria were isolated from rat liver by tissue homogenization and differential centrifugation. They were incubated without or with 1, 3, 10, or 30 µmol/l progesterone in the presence of glutamate plus malate (substrates for respiratory complex I) or in the presence of succinate (substrate for complex II; complex I was blocked by rotenone). Oxygen consumption was measured after addition of inorganic phosphate and ADP, which allows oxidative phosphorylation (state 3), and after quantitative consumption of added ADP (i.e. without oxidative phosphorylation; state 4).
Results: In the presence of glutamate and malate, progesterone dose-dependently inhibited oxygen consumption in state 3 (control = 100%; 1 µmol/l progesterone, 89.5±5.6%, p=0.12, ns; 3 µmol/l, 93.9±5.3%, p=0.30, ns; 10 µmol/l, 86.8±4.6%, p=0.03; 30 µmol/l, 61.1±3.6%, p<0.001). In state 4, the lowest employed concentration of the steroid hormone was sufficient to trigger significant inhibition (control = 100%; 1 µmol/l progesterone, 92.6±2.6%, p=0.04; 3 µmol/l, 91.4±2.4%, p=0.02; 10 µmol/l, 84.3±5.4%, p=0.03; 30 µmol/l, 69.3±4.1%, p<0.001). In succinate-fed mitochondria, inhibitory action of progesterone was still present but less distinct than in such fed with glutamate and malate (with succinate, state 3: 10 µmol/l progesterone, 96.5±1.4%, p=0.06, ns; 30 µmol/l, 93.1±3.0%, p=0.07, ns; state 4: 10 µmol/l progesterone, 94.5±1.7%, p=0.02; 30 µmol/l, 87.9±2.0%, p=0.002). In mitochondria uncoupled by 100 µmol/l 2,4-dinitrophenol (DNP), inhibitory action of progesterone was also obvious (30 µmol/l progesterone: with glutamate and malate, 66.9±3.3%, p<0.001; with succinate, 94.3±1.0%, p=0.003).
Conclusion: Progesterone can impair respiratory function by direct interaction with isolated mitochondria in vitro. A progesterone concentration that can be reached in plasma of pregnant humans and rats is sufficient to induce such effects on mitochondrial function, which could be the mechanism responsible for impaired cell respiration seen in progesterone-exposed tissue specimens. Apart from possible relevance for the metabolic adaptations to pregnancy, direct modulation of mitochondrial function could be a novel pathway, via which progesterone triggers non-genomic effects.

614

Short-term changes in serum sex steroid levels affect postprandial triglyceride metabolism in healthy young men
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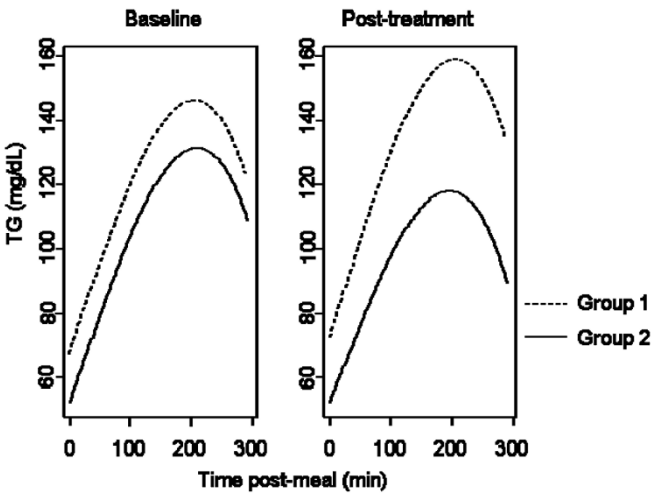
Background and aims: Male hypogonadism is associated with obesity and diabetes, which suggests a relation between sex steroid status and glucose and/or lipid metabolism. However, it is not clear if this is due to direct hormonal effects or results from changes in body composition. By this intervention study, we investigated whether short-term changes in sex steroid levels directly affect post-meal lipid-handling in healthy young men.
Materials and methods: In order to obtain two contrasting groups with high estradiol (E2) and low testosterone (T) vs. low E2 and high T levels, healthy young men (aged 20-40 yrs) were randomized to receive either an aromatase inhibitor (letrozole 2.5 mg daily) plus an E2 patch (75µg/day) (group 1; n=10) or letrozole only (group 2; n=10), for a duration of 7 days. All subjects ingested a mixed-meal (1000 KCal - 45% fat, 36% carbohydrates, 19% proteins) and blood samples were taken up to 5 hours post-meal to assess the postprandial triglyceride (TG)-response. Changes in response between both groups were assessed using longitudinal mixed-effects modeling analyses.
Results: In group 1, mean E2 levels rose 43% and T levels decreased 44%, whereas mean T levels rose 114% and E2 levels decreased 56% in group 2. Fasting TG levels did not change in either group (P>0.12). However, postprandial TG-response was differentially affected by treatment in both groups. Following 7 days of treatment, the increment in postprandial TG levels in group 1 was faster as compared to baseline (+0.54 mg/dL/min vs. +0.50 mg/dL/min; P = 0.010), whereas this was slower in group 2 (+0.44 mg/dL/min vs. +0.50 mg/dL/min; P = 0.036), resulting in a significant treatment effect (P = 0.006) (see Figure).
Conclusion: Short-term administration of letrozole plus E2 in healthy young men appeared to have direct elevating effects on the postprandial TG-response. Contrasting effects were observed in men treated with letrozole only, displaying a lower postprandial TG-response. These data suggest that part of the increase in metabolic risk in obesity in males might be explained by adverse affects on lipid-handling by changed sex steroid levels.



615

Role of sex hormones in insulin resistance - a lesson from the Aromastase Knockout (ArKO) mouse
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Background and aims: Sex hormones such as estrogens and androgens are known to influence development of insulin resistance and adiposity, which

contribute to many disease processes such as type 2 diabetes and the Metabolic Syndrome. Estrogen (E2) and testosterone (T) are known to have bi-phasic effects on insulin resistance in a dose-dependent manner. To research the effects of sex hormones on insulin resistance we studied Aromatase knockout (ArKO) mice which are unable to convert androgens to estrogens.
Materials and methods: Glucose and pyruvate tolerance and insulin sensitivity of 12, 24 and 52 week-old animals were assessed by Glucose Tolerance Test (GTT; ip glucose injection, 1mg/g of body wt), Insulin Tolerance Test (ITT; ip insulin injection, 0·05 or 0.025U/g) and Pyruvate Tolerance Test (PTT; pyruvate injection 1mg/g of body wt) after a 6-8h (GTT and ITT) or overnight fast (PTT). Blood glucose concentrations were measured at 0, 20, 40, 60, 90 and 120 min. Fed, fasted and GTT (20min) blood insulin concentrations were measured.
Results: Sexually dimorphic differences were observed between the male and female ArKO mice. Male ArKO mice by 12 wks of age present with hyperglycemia and significant glucose and pyruvate intolerance and signs of reduced insulin sensitivity. Similar phenotypes are present in 24- and 52-wk-old male ArKO mice. Upon estrogen treatment, these phenotypes were corrected to WT levels. Only ArKO males develop hepatic steatosis which may be a contributing factor in the development of the insulin resistant state. Female ArKO mice by 12 wks of age show signs of hyperglycemia and glucose intolerance with trends continuing at 24wks. They also develop significant pyruvate intolerance and reduced insulin sensitivity. Surprisingly, upon administration of 17β-estradiol (E2, 0.0025mg/day) to 12 and 24wk female ArKO mice, we observed no improvement of the insulin resistance phenotype. Further, upon E2 administration to 12wk WT mice, we saw a similar development of insulin resistance as seen in the plus E2 treated ArKO mice. We hypothesise that high estrogen levels may have a negative effect on insulin sensitivity. An example of a physiological state in which estrogen levels are high is pregnancy. The National Institute of Health (NIH) estimate that 7% of women develop gestational diabetes with 40% increased risk of developing type 2 diabetes later in life. By 52wks no differences were observed between the ArKO and WT females. This maybe attributed to WT females now showing significantly increased basal glucose levels and reduced sensitivity to insulin. WT females at this age have completed natural cycling - a state similar to human 'menopause' with low E2 levels making them more hormonally similar to the ArKO mouse. Upon E2 administration to the 52wk old ArKOs there was an improvement of glucose and pyruvate tolerance and insulin sensitivity. We are currently elucidating the mechanism/s that underpins our observations.
Conclusion: Estrogen may have biphasic effects on insulin sensitivity in female animals.
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616

Estradiol derivative PE0607 decreases visceral obesity and improves insulin sensitivity in ovariectomized rats fed on a high fat diet
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Background and aims: It is believed that estrogen deficiency contributes importantly to the pathogenesis of menopausal metabolic syndrome and symptoms can be ameliorated with estradiol therapy Estrogens have been found to reduce coronary heart disease and to have favorable effects on glycaemic and lipid profiles. However, the risks of adverse health effects must be balanced against the benefits associated with hormone replacement therapy. The aim of the study was to assess the effects of novel estradiol derivative PE0607 (P) on the visceral fat accumulation and insulin resistance development in ovariectomized (OVX) rats fed on a high-fat diet (HFD).
Materials and methods: Wistar rats were divided into three groups: OVX group fed on a regular diet (OVX, n=8), OVX rats fed on a HFD for 4 months (OVX+HFD, n=8), and OVX rats treated with P (20 μg/kg/day per os) during the last 2 months of HFD feeding (OVX+HFD+P). Bilateral ovariectomy was applied to the animals under anaesthesia. At the end of the study fasted rats were subjected to the glucose tolerance test (GTT, 3 g/kg i.p.). Indexes of insulin resistance (IR) were determined using the homeostasis model assessment methods (HOMA) and quantitative insulin sensitivity check index (QUICKI). Plasma levels of non-esterified fatty acids (NEFA), triglycerides (TG) and alanine aminotransferase (ALT) were assessed as parameters of insulin resistance. Visceral fat weight was calculated as the sum of the retroperitoneal, mesenteric, and epigonadal fat pad weights.
Results: HFD induced obesity (body weight was increased by 52%) and severe insulin resistance in OVX rats, as indicated by higher HOMA-IR index (OVX+HFD: 8.91±0.70 vs OVX: 3.39 ±0.30, $p<0.001$) and lower QUICKI ($p<0.01$) indexes. The combined weight of the abdominal fat depots was threefold greater in the HFD animals compared with OVX rats on a regular diet ($p<0.001$). OVX+HFD group also developed glucose intolerance, hypertriglyceridemia, elevated NEFA levels, and ALT activities ($p<0.01$). Administration of P ameliorated HFD-induced glucose intolerance. Area under curve over the GTT was OVX+HFD+P: 1375±101 mM x hour vs OVX+HFD: 1837±145 $p<0.01$; OVX: 1071±176. The treatment with P decreased insulin resistance (HOMA-IR index OVX+HFD+P: 2.97±0.28, $p<0.01$), triglyceride concentration (OVX+HFD+P: 0.75±0.08 vs OVX+HFD: 1.38±0.27 mmol/l, $p<0.01$), NEFA levels and ALT activity ($p<0.02$).The reduction in body weight gain in OVX rats treated with P was accompanied by a significant reduction in visceral fat (OVX+HFD+P: 16.2±1.6 g vs OVX+HFD: 32.3±4.5 g, $p<0.001$) P. In addition, there were no differences in uterine weights between OVX rats treated with P or vehicle.
Conclusion: These data demonstrate that the novel estradiol derivative PEO607 ameliorate adiposity and insulin resistance in OVX rats fed on a HFD without impact on the uterine weight .We suggest that the use of PE0607 may be beneficial for the treatment of obesity and associated metabolic disturbances in postmenopausal women.

PS 44 Systemic inflammation in obesity and diabetes

617

PKCδ augments IL-6 signalling and proinflammatory response

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Background and aims: We have previously shown in differentiated 3T3-L1 adipocytes that insulin antagonizes interleukin-6 (IL-6) signaling and exerts anti-inflammatory effects by significantly reducing the Tyr-705 phosphorylation and nuclear translocation of the intracellular signaling molecule STAT3. Both insulin and IL-6 increase the Ser-727 phosphorylation of STAT3 but through two independent pathways. Ser-727 phosphorylation of STAT3 is important for the transcriptional activity of STAT3. The aim with the present study was to examine the importance of PKCδ for STAT3 phosphorylation and activation of proinflammatory genes in response to IL-6.
Materials and methods: Differentiated 3T3-L1 cells were cultured and pre-incubated with or without the PKCδ inhibitor rottlerin (6 μM) for 30 minutes before stimulation with insulin (100 nM) and/or IL-6 (20 ng/ml) for 30 minutes up to 24 hours. Wt MEFs and PKCδ-/- MEFs were incubated with IL-6 (30 ng/ml) and soluble IL-6 receptor (30ng/ml) for 60 minutes and 24 hours. RNA was extracted and the gene expression was analyzed with real-time RT-PCR (TaqMan) while the proteins were analyzed by immunoblotting. Transcriptional activity of STAT3 was analyzed by using a STAT3 transcription factor assay kit.
Results: Inhibition of PKCδ with rottlerin significantly reduced not only the Ser-727 phosphorylation, but also Tyr-705 phosphorylation of STAT3. As a consequence, both nuclear translocation of STAT3 and the IL-6-induced gene transcription of the inflammatory molecules SAA3 and haptoglobin were reduced, as well as the transcription of the negative feed-back inhibitor of IL-6, SOCS3. Similar to STAT3, we found that PKCδ translocated to the nucleus upon IL-6 addition and that rottlerin reduced this translocation. We also examined the importance of PKCδ for IL-6 signaling and action in Mouse Embryonic Fibroblasts (MEFs) lacking PKCδ expression. Similar to rottlerin, PKCδ-/- MEFs displayed a significantly reduced activation of SAA3, haptoglobin and SOCS3 compared to wt MEFs. These results correlated with an impaired nuclear translocation and a reduced Tyr-705 phosphorylation of STAT3.
Conclusion: Our results show that PKCδ plays a critical role for IL-6 signaling and action. PKCδ seems to be an interesting target for the development of anti-inflammatory agents.
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618

The role of macrophage migration inhibitory factor in obesity-associated type 2 diabetes

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Background and aims: Obesity-associated type 2 diabetes (T2D) develops as a consequence of inappropriate insulin action and is a state of persistent low grade inflammation. Both lipids and immune mediators are involved in maintaining this inflammatory process. Recently, elevated expression of pro-inflammatory cytokine macrophage migration inhibitory factor (MIF) has been associated with obesity and glucose intolerance. Our preliminary data indicate that mice with MIF gene knock-out (MIF-KO) kept on standard diet gain more weight then their wild type (wt) counterparts C57BL/6 mice. Introduction of high fat diet (HFD) provoked considerable obesity in C57BL/6 strain, while MIF-KO mice did not significantly increase their body weight. Furthermore, MIF-KO mice on HFD responded normally to glucose overload during intraperitoneal glucose tolerance test and preserved euglycemia compared to wt mice. Our next goal was to further examine involvement of MIF in the course of obesity-associated T2D induced by HFD.
Materials and methods: MIF-KO and C57BL/6 mice were fed a standard diet (10% fat) or HFD (60% fat) for 14 weeks. Pancreatic islets were isolated by collagenase V digestion and hand-picked. MIF mRNA expression in pancreatic islets from C57BL/6 mice was detected by Real-time PCR. Serum MIF and MIF secreted from cultured pancreatic lymph node cells (PLNC) were measured by ELISA. Serum insulin and insulin secreted from islets stimulated by 1.7 mM or 16.7 mM glucose was measured by ELISA, while triglycerides were determined spectrophotometrically after enzymatic digestion and oxidation.
Results: As expected, MIF serum level was elevated in C57BL/6 mice fed a HFD and accompanied with higher MIF expression in the islets and secretion in PLNC compared to mice on standard chow. This indicates a causal relationship between MIF and the development of obesity-associated T2D. Accordingly, MIF-KO mice which are inherently mildly obese did not respond to HFD as wt mice and maintained normal levels of triglycerides and insulin. In addition, when challenged with glucose MIF-KO mice on HFD secreted less insulin compared to C57BL/6 mice. Also, it was evident that pancreatic islets from MIF-KO mice on HFD preserved normal function compared to wt mice as observed by their better response in glucose-stimulated insulin release test.
Conclusion: Our results indicate that MIF is probably required for optimal manifestation of obesity-associated T2D in mice since lack of MIF ensures normal glucose, insulin and triglycerides levels. These data implicate a potential new approach for T2D treatment.
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619

Transgenic mice overexpressing C reactive protein show reduced insulin secretion and impaired glucose homeostasis

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Background and aims: Epidemiological studies indicate that increased plasma C reactive protein (CRP) levels are associated with type 2 diabetes and glucose metabolism disorders, and even predict the development of these diseases. We thus wished to investigate if the pro-inflammatory molecule CRP directly affects insulin sensitivity and glucose homeostasis *in vivo*.
Materials and methods: Transgenic mice(tgCRP) used in this study express the rabbit CRP transgene under the control of the PEPCK promoter. ELISA assays were used to evaluate CRP and insulin concentration in plasma obtained from transgenic and wild-type (WT) mice. Fed and fasting insulin and glucose levels have been assessed in 3- and 6-months old mice and the mice have also been subjected to intraperitoneal glucose (IPGTT) and insulin tolerance (ITT) and acute-phase glucose-stimulated insulin secretion (A-GSIS) tests. For high fat diet studies, mice were fed a diet fcontaining 60% kcal as fat or 24 weeks after weaning. Statistical differences were assessed by Student's t or two-ANOVA tests as appropriate
Results: We confirmed that TgCRP had serum CRP concentrations ranging between 10 and 20 μg/mL, comparable to those considered to confer high risk for atherosclerosis, insulin resistance, and type 2 diabetes in humans. We observed then a slight, but significant, increase of fasting glucose levels in 3-months-old TgCRP as compared with WT littermates (98±13 and 119±17 mg/dl for WT and TgCRP respectively, $p<0.05$, n=10-12), however fed glucose levels were unaltered and IPGTTs and ITTs showed that young TgCRP had a normal glucose metabolism. By contrast, 6 months-old transgenic mice had significantly increased fasting (108±14 and 128.9±36 mg/dl for WT and tgCRP respectively, $p<0.05$, n=10-12) and fed (135±30 and 160±29 mg/dl for WT and tgCRP respectively, $p<0.05$, n=10-12) glucose and significantly lower insulin(3.3±2.3 ng/ml and 2.33±1.2 $p<0.02$ for WT and TgCRP respectively, n=8-10). IPGTTs and ITTs revealed that 6 months-old TgCRP have developed glucose intolerance ($p<0.05$) and insulin resistance ($p<0.05$). Interestingly, when we performed A-GSIS tests to further elucidate the defects underlying this phenotype, we found that TgCRP mice release significantly less insulin at every time point examined ($p<0.01$), thus suggesting that the primary defect in TgCRP may be reduced insulin secretion. When we subjected mice to HFD for 24 weeks, TgCRP gained more weight than WT littermates (47.6±7.6 and 51.5±3.69 grams for WT and TgCRP respectively, $p<0.05$, n=6-8), and had significantly higher fed (204±55.32 and 257±50.28 mg/dl for WT and TgCRP respectively $p<0.05$, n=6-8) and fasting (133.6±31.9 and 149±30.38 mg/dl for WT and TgCRP respectively, $p<0.05$, n=6-8) glucose levels and significantly lower fasting insulin levels (2.2±1.3 and 2±1 for WT and TgCRP respectively $p<0.05$, n=6-8); IPGTT tests revealed that TgCRP develop a significantly higher glucose intolerance than their WT littermates ($p<0.05$, n=6-8).

Conclusion: Our data suggest that CRP may directly affect glucose metabolism and, notably, insulin secretion *in vivo*. TgCRP may thus represent a model to study the progression from a chronic low-level, inflammatory state to full-blown type 2 diabetes and to identify potential therapeutical intervention points to stop the progression of the disease.
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620

C-reactive protein mediates the association of liver fat and carotid intima media thickness in men with the metabolic syndrome and/or type 2 diabetes

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Background and aims: Liver fat content is closely related with features of the metabolic syndrome (MetS), including central obesity, dyslipidaemia and glucometabolic disorders. Liver fat is also associated with increased risk of cardiovascular disease (CVD). Mechanisms underlying this association are incompletely understood. The liver-derived pro-inflammatory marker C-reactive protein (CRP), has been associated with increased CVD risk. We assessed the inter-relationships of liver fat, subclinical atherosclerosis and CRP in men with uncomplicated type 2 diabetes (T2DM) and/or the MetS and healthy controls.
Materials and methods: Liver fat was quantified by proton magnetic resonance spectroscopy in 16 men with known T2DM (mean±SD age 55.4±4.8 yrs), 36 men with the MetS, according to IDF criteria, (55.5±7.0 yrs) and 21 age-matched healthy men (56.8±7.4 yrs). High liver fat content was defined as liver fat percentage ≥5.56%. Carotid intima media thickness (cIMT) was measured by ultrasound. Relations of liver fat, cIMT and high sensitivity CRP were analyzed by univariate and multivariable regression.
Results: Median (IQR) liver fat percentage was highest in T2DM, as compared to MetS and controls [17.8% (9.4-39.8); 8.2% (4.2-14.2); 5.4% (2.4-8.4), respectively; ANOVA $P<0.001$]. Subjects with high versus low liver fat content had higher cIMT ($P=0.050$) and CRP ($P=0.001$). In all subjects, liver fat content was associated with cIMT (r=0.253; $P=0.039$) and CRP (r=0.425; $P<0.001$). This association between liver fat content and cIMT disappeared after adjustment for CRP. The association of cIMT with CRP (r=0.322; $P=0.011$) remained unchanged after adjustment for age, BMI, waist, blood pressure, HbA1c, fasting plasma glucose, or triglycerides.
Conclusion: Liver fat content is associated with carotid artery subclinical atherosclerosis in men with the MetS and/or uncomplicated T2DM. Since this association disappeared after correction for plasma CRP we suggest that this circulating inflammatory marker mediates the relationship between liver fat and subclinical atherosclerosis in this high risk population.

621

Plasma C-reactive protein, apolipoprotein B and triglycerides/HDL-cholesterol ratio as cardiometabolic risk factors in adolescents with and without metabolic syndrome

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Background and aims: The prevalence of the metabolic syndrome (MS) has increased worldwide. That imposes a substantial risk for type 2 diabetes and premature cardiovascular disease in adults. However to date no unified criteria exist to assess risk or outcomes in children and adolescents. The objetive was to establish the prevalence of MS and risk factors for cardiovascular disease in adolescents.
Materials and methods: Adolescents from high school were studied (n=943), 429 females and 514 males, ages range from 11 to 14 years old. Weight, height, body mass index (BMI), waist circumference (WC), and blood pressure were determined in all patients. Glycemia, lipid and lipoprotein profile, apolipoprotein B (Apo B) and high sensitivity C-reactive protein (hs-CRP) levels were measured after an overnight fast of 12 hours. Triglycerides/HDL-Cholesterol ratio (TG/HDL) was calculated.. Metabolic syndrome was diagnosed according to the NCEP: ATP III criteria modified by Cook et al. A parent or guardian gave consent for participation. The protocol was approved by the ethics committee.
Results: Prevalence of metabolic syndrome was 5.45% in the male group and 1.63% in the female group. MS group presented higher TG/HDL ratio than those without MS, median (range): 3.58 (0.94-7.4) vs 1.38 (0.63-7.59), p<0.001; no differences in hs-CRP were observed between groups: 1.7 mg/L (0.1-8.3) *vs* 1.20 mg/L (0.1-8), respectively. Apo B (mean±SD) was 74.1 ± 6.3 mg/dl in MS group and 46 ± 15.7 mg/dl in those without MS (p= 0.001). TG/HDL ratio positively correlated with BMI (r=0.18, p <0.001), WC (r=0.24,p<0.001), and Apo B (r=0.24, p<0.001). Although no differences between groups were observed, hs-CRP correlated with WC (r=0.14, p<0.001) and BMI (r=0.17, p< 0.001).
Conclusion: Even when prevalence of MS in our patients was low, in those patiens who had MS atherogenic lipoprotein parameters were higher. However the inflamatory marker was not different between groups. The risk to develop cardiovascular disease was clearly higher in the group with MS than in the group without MS. These findings emphasize the importance to evaluate cardiovascular risk factors in adolescents to assess strategies to prevent future disease.

622

Metabolic endotoxaemia and saturated fat contributes to circulating NGAL concentrations in subjects with insulin resistance

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Background and aims: Lipocalin-2 (NGAL) is an innate immune system protein linked to insulin resistance and obesity, but the mechanisms behind these associations are poorly known. The aims of this study were 1) to evaluate the cross-sectional association among serum NGAL concentration, circulating inflammatory markers and insulin sensitivity; 2) To evaluate the effects of weight loss-induced changes in insulin sensitivity on circulating NGAL concentration and diurnal NGAL rhythm; 3) to test the acute effects of saturated fatty acids on circulating NGAL concentration according to insulin resistance status; and finally, 4) to investigate the effects of LPS on NGAL concentration in adipose tissue explants and whole blood.
Materials and methods: We studied 4 cohorts: 1) a cross-sectional study in 194 subjects (109 with normal glucose tolerance and 85 with type 2 diabetes); 2) the changes in NGAL concentration induced by diet and weight loss in 36 obese women (with circadian rhythm in 8 of them); 3) the effects of acute fat intake on circulating NGAL concentration in 42 morbidly obese subjects; and 4) LPS-induced NGAL secretion e*x vivo* (whole blood and adipose tissue explants).
Results: In NGT subjects, circulating NGAL concentration was significant lower than in T2D subjects (61.1 ± 25.9 vs. 99.04 ± 52.3 ng/ml, p<0.001). Serum NGAL concentration was significantly associated with fasting triglycerides, sTNFR2, and lipopolysaccharide (LPS) binding protein) in patients with type 2 diabetes. In obese subjects, the intake of saturated fatty acids contributed independently to 14 % of NGAL variance. Weight loss changed significantly the circadian rhythm of NGAL. The acute increase in circulating NGAL after fat-overload was significantly associated with fasting insulin (r=0.52, p<0.001), HOMA-IR (r=0.36, p=0.02) and post-load triglyceride concentrations (r=0.38, p=0.018).). In ANOVA analysis, we found a significant association between the changes in circulating NGAL and HOMA-IR status (p=0.03). LPS-induced NGAL secretion from adipose tissue explants did not change significantly but LPS led to a significant increase in NGAL concentration in whole blood from patients with type 2 diabetes (1.86 ± 0.12-fold, p= 0.003).
Conclusion: In summary, metabolic endotoxemia and saturated fat contributes to circulating NGAL concentration in patients with insulin resistance.
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623

Inflammation markers and metabolic characteristics of subjects with 1-hour hyperglycaemia

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Background and aims: Chronic subclinical inflammation may be associated to a worsening of glucose metabolism and cardiovascular disease risk factors (CV risks) such as pre-diabetes (pre-DM). Recently, 1-hour hyperglycaemia (1hPG) after an oral glucose load (OGTT) with a cut point of 155 mg/dl, has been indicated as a risk factor to develop type 2 diabetes. Aim of this study is to evaluate the association of 1hPG and inflammation markers in subjects with normal glucose tolerance (NGT) and pre-DM.
Materials and methods: We examined a consecutive series of 1549 subjects aged 44.8±14.2 years with a body mass index (BMI) of 34.8±7.3 kg/m^2 with no history of diabetes or inflammatory diseases and without drugs interfering with glucose or lipid metabolism, seeking treatment for overweight or obesity. Glucose tolerance was determined after overnight fasting by a standard OGTT (75 g) with blood samples at 0', 60', 120' for glucose and insulin; the cut point of 1hPG>155 mg/dl was applied for each subject, subdividing all patients with NGT and pre-diabetes (impaired fasting glucose and impaired glucose tolerance) above or below the cut off of 1hPG: NGT-high, NGT-low, pre-DM-high and pre-DM-low. Insulin resistance were evaluated by HOMA2-IR computer model. A lipid profile for total and HDL cholesterol (HDL-C) and triglycerides was performed. Triglycerides/HDL cholesterol ratio (Tg/HDL-C) >3.5 and total cholesterol/HDL-C ratio (TC/HDL-C) >5 were considered for CVD risk; moreover, uric acid, and inflammatory markers such as fibrinogen and leucocytes count (WBC) were determined.
Results: In the sample studied, according to American Diabetes Association criteria, 46.2% subjects were NGT, 40.5% with pre-diabetes and 13.2% with type 2 diabetes. NGT and pre-DM subjects with 1hPG>155 mg/dl showed a significant increase (p<0.05) of inflammatory markers versus those patients below 1hPG cut point. In age-sex-BMI-adjusted analysis, 1-hour hyperglycaemia is associated with a significant high levels of WBC count and fibrinogen. Tg/HDL ratio was significantly higher in NGT-high vs NGT-low (3.6±2.8 vs 2.8±2.4, p<0.05) such as TC/HDL ratio (4.6±1.5 vs 4.2±1.4, p<0.05). In patients with pre-DM, those with pre-DM-high showed a significant higher levels of Tg/HDL ratio (4.2±3.8 vs 3.5±2.4, p<0.05) and TC/HDL ratio (5.0±1.6 vs 4.5±1.5, p<0.05) than pre-DM-low individuals. The distribution of subjects with high levels of lipid ratios shows a significant increase of prevalence of 1hPG-high versus 1hPG-low groups. In particular, in the category of NGT, subjects with Tg/HDL ratio>3.5 were 38.6% vs 17.7% (1hPG-high vs 1hPG-low) and TC/HDL>5 patients were 34.0% vs 22.3%, respectively. The two categories of 1hPG for the pre-DM group showed 41.3% vs 30.3% of patients with Tg/HDL ratio>3.5 and 43.7% vs 34.0% of subjects with TC/HDL>5. In NGT and pre-DM subjects, no significant difference has been shown for HOMA2-IR levels between 1hPG-high and 1hPG-low groups.
Conclusion: High 1hPG in normal glucose and pre-DM state is associated to an higher levels of inflammatory markers and lipid ratios exposing those subjects to an increase CV risk, despite no significant difference in insulin resistance levels.

624

Increased levels of proinflammatory cytokines are associated with impaired immune activity of natural killer (NK) cells of prediabetic subjects (PS)

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Background and aims: Inflammatory mechanisms are believed to be involved in the pathogenesis of diabetic complications. Diabetic hyperglycemia may suppress the function of peripheral blood NK cells. Recent experimental studies have shown significant differences in immune activity of NK cells of Type 2 diabetic patients in comparison to healthy subjects. The aim of this study was to asses the relation between the plasma concentration of selected proinflammatory cytokines (interleukin 1; IL-1 and interleukin 6; IL-6) and the number and activity of NK cells obtained from PS.
Materials and methods: The study group included 12 newly-diagnosed (according to WHO definition) PS, naive to any hypoglycaemic drugs and 8 normoglycaemic control subjects (CS) matched for sex, BMI and waist circumference. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll gradient centrifugation. Immunofluorescent phenotyping of NK (CD16$^+$) cells in PBMC was performed using specific murine anti-human CD16 PE-conjugated monoclonal antibodies. The K562 human erythroleukemia cell line was used as the standard target for human NK cytotoxicity assay. K562 were labelled with DIO(3,3-dioctadecyloxacarbocyanine perchlorate). Target and effector cells were added to reach effector/target rations: 50:1 ,12:1. Dead target cells were stained with PI (propidium iodide). After 4 hours of incubation data were collected for analysis on the Becton-Dickinson FACScalibur flow cytometer. The data was analyzed using Cell Quest software. Concentrations of IL-1 and IL-6 were determined using fluorescent cytometric microbead assay. Plasma aliquots were incubated with color-coded fluorescent microbeads labeled with antibodies against respective cytokines. The fluorescence intensity was determined using dual-laser detector and cytokines concentrations were read from standard curve.
Results: PS exhibited higher insulin resistance reflected by HOMA-IR (3.21±2.32) compared to control group (1.96±1.18, p=0.03). Plasma concentrations of investigated cytokines were significantly higher in the PS compared to CS (IL-1: 1.36±1.27 vs 0.35±0.31 pg/ml, p=0.01; IL-6: 4.09±3.30 vs 2.12±1.13 pg/ml, p=0.03). Correlations between cytokines and HOMA-IR were weak and non-significant (all p>0.05). The PS in comparison to healthy subjects had an increased number (12,45±5,8% vs 9,63±4,7%) but decreased activity (4,4±2,0% vs 9,2±3,7%) of NK cells (p=0.01). Correlations between cytokines and both the number and activity of NK cells were statistically significant (p<0.05).
Conclusion: In the prediabetic state the increased number and decreased activity of peripheral NK cells are associated with high excretion of proinflammatory cytokines (IL-1, IL-6). This supports the hypothesis that inflammation is involved in the pathogenesis of early impairment of NK cell killing function. Inflammation markers are likely to be useful in the screening of metabolically vulnerable individuals.

625

Matrix metalloproteinases in type 2 diabetes and in non-diabetic controls: effects of short-term and chronic hyperglycaemia

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Background and aims: Matrix metalloproteinases (MMPs) remodel extracellular matrix in various physiological and pathological conditions, including cancer, inflammatory states and cardiovascular disease. The role of MMPs in type 2 diabetes (DM) is not clear as increased activation of MMPs in vasculature contrasts with decreased activity of MMPs in kidneys, thus contributing to the development of diabetic nephropathy. In our study we have aimed to assess the effects of transient *versus* chronic hyperglycaemia on concentrations of MMPs.
Materials and methods: We measured serum MMP-2, MMP-9 in 22 subjects with type 2 DM, age (mean±SD) 56.7±16.8 years, BMI 31.8±4.6 kg/m^2, HbA1c 8.45±1.78% and in 32 Controls, age 39.2±16.0 years, BMI 35.2±8.5 kg/m^2. In 15 subjects with type 2 DM we also measured MMP-2 and -9 at discharge from hospital and after three months (n=8). In a subgroup of Controls MMP-2 and -9 were also measured during 75 gram oral glucose tolerance test (OGTT).
Results: Concentrations of MMP-2 and MMP-9 were lower in subjects with type 2 DM (219±62ng/ml *versus* 305±63ng/ml and 716±469ng/ml *versus* 1285±470ng/ml, for MMP-2 and MMP-9, respectively, p<0.05). Control subjects were younger (p<0.05), however, without statistical difference in BMI between the groups. MMP-9 concentrations fell at 120 minutes of OGTT from 1675±372 ng/ml to 1276±422 ng/ml, p<0.05). In diabetic subjects there was a correlation between MMP-9 and HbA1c (r=0.51, p<0.05). In patients with diabetes there was no difference in MMP-2 at the time of admission *versus* discharge from hospital with a trend towards lower MMP-9 (p=0.08, n=15) at discharge, that became significant at three months post-discharge (p<0.05).
Conclusion: Concentrations of MMP-2 and MMP-9 were lower in subjects with type 2 diabetes than in non-diabetic controls. Regulation of MMPs appears to be, however, complex as hyperglycaemia during OGTT results in a decrease in MMP-9, while chronic hyperglycaemia, reflected by HbA1c, correlates with MMP-9 concentrations in subjects with type 2 diabetes.
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626

Serum IL-18 concentration is increased in obese subjects with impaired glucose tolerance and related to the markers of atherogenesis

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Background and aims: IL-18 is considered as a proinflammatory and proatherogenic factor. Therefore the aim of the present study was to asses the serum IL-18 concentration in relation to insulin sensitivity and markers of endothelial dysfunction in obese subjects with normal (obese-NGT) and impaired glucose tolerance (obese-IGT).
Materials and methods: The study group consisted of 87 lean subjects with NGT (control group), 120 obese-NGT and 41-obese IGT. In the entire group OGGT, euglycemic hyperinsulinemic clamp and estimation of serum concentration of IL-18, soluble intercellular adhesion molecule 1 (sICAM-1) and soluble E-selectin (sE-selectin) were performed.
Results: Serum IL-18 concentration was the highest in obese-IGT group (327.9±132.8 pg/ml vs 282.9±123.9 pg/ml, p= 0.01 in obese-NGT and 238.3±121.3 pg/ml, p=0.002 in the control group). Obese-IGT subjects had also the lowest insulin sensitivity and the highest concentration of sICAM-1 and sE-selectin (all p<0.05 for comparisons with obese-NGT and control group). In the entire group serum IL-18 was inversely correlated with insulin sensitivity (r=-0.32, p<0.0001), HDL-cholesterol (r=-0.022, p<0.0001) and positively related to systolic (r=0.19, p=0.003) and diastolic blood pressure (r=0.18, p=0.004), fasting glucose (r=0.25, p<0.0001), TG (r=0.39, p<0.0001), sICAM-1 (r=0.41, p<0.0001), sE-selectin (r=0.325, p<0.0001). All these correlation were also present in all subjects with NGT. In the obese-IGT group, serum-IL-18 were related to fasting glucose (r=0.47, p=0.002), insulin (r=0.325, p=0.046), sICAM-1 (r=0.72, <0.0001) and sE-selectin (r=0.33, p=0.037).
Conclusion: Our data indicate that IL-18 might contribute to the proatherogenic state observed in IGT.
Supported by: Medical University of Bialystok, Poland

PS 45 Adipose tissue and adipocytes

627

Expression of the bone morphogenetic protein receptor 2 (*BMPR2*) mRNA in adipose tissue and *BMPR2* genetic variation are related to human obesity

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Background and aims: The human bone morphogenetic protein receptor 2 (BMPR2), a family member of transmembrane serine/threonine kinases, is one of the specific BMP-receptors essential for BMP signalling during adipogenesis, osteogenesis and myogenesis. We hypothesized that *BMPR2* mRNA expression in adipose tissue and genetic variation could be related to the pathophysiology of human obesity.
Materials and methods: *BMPR2* mRNA expression was measured in 198 human paired samples of visceral and subcutaneous adipose tissue. The mRNA levels were compared between lean and obese subjects. To determine, whether genetic variants within the *BMPR2* are associated with mRNA expression in fat and whether they are related to measures of obesity and fat distribution, we sequenced the *BMPR2* in DNA samples from 48 non-related subjects from Eastern Germany. Ten representative variants including HapMap tagging single nucleotide polymorphisms (SNPs) were genotyped for subsequent association studies on quantitative traits in 1808 subjects from Eastern Germany with a wide range of BMI distribution and glucose tolerance.
Results: Expression of *BMPR2* mRNA was significantly increased both in visceral and subcutaneous fat of 51 overweight (BMI 25 - 30 kg/m^2) and 91 obese (BMI > 30 kg/m^2) subjects compared with 56 lean subjects (BMI < 25 kg/m^2) (P<0.001). In a case control study including 461 lean and 678 obese subjects, two intronic SNPs (rs6717924 and rs13426118) were significantly associated with obesity (P<0.05, adjusted for age and sex). Consistent with this, we found significant associations of *BMPR2* SNPs with BMI, % body fat and circulating serum leptin (adjusted P<0.05) in 896 non-diabetic subjects. Finally, the rs6717924 was significantly associated with *BMPR2* mRNA expression levels in visceral fat (P=0.05, adjusted for age, sex and BMI).
Conclusion: Our data suggest a role of *BMPR2* in the pathophysiology of human obesity. The association of genetic variants with obesity might be mediated via effects on *BMPR2* mRNA expression in fat.

628

Thyroid hormone responsive Spot 14 increases during differentiation of human adipocytes and its expression is down-regulated in obese subjects

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Aims: Thyroid hormone responsive spot 14 (THRSP or S14) has been postulated to be involved in lipogenesis but very limited information regarding its role in adipogenesis and human adiposity is available. We aimed to evaluate S14 expression in human adipocytes and in fat samples from a cohort of subjects who varied widely in terms of obesity and body fat distribution.
Materials and methods: We evaluated S14 during differentiation of human pre-adipocytes into mature adipocytes using an automated confocal imaging approach. We also studied the relative levels of S14 mRNA (assessed by RT-PCR) in stromal-vascular cells (SVCs) and mature adipocytes (MAs) isolated from human fat biopsies. In parallel, we performed a cross-sectional study in which S14 gene expression levels were measured in 161 visceral and 87 subcutaneous adipose tissue samples.

Results: We report the occurrence of S14 up-regulation during the early stages of differentiation of human pre-adipocytes accompanied by cytoplasmic accumulation of fatty acid synthase. These observations were most prominent in those individual cells exhibiting the more marked differentiation features. In agreement with these findings, S14 gene expression increased by ~130-fold from pre-adipocytes to mature adipocytes. Interestingly, S14 levels were decreased in visceral adipose tissue from overweight (-42.0%) and obese subjects (-56.5%) compared with lean subjects ($p<0.05$ and $p<0.0001$, respectively). S14 gene expression was inversely associated with obesity measures such as body mass index ($p=0.001$), percent fat-mass ($p=0.001$), waist-to-hip ratio ($p=0.02$) and with systolic blood pressure ($p=0.03$). Otherwise, increased S14 mRNA levels were found in stromal-vascular cells/pre-adipocytes (3.8-fold, $p<0.05$) and in whole adipose tissue samples (1.9-fold, $p<0.0001$) from subcutaneous compared with visceral fat depots.
Conclusion: These results suggest that S14 is involved in human adipogenesis. The inverse association between S14 gene expression levels and obesity measures hints at a negative feed-back among adiposity, adiposity-related disorders and S14 gene expression levels.
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629

Insulin increases natriuretic peptide clearance receptor expression in the subcutaneous fat depot in obese subjects: a clamp study
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Background and aims: Atrial natriuretic peptide (ANP) is a cardiac hormone that protects cardiovascular integrity through different mechanisms. Moreover ANP has multiple metabolic properties and it was recently discovered to induce lipolysis. Obese subjects have reduced levels of circulating ANP for unknown reasons. An intensive weight reduction enhanced circulating ANP in obesity. Our recent observation suggested that insulin suppresses ANP levels in humans. To investigate a possible regulation of natriuretic peptide receptors (NPRs) expression by insulin, we conducted hyperinsulinemic, eu- and hyperglycemic clamp experiments on obese subjects and determined the concentrations of circulating midregional proANP53-90, a stable prohormone fragment co-released with mature ANP from proANP, and investigated the expression of three types of NPR´s in subcutaneous fat tissue.
Materials and methods: Hyperinsulinemic, euglycemic (EC) (4.4 mmol/l for 240 min, n=10) and hyperinsulinemic, hyperglycemic clamps (7.8 mmol/l for 240 min, n=7) were conducted on healthy obese non diabetic men. Natriuretic peptide receptors A, B and C were determined by QPCR in biopsies of subcutaneous fat tissue (-40 and 240 min of the clamp). Insulin and MR-proANP53-90 levels were assessed at -70, 120 and 210 min of the clamp.
Results: MR-proANP53-90 levels decreased under insulin infusion at 120 and 210 min in both clamps ($p<0.05$ for EC and $p<0.01$ for HC). Natriuretic peptide clearance receptor expression (NPRC) was increased 1.75 fold under insulin infusion in EC ($p=0.04$) and 1.3 fold in HC ($p=0.02$). A trend to an increase of NPRA expression was observed in HC ($p=0.07$) but not in EC ($p=0.5$). NPRB expression did not change in both clamps.
Conclusion: Acute insulin infusion increases expression of NPRC in subcutaneous fat tissue independent of glucose concentration in obese subjects. Insulin thus may increase the elimination of ANP via regulation of NPRC and suppresses the secretion as shown by decreased MR-proANP53-90.
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630

Adipocyte lipid-binding protein (ALBP) in subjects with different categories of glucose intolerance during acute hyperinsulinaemia
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Background and aims: Adipocyte lipid-binding protein (ALBP) has been shown to regulate lipid metabolism and to affect thereby insulin sensitivity. However, its regulation in human obesity and different stages of glucose intolerance has not been clarified yet. Our aims were to evaluate plasma concentrations of ALBP and its expressions in subcutaneous adipose tissue (SAT) during acute hyperinsulinaemia in subjects with different categories of glucose intolerance.
Materials and methods: 12 subjects with impaired glucose homeostasis (IGH), 11 patients with type 2 diabetes (D) and 11 age-matched healthy men - controls (C), underwent 2-hour hyperinsulinaemic (1 $mU.kg^{-1}.min^{-1}$) euglycaemic (5 mmol/l) clamp to verify insulin sensitivity. Plasma concentrations of ALBP were measured at 0 and 120 min of the clamp. Needle biopsy of abdominal subcutaneous fat was performed (0 and 30 min of the clamp) to assess the ALBP expression using the real-time PCR method. As a reference gene human cyclophilin was used. For statistical analysis ANOVA with repeated measures was used.
Results: Insulin sensitivity estimated as metabolic clearance rate of glucose (MCR) was significantly higher in C compared to D and IGH, whereas IGH did not differ from D: MCR (IGH: 4.47±0.66 vs. D: 4.88±0.49 vs. C: 9.12±1.0 $ml.kg^{-1}.min^{-1}$; $p<0.001$ vs. C). Other anthropometric and metabolic parameters (fasting serum lipids, waist circumference, BMI) were comparable between IGH and D, and lower in C compared to other groups. Plasma concentrations of ALBP were lowest in C compared to D and IGH ($p<0.001$) at baseline as well as during the clamp, there were no significant differences between D and IGH. Relative expressions of ALBP were highest in D compared to IGH and C ($p<0.001$) both at baseline and during the clamp; the differences between IGH and C were not significant. ALBP plasma concentrations significantly correlate with its expression in SAT ($r=+0.45$; $p<0.05$), BMI ($r=+0.64$; $p<0.001$), waist circumference ($r=+0.70$; $p<0.001$), fasting plasma glucose ($r=+0.49$; $p<0.01$) and fasting insulinaemia ($r=+0.38$; $p<0.05$) and negatively correlate with MCR ($r=-0.42$; $p<0.05$). Significant positive correlations were found between ALBP expression and BMI ($r=+0.46$; $p<0.01$) or fasting plasma glucose ($r=+0.60$; $p<0.001$).
Conclusion: Our results support the hypothesis that ALBP expression and secretion are differentially regulated in different source tissues and depots during the progression of glucose intolerance: while ALBP plasma concentrations were comparable in IGH and D, the ALBP expressions in SAT were higher in D, which suggests the involvement of other important source of circulating ALBP in IGH than SAT. Our results also show the association of plasma ALBP with parameters of obesity, insulin resistance and dysregulation of glucose metabolism.

ALBP plasma concentrations (ng/ml) and relative expression in SAT. Data expressed as mean±SEM.

Group	p-ALBP (ng/ml)		Relative expression (AU) ALBP mRNA/cyclophilin mRNA	
	0 min	120 min	0 min	30 min
IGH	24.14±2.84	25.31±3.50	1062±114.3	734±70.03
D	22.61±2.77	19.68±1.99	2024±194.8	2382±330.3
C	12.92±1.89	13.04±1.76	769.4±77.46	730±86.23

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631

Histone lysine demethylase LSD1 is required for adipogenesis
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Background and aims: Obesity has reached epidemic proportions worldwide, thus prompting a renovated interest on the mechanisms regulating the differentiation and function of the adipose cells. However, although the hormonal and transcriptional cascades involved in adipogenesis have been thoroughly examined, much less is known about the role of epigenetic phenomenons on the differentiation of adipocytes. We have previously shown that the promoters of key adipogenic genes display significant levels of dimethylation at the Lys 4 of histone H3 (H3K4Me2) in preadipocytes, where those genes are not yet expressed, even though H3K4Me2 is considered to be an activating epigenetic mark. Thus, we were interested in elucidating the role of histone methylation and the enzymes involved in regulating its turnover on adipogenesis.
Materials and methods: We knocked down the expression of different regulators of histone methylation in 3T3-L1 preadipocytes by means of siRNA transfections. We studied the effects of knocking down those regulators upon adipogenesis, as well as upon the expression (measured by real time RT-PCR) and chromatin environment (by chromatin immunoprecipitation assays) of key adipogenic promoters.

Results: We focused on the histone demethylase LSD1, which has been shown to demethylate both the positive signal H3K4Me2 and the repressive mark H3K9Me2, thus acting either as a repressor or an activator, depending on context. Our results show that adipogenesis in the absence of LSD1 is severely impaired, as measured by lipid accumulation and the expression of mature adipocyte-specific genes. This outcome is associated with decreased H3K4Me2 (37%, p<0.0005) and increased H3K9Me2 (235%, p<0.0005) levels at the promoter of the key adipogenic transcription factor *Cebpa*, which is silenced in preadipocytes but whose expression increases exponentially during adipogenesis. Our data suggests that LSD1 acts as a H3K9 demethylase in this promoter, maintaining low levels of this repressive mark and probably counteracting the action of a H3K9 methyltransferase. We tested the possible involvement of the H3K9 methyltransferase SETDB1, since it has previously been shown to block adipogenesis in pluripotent precursor cells. In 3T3-L1 preadipocytes, both LSD1 and SETDB1 are bound to the *Cebpa* promoter, but the recruitment of LSD1 increases after the start of differentiation, whereas the recruitment of SETDB1 decreases. Knocking down SETDB1 increases H3K4Me2 (202%, p<0.0005) and decreases H3K9Me2 (48%, p<0.001) at the *Cebpa* promoter, thus producing the opposite results to LSD1 knock down and suggesting that both factors might be involved in the same regulatory pathway.
Conclusion: Our data suggest that the expression and function of the proteins involved in the regulation of histone methylation play a crucial role in the correct differentiation of adipocytes. Specifically, the histone demethylase LSD1, which is currently being explored as a possible target for cancer treatment and has also been shown to be involved in diabetes and inflammatory pathways, is shown to be crucial for adipogenesis, thus offering new insights into the function of this pleiotropic transcriptional regulator and providing new targets for the treatment of diabetes and obesity.
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632

Orexin regulates glucose and lipid metabolism - *in vitro* study on isolated primary rat adipocytes and 3T3-L1 cells
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Background and aims: Orexin regulates energy expenditure, food intake and sleeping behavior. Lack of orexin/orexin receptors is associated with increased body mass index and type 2 diabetes mellitus. Recently, we and others demonstrated that orexin receptors (orexin receptor 1 and 2) are expressed in the endocrine pancreatic islets and in adipose tissue in rodents. It is unknown, whether orexin regulates adipose tissue functions. We therefore studied the role of orexin in regulating the lipid and carbohydrate metabolism in adipocytes.
Materials and methods: We used isolated primary rat adipocytes and 3T3-L1-cells that were differentiated into adipocytes. We studied the short - (8 hours) and long-term (up to 72 hours) effects of orexin A (OXA) on lipogenesis, lipolysis, glycerol secretion, glucose uptake and ATP levels. Furthermore, we characterized the effects of OXA on adiponectin and resistin secretion and expression using RIA and Western blot analysis.
Results: We detected orexin receptor expression in both, differentiated 3T3-L1 cells and primary rat adipocytes, with OX1R, as the predominant receptor subtype. Isolated adipocytes showed higher rate of lipogenesis after 2 hours of exposure to OXA, as compared to non-treated cells. In addition, OXA potentiated the lipogenic effect of insulin. OXA increased intracellular triglyceride content after 24 and 48 hours of incubation and potentiated the effect of insulin. OXA reduced glycerol secretion from adipocytes. OXA time-dependently increased glucose uptake by adipocytes and the intracellular ATP content. In addition, OXA increased the secretion and expression of adiponectin, while the secretion of resistin was attenuated.
Conclusion: We identify orexin A as an anabolic factor which enhances glucose uptake and lipogenesis. In addition, orexin enhances the effects of insulin on lipid and glucose metabolism, suggesting possible insulin sensitizing activity. Furthermore, orexin stimulates adiponectin and inhibits resistin secretion. In summary, the effects of orexin on adipose tissue may represent mechanisms, by which orexin lowers hyperglycemia, an effect previously reported in animal models of type 1 and type 2 diabetes mellitus.

633

Adiponectin independently improves endothelial dysfunction in obese rats
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Background and aims: Hypoadiponectinemia has been proved to be closely related to vascular endothelial dysfunction and is thought to be an independent risk factor for cardiovascular disease. The aim of this study was to determine whether adiponectin might independently improve endothelial dysfunction in aorta isolated from high-fatty diet induced obese rats.
Materials and methods: Two weeks aged male SD rats were assigned to control group (fed with normal chow) and OB group (fed with high-fat food). Aortic rings were extirpated at the end of six weeks and the rings obtained from OB group were sub-divided into three groups and incubated with vehicle (OB-C group), globular adiponectin (OB-gAd group), or adiponectin plus L-NAME, the inhibitor of NO synthase, (OB-gAd-NAME) for 2 hr. The rings were placed into an organ bath and endothelial dependent or independent relaxation was tested with acetylcholine (Ach) or sodium nitroprusside (SNP). Total NO production (NOx) by aortic segments was determined by measuring the concentration of nitrite.
Results: Severe endothelial dysfunction (maximal vasorelaxation in response to ACh: 24.5±6.1 vs. 84.1±3.2% in control, $P< 0.05$) was observed in OB-C group aortic segments, and treatment with gAd significantly improved endothelial function (49.6±7.4%, $P<0.05$). Concentration-dependent vasorelaxation in response to SNP, an endothelium-independent vasodilator, remained unchanged in these vessels. Total NO production was decreased in OB-C group vessels (6.39μmol/mg dw, vs. 13.47μmol/mg dw in control, $P< 0.01$) and treatment with gAd increased total NO production in these vessels (10.53μmol/mg dw, $P< 0.05$). Addition of L-NAME completely blocked vasorelaxation in response to ACh and NOx production in those vessels pretreated with gAd.
Conclusion: Impaired endothelial dependent vasodilatation is one of the hallmarks of vascular injury in patients with metabolic syndrome. The present study demonstrated that adiponectin is a unique cytokine that may directly and independently improves endothelial function by enhancing NO production. Reduced adiponectin production and/or development of adiponectin resistance in patients with metabolic syndrome may play a critical pathogenic role in atherosclerosis.
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634

TWEAK/Fn14 axis is related to hypoxia and endoplasmic reticulum stress markers
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Background and aims: Inflammation is a response to oxidative stress, endoplasmic reticulum (ER) stress and local hypoxia. These processes are highly integrated and work in vicious cycles. Tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) is a cytokine that controls many cellular activities and is a new player in the inflammatory processes. TWEAK acts by binding to Fn14, a highly inducible cell-surface receptor that is linked to several intracellular signalling pathways, including NF-κB pathway. Fn14 mRNA has been found to be increased in severely obese type 2 diabetic patients. It has been reported a preponderant role of TWEAK and Fn14 in brain ischemic injuries in human patients. TWEAK can stimulate angiogenesis. We hypothesize that TWEAK and Fn14 could act as pro-inflammatory cytokines in human obesity; been in time with micro-hypoxic events in adipose tissue. We aimed to explore the role of TWEAK mRNA expression in obesity and to investigate its relation to hypoxia and ER stress genes markers.
Research design and methods: *Obese cohort:* 19 lean, 28 overweight, and 15 obese non-diabetic subjects. *Severely obese cohort:* 23 severely obese subjects. *Type 2 diabetes (T2D) cohort:* composed by 11 type 2 diabetic obese subjects. RNA from subcutaneous (SAT) and visceral (VAT) from 96 paired samples were obtained. Gene expression relative and quantification was assayed for: *Fn14*, *TWEAK*, *VISFATIN HYOU1*, *FIAF*, *HIF-1a*, *VEGF*, *GLUT-1*, *GRP78*

and *XBP-1*. Statistical analysis was performed by using the SPSS statistical package.
Results:
TWEAK/Fn14 expression
In obesity: *TWEAK* mRNA levels were significantly high expressed in SAT (p<0.001) whereas *Fn14* was predominantly expressed in VAT samples (p<0.001). *TWEAK* and *Fn14* expression levels both in SAT and VAT depots did not change among obesity groups.
The presence of T2D did not alter the expression of *TWEAK* and *Fn14* in any depot.
In severe obesity: *TWEAK* and *Fn14* mRNA were significantly over-expressed when compared to its control group independently of the adipose depot.
TWEAK /Fn14 expression in relation to hypoxia and ER
In obese and T2D cohorts
Regression analysis showed that SAT *XBP-1* expression levels (B=0.514 p<0.001) predicted the SAT *TWEAK* expression. The presence of T2D did not affect the results of the model. VAT *TWEAK* was predicted by WHR (B=0.007 p=0.006), and SAT *FIAF* (B=0.110 p=0.032).
SAT *Fn14* expression was only predicted by SAT HIF-1α (B=1.3 p<0.001).
VAT *Fn14* expression was predicted by WHR (B=3.7 p=0.003, FIAF (B=-0.42 p=0.02, GRP78 (B=1.6 p=0.041) and XBP-1 (B=3.3 p<0.001).
In severe obese cohort
Regression analysis showed that SAT *Fn*14 expression was predicted by SAT *VISFATIN* (B=2.3 p<0.001), *SAT GLUT*-1 (B=5.8 p=0.001) and SAT *VEGF* (B=-5.5 p=0.015). VAT *Fn14* expression was predicted by VAT *XBP-1* (B=24.4 p<0.001)
Conclusion: Tweak-Fn14 system seems to be closely associated with hypoxia and ER stress gene markers in both adipose tissue depots. Likewise, the presence of visceral adiposity determines in part the TWEAK-Fn14 adipose tissue expression, with independence of the glucose metabolic status.
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PS 46 Regulation of adipose tissue inflammation

635

Control of adipose tissue inflammation in obesity and diabetes through pseudokinases
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Background and aims: Inflammation of adipose tissue represents a major hallmark of obesity-related type II diabetes and the Metabolic Syndrome. Despite its critical importance for the development of insulin resistance and associated complications, major molecular pathways in adipose tissue inflammation still remain unknown.
Materials and methods: We employed expression profiling studies in cultured and primary white adipocytes and white adipose tissue (WAT) depots of various obesity and type II diabetes mouse models to identify dysregulated marker genes under these conditions. Subsequently, molecular pathways leading to aberrant gene expression under diabetic conditions and functional consequences of genetic dysfunction were investigated by employing genetic and acute gene ablation technology and metabolic monitoring.
Results: Expression profiling in mouse models of systemic inflammation demonstrated the transcriptional induction of the tribbles pseudokinase TRB1 in WAT in response to acute pro-inflammatory stimuli. Furthermore, we found that TRB1 mRNA is also significantly upregulated in WAT of obese db/db and ob/ob animals, suggesting that TRB1 represents a novel marker gene for obesity-related WAT inflammation. Indeed, levels of TRB1 but not of highly-related family members, TRB2 and TRB3, were strongly induced in fully differentiated adipocytes in response to lipopolysaccharide-conditioned macrophage supernatant, thereby demonstrating the cell autonomy of this effect. Intriguingly, the induction of TRB1 through conditioned macrophage supernatant in differentiated adipocytes could be blocked by interference with specific cytokine-dependent signaling pathways, leading to the improvement of cellular inflammation in white adipocytes.
Conclusion: These results indicate that the induction of TRB1 is a novel adipocyte-specific feature of pro-inflammatory conditions as associated with obesity-related type II diabetes, thereby providing a novel molecular checkpoint and potential therapeutic target in adipose tissue dysfunction under these conditions.
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636

Txnip expression associates with hyperglycaemia-induced inflammation in human peripheral blood mononuclear cells and adipocytes
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Background and aims: Subjects with type 2 diabetes mellitus (T2DM) are characterized by the presence of a chronic low grade inflammation mainly originating from expanding adipose tissue. Obesity-induced enlargement of adipose tissue promotes the infiltration of monocytes and lymphocytes, leading to an increased production of pro-inflammatory cytokines. Hyperglycaemia plays a central role in the progression of T2DM and is known to induce an inflammatory response and a disturbed redox balance. Thioredoxin-interacting protein (Txnip) controls the cellular redox state and appears to be an important regulator of glucose homeostasis. In this study we investigate the role of Txnip in the production of pro-inflammatory cytokines in human adipocytes and peripheral blood mononuclear cells (PBMCs) under normo- and hyperglycaemic conditions.
Material and methods: Differentiated primary adipocytes, total adipose tissue, PBMCs and isolated monocytes of human origin were cultured for 48 hours under normoglycaemic (5mM) or hyperglycaemic (25mM) conditions. Medium, RNA, and protein lysates were collected to determine the expression level of Txnip and pro-inflammatory cytokines.
Results: Txnip mRNA and protein levels were abundantly expressed in adipose tissue and were elevated 3-fold after exposure to high glucose. Fractioning of adipose tissue into mature adipocytes and stromal vascular cells

revealed that Txnip expression was mainly originating from adipocytes. Differentiation of primary human pre-adipocytes towards mature adipocytes under hyperglycaemic conditions led to a 3-fold higher expression of Txnip protein and mRNA as compared to normoglycaemic treatment. Furthermore, exposure of primary human mature adipocytes to high glucose also increased Txnip protein and mRNA levels. In line with the effects observed in adipocytes, also incubation of isolated human PBMCs and monocytes under high glucose conditions induced Txnip mRNA and protein expression levels, with Txnip protein being elevated more than two fold under hyperglycaemic compared to normoglycaemic conditions. In both adipose tissue and PBMCs, Txnip expression completely disappeared when cultivated in the absence of glucose.
Concurrent with the induction of Txnip, hyperglycaemia showed clear increases in mRNA expression of the pro-inflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-8 (IL-8) by mature adipocytes. In PBMCs and monocytes, hyperglycaemia resulted in a 2-fold increase in the intracellular level of interleukin-1β (IL-1β), and a 5-fold induction of IL-1β mRNA as compared to normoglycaemic conditions. Secretion levels of TNF-α and IL-8 were also elevated by high glucose.
Conclusion: Our results show that Txnip is present in human adipose tissue and PBMCs and that its activation strongly dependents on glucose exposure. Hyperglycaemic conditions induce a strong pro-inflammatory response in both adipocytes and peripheral blood cells, and this is associated with a strong increase in Txnip levels. Our results suggest that Txnip may be involved in the hyperglycaemia-induced production of pro-inflammatory cytokines.

637

Fas (CD95) induces lipolysis through down-regulation of lipin-1 and subsequent activation of the MAPK pathway in 3T3-L1 adipocytes
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Background and aims: Fas (CD95) is a member of the tumour necrosis receptor superfamily that plays a crucial role in the induction of apoptosis. In addition, activation of Fas can also induce non-apoptotic signalling pathways including cell proliferation. We previously found that Fas-deficient and adipocyte-specific Fas knockout mice are partly protected from high fat diet induced insulin resistance. The aim of this study was to elucidate the molecular mechanisms behind this protective effect; in particular the impact of Fas activation in adipocytes on lipolysis.
Materials and methods: Fully differentiated 3T3-L1 adipocytes were treated with 2 ng/ml Fas ligand (FasL) in the presence or absence of the MEK inhibitor U0126, or with various concentrations (1 - 100 μM) of phosphatidic acid. Protein and mRNA was collected for western blot and real-time PCR analysis. Lipolysis was assessed by measuring free glycerol release.
Results: Treatment of adipocytes with FasL influenced lipolysis in a time dependent fashion with an initial decrease after 2 hours and an approximately 2-fold increase after 12 hours. Fas activation induced phosphorylation of the p44/42 MAP kinases (ERK1/2) with a peak after 6 hours and FasL-induced lipolysis was completely blocked by ERK-inhibition. Interestingly, FasL reduced mRNA-expression and protein levels of lipin-1, a phosphatase involved in the generation of triacylglycerols.This effect was independent of ERK1/2 activation. It was previously shown that down-regulation of lipin-1 leads to accumulation of its substrate phosphatidic acid (PA) in adipose tissue. We show here that administration of PA to adipocytes increases phosphorylation of ERK1/2 and induces lipolysis.
Conclusion: These results suggest that Fas activation induces lipolysis in a lipin and ERK1/2-dependent manner and, thus, might have an important impact on adipocyte and whole-body metabolism.
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638

AMP-activated protein kinase (AMPK) modulates lipopolysaccharide (LPS)-induced cytokine expression and lipolysis in human adipocytes
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Background and aims: In adipose tissue of obese patients, low-grade chronic inflammation is mirrored by elevated levels of pro-inflammatory cytokines which enhance lipolysis. This contributes to the elevation of circulating triglyceride and non-esterified fatty acid concentrations in type 2 diabetic individuals. It also correlates with deposition of fat in liver and skeletal muscles, promoting insulin resistance. Therefore, we investigated modulators of inflammation and lipolysis in adipose tissue using a human adipocyte model and inducing inflammation with endotoxin (lipopolysaccharide, LPS).
Materials and methods: Human mesenchymal stem cells and human pre-adipocyte-derived adipocytes obtained from surgical biopsies were differentiated *in vitro* into adipocytes, and the effects of 24 h stimulation with LPS (100 ng/ml), metformin (1mM), AICAR, compound C (5μM) and their respective combinations on inflammatory cytokine (IL-6, IL-1beta and IL-8) mRNA expression and glycerol release (readout of lipolysis) were determined.
Results: LPS increased mRNA expression of IL-6 to 1269±184% of control (P<0.001), IL-8 to 32780±6437% of control (P<0.001) and IL-1beta to 1027±91% of control (P<0.001). Induction of IL-6 mRNA by LPS was significantly reduced by pre-incubation with metformin and AICAR, 5'AMP-activated protein kinase (AMPK) activators, and increased by compound C, an AMPK inhibitor. In contrast, induction of IL-1beta and IL-8 mRNA by LPS was significantly increased by metformin and AICAR. Glycerol release increased from 41.6±2.5μg/ml in basal state to 82.1±2.2μg/ml in LPS-treated cells (P<0.001). LPS-induced lipolysis was significantly inhibited by metformin and AICAR and slightly increased by compound C.
Conclusion: Compound C increased and both, metformin and AICAR inhibited LPS-induced IL-6 expression and lipolysis. This indicates that AMPK modulates the inflammatory and lipolytic effects of LPS in human adipocytes.
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639

Involvement of receptor-mediated processes in proinflammatory adipocyte activation by heat shock protein 60
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Background and aims: Adipocytes are a source of a variety of inflammatory mediators which allow the cells to exert immunoregulatory activities resembling those of innate immune cells. Adipocyte-derived proinflammatory mediators contribute to the development of chronic inflammation, thereby promoting the induction and progression of insulin resistance/metabolic-syndrome and diabetes. Currently, the physiological signals inducing the release of inflammatory mediators by adipocytes are largely unknown. As heat shock proteins (Hsp) have been identified as potent regulators of the inflammatory activities of innate immune cells, our present study was designed to investigate the interaction of adipocytes with Hsp60 as the decisive initial event in the induction of proinflammatory adipocyte mediators.
Material and methods: Adipocytes of the murine line 3T3-L1 were incubated with recombinant Hsp60 and the release of interleukin (IL)-6, CXCL1 and monocyte chemoattractant protein (MCP)-1 from these cells was determined by multiplex beads system. Analyses of Hsp60-signalling was performed by immunoblotting and by the application of inhibitors against specific signalling proteins. Characterization of Hsp60-binding to adipocytes and identification of responsible binding epitopes was studied by FACS analyses with fluorescent-labelled Hsp60 and with specific antibodies.
Results: Our experiments demonstrate that Hsp60 stimulates the release of the proinflammatory cytokines IL-6, CXCL1 and MCP-1 in a time- and con-

centration-dependent manner from 3T3-L1 adipocytes. Analyses of Hsp60-signalling in these adipocytes revealed that members of the MAPK-family (ERK1/2, p38) and the transcription factor NFκB are involved in Hsp60-mediated induction of the mediators IL-6, CXCL1 and MCP-1. Binding-studies with fluorescence-labelled Hsp60 demonstrated that the interaction of Hsp60 with adipocytes exhibits basic features of receptor-mediated interactions. Hsp60-binding to adipocytes was saturable and reached its maximum at 3.5 μM. Binding was inhibitable only by the unlabelled ligand (52%), but not by unrelated proteins, thereby proving the specificity of Hsp60-binding. Further approaches to characterize Hsp60-receptor structures on adipocytes revealed the presence of Toll-like receptor (TLR)4 on adipocytes. TLR4 has been found to be expressed on macrophages and to interact with Hsp60, therefore suggesting TLR4 as a potential receptor candidate for Hsp60 on adipocytes. In order to identify the Hsp60-epitope involved in binding, we investigated the effect of specific antibodies directed against defined epitopes of the Hsp60-molecule. Incubation of Hsp60 with antibodies directed against the N-terminus of the Hsp60-molecule (amino acids 1-200; 5-25 μg/ml) inhibited Hsp60-binding to adipocytes (47-80%) indicating that the N-terminal region of Hsp60 is involved in receptor binding.
Conclusion: Our experiments demonstrate that the Hsp60 stimulated release of proinflammatory mediators from adipocytes involves a receptor-mediated interaction of the heat shock protein with the cells. These findings implicate that Hsp60-receptor interactions could serve as a target to modulate the proinflammatory activity of adipocytes and to develop therapeutic strategies for patients suffering from chronic, diabetes-associated inflammatory diseases.
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640

NF-κB dependent regulation of IP-10 in 3T3-L1 and human primary preadipocytes and adipocytes
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Background and aims: Chemokine secretion by adipocytes and preadipocytes has been postulated to initiate leukocyte infiltration and might mediate the establishment of obesity-related low-grade inflammation. In this context, interferon-γ-inducible protein 10 (IP-10/CXCL10) is a novel interesting candidate, as increased levels of IP-10 expression have been associated with obesity and type 2 diabetes in humans and it is expressed in primary human adipocytes. The aim of this work was to study the role of NF-κB in the regulation of IP-10 in murine 3T3-L1, primary human preadipocytes and adipocytes and and to characterize its functional role in adipose tissue.
Materials and methods: Cell culture of *in vitro* differentiated murine 3T3-L1, primary human preadipocytes, adipocytes and primary mouse splenocytes; retroviral infection, transfection, luciferase-assay, ELISA, western-blot, qRT-PCR, migration-assays.
Results: In order to specifically inhibit the NF-κB activity, we stably expressed a transdominant mutant of IκBα in 3T3-L1 cells. This mutant completely suppressed the basal and induced IP-10 secretion and expression. In primary mature adipocytes IL-1β stimulated IP-10 mRNA expression was completely blocked by a chemical NF-κB-inhibitor. Moreover, adipocytes secrete and express significantly higher levels of IP-10 compared with preadipocytes. In addition, we could demonstrate that induction of T cell migration by conditioned medium from IL-1β-induced adipocytes was inhibited by overexpression of IκBα-mut as well as by neutralization of IP-10.
Conclusion: This study demonstrates that the NF-κB pathway is essential for the regulation of IP-10 in 3T3-L1 and primary human adipocytes. Adipocytes rather than preadipocytes contribute to obesity-associated elevated IP-10 levels. Furthermore, IP-10 release from adipocytes may act as a potent chemoattractant for lymphocyte migration towards adipose tissue.
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641

Interleukin-1F6 is expressed in adipose tissue and inhibits adipocyte differentiation
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Background and aims: Inflammation appears to be an important link between obesity and insulin resistance. Nutrient excess and subsequent storage in adipose tissue result in expansion of the adipose tissue mass and adipocyte size, which is positively correlated with the number of infiltrating macrophages. Adipose tissue macrophages (ATMs) in obese individuals express many genes characteristic for M1 macrophages, which have a pro-inflammatory cytokine profile, while most ATMs in lean individuals are M2 macrophages (anti-inflammatory). ATMs can release proinflammatory cytokines such as the Interleukin (IL)-1family members IL-1 and IL-18, and an increasing amount of evidence shows that these cytokines are involved in the interplay between obesity and insulin sensitivity. IL-1 family of cytokines consists of several members in addition to Il-1 and IL-18, among which IL-1F6 has known pro-inflammatory properties. However, little is known about the potential metabolic effects of IL-1F6 in adipose tissue. The aim of this study was to assess whether IL-1F6 is expressed in adipose tissue and whether IL-1F6 influences adipocyte differentiation.
Materials and methods: Human abdominal subcutaneous tissue and primary human MI or type MII differentiated macrophages were used to determine the expression of IL-1F6. MI or MII differentiation of Macrophage was induced using either GM-CSF or M-CSF (100 ng/ml). Because obesity is associated with a chronic low-state inflammatory reaction, we tested whether IL-1F6 gene expression is induced by inflammation, using LPS. To determine the effects of IL-1F6 on adipocyte differentiation, primary human pre-adipocytes were differentiated towards adipocytes in the presence or absence of recombinant IL-1F6 (100 ng/ml). Adipocyte differentiation was analyzed by real-time PCR gene expression levels of adipogenic genes including AP2, PPARgamma, adiponectin and GLUT4, as well as by oil red O staining of lipid uptake.
Results: IL-1F6 is expressed in human adipose tissue. Fractioning of human adipose tissue revealed that IL-1F6 is primarily expressed in the stromal vascular fraction containing the ATMs. Although the stromal vascular fraction showed to be the primary source of IL-1F6, IL-1F6 was also present in adipocytes, be it at low levels. Treatment with endotoxin significant increased the expression of IL-1F6 in both MI and MII macrophages, as well as in SGBS preadipocytes. Recombinant IL-1F6 inhibited adipocyte differentiation, as showed by significantly reduced expression of AP2, PPARgamma and adiponectin, but not of GLUT4. Reduced differentiation was confirmed using microscopy and oil red o staining.
Conclusion: The proinflammatory IL-1 family member IL-1F6 is expressed in human adipose tissue. Although the primary source of IL-1F6 in adipose tissue is the stromal vascular fractions, both adipocytes and macrophages are able to produce IL-1F6. The presence of IL-1F6 inhibits human adipocyte differentiation, and future studies aimed to evaluate its effects on insulin sensitivity are warranted.

642

Remodeling of adipose tissue from lipodystrophic patients with LMNA mutations: presence of fibrosis and mitochondrial alterations but no inflammation
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Context: A low-grade inflammation of adipose tissue has been reported in obesity, associated with metabolic complications of obesity. However, in genetic lipodystrophies, the histological features of adipose tissue have not been described up to now. We then performed the histological characterization of adipose tissue from lipodystrophic patients bearing *LMNA* mutations.
Objective, design and patients: We comparatively studied the histological, ultrastructural and protein expression features of subcutaneous fat obtained during plastic surgery in three patients with *LMNA*-linked lipodystrophy, in

a patient with mitochondrial (mt)DNA-linked lipomatosis, in three patients with HIV antiretroviral-linked lipodystrophy and in eight healthy non-diabetic non-obese controls.
Results: *LMNA*-mutated, mtDNA-mutated and HIV-infected lipodystrophic fat was characterized by decreased adipocyte size, increased intercellular fibrosis and mitochondria density as compared to control samples. An ultrastructural analysis revealed irregular cell outlines with thickened peripheral rims of cytoplasm and interstitial edema with compact and thick fibrils invading the intercellular area in fat from patients bearing *LMNA* mutations. However, *LMNA*-mutated fat was not infiltrated with macrophages and did not show inflammatory alterations, in contrast to adipose tissue from mtDNA-mutated or HIV-infected patients. We also showed abnormal prelamin A accumulation, the precursor protein of lamin A, and altered protein expression of adipogenic transcription factors and mitochondrial respiratory chain proteins.
Conclusion: Lipodystrophic adipose tissue from patients with *LMNA* mutations presents a unique pattern of structural alterations with small adipocytes, intercellular fibrosis and mitochondrial disturbances in the absence of inflammation. We suggest that lamin mutation and/or prelamin A accumulation could impair adipocyte differentiation and lead to adipose tissue fibrosis.
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643

Clinical impact of inflammatory cytokines produced in adipose tissue of septic patients
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Background and aims: Cytokines produced in adipose tissue were recently implicated in the generation of insulin resistance and diabetes. High levels of circulating inflammatory cytokines in circulation might also contribute to the genesis of glucose intolerance as observed in septic patients. However, the role of cytokines produced in adipose tissue was not studied in sepsis. The aim of this study was to investigate possible interactions between blood glucose and cytokines produced in adipose tissue in patients with severe sepsis. Other clinical variables were analyzed in parallel.
Materials and methods: Nine patients with severe sepsis from a medical intensive care unit were investigated over 26 hours. Informed consent was provided from the closest relative. A standard open-flow microperfusion catheter inserted in the subcutaneous adipose tissue (SAT) of the abdominal wall was used for continuous sampling of interstitial fluid effluent. Arterial blood samples were obtained simultaneously at two-hour intervals, along with clinical variables as part of the intensive care monitoring. Blood glucose was measured in a standard point-of-care device. Cytokines (TNF-alpha, IL-1beta, IL-6 and IL-8) present in SAT effluent samples and serum were measured using a multiplexed ELISA system.
Results: Distinct time profiles were registered for each cytokine in SAT effluent samples. Cytokine concentrations were higher in SAT than in serum for IL-1beta (10.0 [3.5; 13.9] vs. 0.7 [0.6; 1.4] pg/ml; median [25; 75 percentile]; $p<0.05$), IL-6 (1.66 [1.17; 2.33] vs. 0.06 [0.05; 0.08] ng/ml; $p<0.01$) and IL-8 (0.83 [0.51; 1.40] vs. 0.08 [0.02; 0.12] ng/ml; $p<0.01$). No important correlation was found for any of the measured cytokines and blood glucose. A weak negative correlation was observed between blood glucose and diastolic blood pressure (DBP; Pearson -0.21, $p=0.03$). Significant negative correlations were observed between SAT inflammatory cytokines and mean DBP (IL-1beta: Pearson -081, $p=0.008$; IL-6: Pearson -084, $p=0.005$; IL-8: Pearson -073, $p=0.025$). Regression analysis uncovered a strong interaction between DBP and IL-6 produced in SAT (B= -0.01; $p=0.002$; R-square=0.78). No other significant correlation between clinical variables and cytokines were observed.
Conclusion: Cytokines measured in SAT effluent from septic patients using OFM technique are probably locally produced, as suggested by the serum-SAT gradients, and seem to exert no major influence on blood glucose levels. Nonetheless, IL-6 might be implicated in the regulation of blood pressure in the studied population.
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PS 47 "Metabolic syndrome" - clinical and experimental

644

APOE*3Leiden.CETP transgenic mice as model for the metabolic syndrome
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Background and aims: Metabolic syndrome is characterized by the co-occurrence of several risk factors i.e. increased body weight (bw) and insulin resistance (IR) with increased plasma glucose and insulin levels and at the same time adverse changes in plasma lipids as observed in diabetic dyslipidemia, with increased triglycerides and apoB-containing lipoproteins and decreased HDL.
We evaluated whether the APOE*3Leiden.CETP (E3L.CETP) mouse is a useful animal model to study the metabolic syndrome.
Materials and methods: E3L.CETP mice, a well-established model for hyperlipidemia and atherosclerosis, responding to all clinically used hypolipidemics, were put on a high fat diet and fructose in drinking water for 12 weeks to induce diet-induced obesity and IR and were subsequently treated with either rosiglitazone (10 mg/kg bw/d) or resveratrol (75 mg/kg/bw/d) for 5 weeks. Effects on bw, plasma parameters and insulin sensitivity (via hyperinsulinemic euglycemic clamps) were assessed.
Results: Dietary treatment resulted in a human-like lipoprotein profile with a TC/ HDL-C ratio of 3. Rosiglitazone significantly decreased triglycerides (-49%), cholesterol (-55%), glucose (-22%) and insulin (-82%) and increased insulin sensitivity (3.5-fold increase in glucose infusion rate). Resveratrol significantly reduced plasma triglycerides (-24%) and cholesterol (-44%) and increased HDL (+25%). All $p< 0.05$.
Conclusion: The data indicate that the E3L.CETP mouse is a good translational animal model that combines all important features that underlie the metabolic syndrome and mimic the human response to clinically used treatments. We conclude that this mouse is a promising model to investigate the effects of new drugs that affect insulin resistance and dyslipidemia.

645

The role of body mass index and cardio respiratory fitness in predicting metabolic risk factors in adolescents: a longitudinal study
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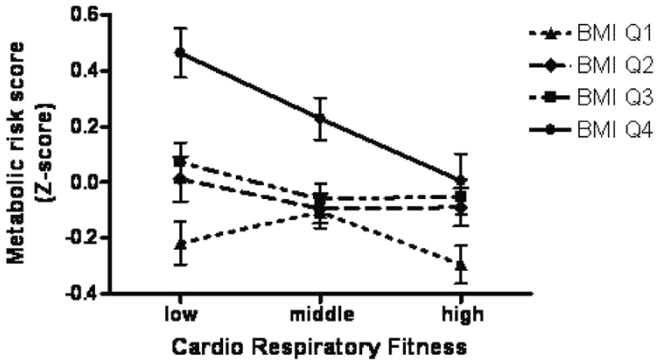
Background and aims: The aim of this study was to examine whether Body Mass Index (BMI) is predictive for a clustering of metabolic risk factors in adolescents and whether this association is influenced by cardio respiratory fitness (CRF).
Materials and methods: Data on BMI were collected at age 11 (march 2001-july 2002) and repeated at age 16 (September 2005-dec 2007). At age 16 fasting blood samples were taken and CRF was estimated using the Shuttle Run Test. All participants from the 'Tracking Adolescents' Individuals Lives Survey' (TRAILS) with measures for metabolic risk and CRF at age 16 were included in this analysis (n=567). A clustered metabolic risk score was calculated as the mean of waist circumference, triglycerides, HDL-cholesterol and mean arterial pressure.
Results: At age 16 clustered metabolic risk score for females was lower than for males ($p<.001$). Clustered metabolic risk score at age 16 was higher for the upper quartiles of BMI measured at age 11 ($p<0.001$) compared to the lower quartiles. After adjustment for pubertal stage, sex, school and wearing HR-monitoring during SRT, CRF was associated with clustered metabolic risk score in the highest BMI quartile at age 11 (standardized β=-3.54; 95%CI: -.045- -.013) but not in the lower BMI quartiles. This is graphically depicted in the figure. For males CRF is negatively correlated to waist circumference (Pearson's $r=0.25$, $p<.01$), triglycerides ($r=0.121$, $p<.05$) and glucose ($r=0.14$, $p<.05$). For females CRF is negatively correlated to waist circumference ($r=0.226$, $p<0.01$) and positively related to HDL-cholesterol ($r=0.155$, $p<.01$).

Figure. A high CRF is related to reduced clustered metabolic risk score at age 16 in those with increased BMI at age 11. Differences on the clustered metabolic risk score was only significantly reduced between the low and high CRF tertiles for those with increased BMI.

Conclusion: A higher BMI at age 11 is associated with higher clustered metabolic risk scores at age 16. CRF has a positive influence on metabolic risk in adolescents with a higher BMI. The positive effect of CRF on males on clustered metabolic risk score is due to lower waist circumference, triglycerides and glucose. In females the positive effect of CRF on clustered metabolic risk score is due to lower waist and a higher HDL-cholesterol.



646

Significant decrease of fetuin-a after dramatic weight loss in morbidly obese women

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Background and aims: Patients with morbid obesity have a high risk for cardiovascular morbidity, mortality and diabetes. The only effective method to reduce weight and to lower the high cardiovascular risk is bariatric surgery. Recent studies demonstrated that the liver-secreted protein fetuin-A is elevated in insulin resistance, is an independent predictor of type 2 diabetes and is associated with atherosclerosis. Thus it was of interest for us to evaluate Fetuin-A levels in patients with morbid obesity versus control subjects as well as before and after weight loss induced by gastric bypass.

Materials and methods: We included 47 morbid obese patients (MO, 40 women, BMI 46.6±7.3 kg/m²) and 28 healthy controls (13 women, BMI 26.1±3.3 kg/m²) in a cross sectional study. 31 women were investigated before and one year after gastric bypass surgery. Apart from weight and cardiovascular risk-markers (blood pressure, lipids), a glucose tolerance test (75g oGTT) was performed and renal, liver as well as inflammation parameters were assessed. Insulin resistance (IR) was calculated by using HOMA.Fetuin-A levels were determined in serum samples by a commercial ELISA.

Results: Fetuin-A levels were significantly increased in MO: 795.0±191.5 vs healthy controls: 393.2±146.5µg/ml; p <0.001. In the longitudinal study Fetuin-A (827.2± 181.8 vs 753.9 µg/ml; p= 0.033) BMI (46.6±7.3 vs 31.6±6.8 kg/m²; p < 0.001), weight (133.5± 20.8 vs.90.6±20.3kg;p<0.001) and HOMA (6.6±6.3 vs. 2.0±1.2 p<0.001) decreased significantly 1 year after gastric bypass. Correlation analysis of pre-surgery Fetuin-A values revealed that Fetuin-A was associated with HOMA-IR (R=-0.413; p=0.029), diastolic blood pressure (R=-0.392; p=0.036) and 1hour postprandial blood glucose (R=-0.385; p=0.047). In a multivariate model HOMA-IR (beta=-0.408; p=0.024) and diastolic blood pressure(beta=-0.382; p=0.033mmHg) predicted independently from each other Fetuin-A.Correlation analysis of post-surgery Fetuin-A showed that Fetuin-A levels were associated with bilirubin (R=0.390; p =0.040) and 2 hour postprandial Insulin levels (R=-.479; p=0.028). In a regression analysis Insulin 2 hour postprandial levels remained the only significant predictor for post-surgery Fetuin-A (beta=-0.479; p= 0.028).

Conclusion: This is the first study demonstrating a significant increase in Fetuin-A values in patients with morbid obestity compared to nonobese control subjects. Fetuin-A levels were reduced after bariatric surgery. Since Fetuin-A was shown to be associated with the future development of type 2 diabetes and atherosclerosis, the observed significant decrease in Fetuin A after dramatic weight loss might help to understand the beneficial effects of gastric bypass surgery.

647

Influence of "hypertrigliceridaemic waist" phenotype on postprandial lipaemia in obese type 2 diabetic patients

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Background and aims: Obesity, especially visceral has been shown to be associated with abnormal postprandial lipid response. "Hypertrigliceridemic waist" (HW) phenotype is suggested to be a reliable marker of visceral obesity (waist girth ≥90cm in men and ≥85cm in women, with plasma triglycerides (TG) concentration ≥2mmol/l). Type 2 diabetes (T2D) represents frequent comorbidity in obesity and is closely associated with abnormalities in postprandial lipid metabolism mostly through insulin resistance. The aim of our study was to analyze the influence of presence of HW phenotype and postprandial lipemia in obese patients with T2D. The following groups of patients were included in the study: obese T2D patients with HW phenotype (Group A, n=30), obese T2D without HW phenotype (Group B, n= 25) and nonobese T2D without HW phenotype (group C, n=30). Patients were aged 40-70 years, matched by gender, duration of diabetes with optimal glycemic control.

Materials and methods: Postprandial lipids, apolipoproteins (Apo), NEFA and insulin response were measured after mixed meal (51g fat) at 2-h intervals during 8hrs. Results were expressed as AUCs determined by the trapezoid method. Total cholesterol (TC), HDL, LDL and TG were determined by enzymatic methods. Apolipoproteins (Apo A1, ApoA2,ApoB,ApoE) were determined by nephelometric and NEFA by colorimetric methods. Insulin levels were measured by RIA and insulin resistance was evaluated in all groups in fasting state using HOMA method.

Results: We found that the postprandial TG increase were the highest in group A, being significantly higher in group A than in B and in group B than in C (AUC A: 2020.4±133, B:1575.6±100.4, C:1263±100.2mmol/l/min, A vs B p<0.01 and B vs C p<0.05). Simultaneously, postprandial response of TC were the highest in group A, being significantly higher in group A than in group B, but without significant difference beetwen groups B and C (AUC A:3088.9±213, B:2879±150, C:2682±145mmol/l/min; A vs B p<0.01 and B vs C p=NS). There was no differences in postprandial response of HDL, LDL and NEFA between groups. However, significantly higher postprandial increased of Apo B and Apo E was found in group A than in groups B and C (ApoB AUC: A:633.9 ± 52, B:591.5± 45, C: 514 ± 50 g/l/min; A vs B vs C p<0.05; ApoE AUC: A:30662±538, B:26232±999, C:20460±538mg/l/min; A vs B vs C p<0.01) without differences regarding levels of ApoA1 i ApoA2. Insulin response was significantly higher in group A compare to group B, but there was no difference among groups B and C (AUC: A:31400±254; B:22900,±129; C:19800±117mI/ml/h;A vs B<0.01, B vs C p=NS). Regarding the insulin resistance, we found the highest HOMA-IR in group A but, there was no differences beetwen groups B and C (A: 11.9±2.3, B: 5.65±1.8, C: 5.2±1.2Avs B p<0.01, AvsC p<0.01, B vs C p=NS). Moreover, amplified postprandial TG response ccorrelated with HOMA-IR and postprandial insulin response (r=0.44, p<0.05 and r=0.42 p<0,05) respectively, only in group A. We did not find that correlation in the other groups.

Conclusion: Our results have demonstrated that in obese T2D patients presence of hypertrigliceridemic waist phenotype was associated with enhancement of abnormal postprandial lipid, especially TG, response. The data imply that this enhancement was strongly influenced by increased of postprandial insulin response and insulin resistance in these T2D patients.

648

The association of serum fatty acid-binding protein 4 with progression of metabolic syndrome status in apparently healthy Korean adults

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Background and aims: Adipocyte fatty acid-binding protein (A-FABP), also known as aP2 or FABP4, is abundantly expressed in adipocytes and plays a role in glucose homeostasis. We analyzed the relationship of serum FABP4 level with the progression of metabolic syndrome in apparently healthy Korean adults.

Materials and methods: In 494 Korean adults (mean age 40.8 years, male 65.8%), selected among the participants in a medical check-up program in

a health promotion in 2003, serum FABP4 levels were measured by ELISA. Anthropometric measurements were performed in all subjects and in fasting status, fasting glucose, insulin and lipid profiles were measured. Insulin resistance was assessed by HOMA-IR and the presence of metabolic syndrome was assessed according to AHA/NHLBI criteria with BMI substituted for waist circumference. After 4 years, the metabolic syndrome status was assessed in association with baseline serum FABP4 level in the same subjects.
Results: In 4 years, 395 subjects (80%) maintained their initial status (normal to normal, or metabolic syndrome present to metabolic syndrome present; non-progressor), 81 subjects (16.4%) progressed to more serious stage (normal to metabolic syndrome; progressors) and 18 subjects (3.6%) regressed to metabolic syndrome to normal (regressors). When baseline serum FABP4 levels were compared among the three groups, progressors showed significantly higher baseline mean value of logarithmically transformed (log) FABP4 compared with the non-progressors in 4 years of follow-up (2.42±0.45 vs. 2.16±0.45 ng/mL, p<0.001), and these significant differences were consistent even after adjustment for age and BMI (p=0.029). When logistic regression analysis with the progression to metabolic syndrome as the dependent variable, was performed after excluding regressors (n=18), with age, high baseline log FABP4 level, male gender, high BMI and low SBP were the significant determinants for the development of metabolic syndrome (odds ratio for log FABP4 levels, 2.549, p<0.001).
Conclusion: Baseline serum FABP4 level is assumed to be the significant predictor for future progression of metabolic syndrome in apparently healthy adults even after adjusting confounding factors in 4 years of follow-up in this ethnic groups.
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649

Intestinal triglyceride concentration of morbidly obese persons is lower in those with type 2 diabetes mellitus
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Background and aims: High levels of apolipoprotein B-48-containing lipoproteins in the fasting state seem to be associated with increased cardiovascular disease risk. We studied the association between insulin resistance and the lipid content in plasma and the small intestine of morbidly obese persons with or without diabetes.
Materials and methods: The study was undertaken in 17 morbidly obese persons, 8 of whom had type 2 diabetes. The HOMA-IR was calculated to determine insulin resistance and measurements were made of the plasma apolipoprotein B-48 and chylomicrons. Jejunum samples were obtained during gastric bypass in order to quantify their content of triglycerides (biochemical techniques and Oil Red-O staining) and apolipoprotein B (immunohistochemistry).
Results: The morbidly obese persons with diabetes had greater plasma concentrations of triglycerides (P=0.003), chylomicron triglycerides (P<0.001) and apolipoprotein B-48 (P=0.012). The samples of jejunum obtained from the morbidly obese persons with diabetes had a lower jejunal triglyceride content (P=0.012) and a lower apolipoprotein B signal (P=0.008). The HOMA-IR correlated positively with the plasma levels of apolipoprotein B-48 (r=0.688, P=0.003) and chylomicron triglycerides (r=0.700, P=0.003), and negatively with the jejunal triglyceride content (r=-0.774, P=0.001) and the jejunal apolipoprotein B signal intensity (r=-0.747, P=0.001). The jejunal triglyceride content also correlated positively with the jejunal apolipoprotein B signal intensity (r=0.776, P<0.001) and negatively with the plasma apolipoprotein B-48 (r=-0.674, P=0.004).
Conclusion: The lipid content of the jejunum was significantly reduced in morbidly obese persons with diabetes and was inversely related with the plasma levels of chylomicrons, apolipoprotein B-48 and the HOMA-IR.

650

Associations between components of the metabolic syndrome and fatty liver are mediated by insulin resistance and endothelial dysfunction, but not by inflammation (the CODAM study)
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Background and aims: The metabolic syndrome (MetS) is associated with presence of fatty liver and this association may be related to insulin resistance and inflammation and possibly also to endothelial dysfunction, e.g. via effects on liver metabolism. We have therefore investigated whether plasma alanine aminotransferase (ALT), a surrogate marker of fatty liver, is associated with individual components of the MetS and, if so, the extent to which these associations are mediated by insulin resistance, inflammation and/or endothelial dysfunction.
Methods: Analyses were done in the CODAM (cohort study on diabetes and atherosclerosis Maastricht) population with only subjects who consumed < 2 units of alcohol/day and included 397 subjects (53.1% male, age 59.2 ± 7.2 yrs). All independent variables were transformed into Zscores (log-transformed when appropriate). An inflammation score, i.e. average of the Z-scores of the inflammation markers CRP, IL6, ceruloplasmin, haptoglobin, serum amyloid A and ICAM, was calculated. Likewise, a score for endothelial dysfunction (E-selectin, vWF and VCAM) was calculated. HOMA2*ir* was used as a measure of insulin resistance.We used linear regression analyses to determine the associations of components of the MetS, and the inflammation, insulin resistance and endothelial dysfunction scores, with ALT (dependent, all adjusted for age, sex and presence of type 2 diabetes). Next, we investigated whether the associations of the MetS components with ALT were mediated by insulin resistance, inflammation and/or endothelial dysfunction.
Results: Prevalence of MetS (NCEP-ATP 2005) and type 2 diabetes was 56.7% and 26.2%, respectively. All components of the MetS (beta [95%CI] for waist was 0.057 [0.041; 0.074], BP; 0.038 [0.020; 0.055], HDL; -0.034 [-0.051; 0.016], TG; 0.048 [0.032; 0.094], glucose 0.046 [0.023; 0.070]) as well as scores for insulin resistance (0.086 [0.070; 0.102]), inflammation (0.036 [0.013; 0.060]) and endothelial dysfunction (0.081 [0.054; 0.109]) were associated with logALT. The associations between MetS components and ALT were strongly attenuated by insulin resistance, with the strongest effect on glucose, followed by HDL, waist, TG and BP (decrease in beta of the MetS components was 89.1, 76.5, 73.7, 58.3, and 47.4%, respectively). They were somewhat attenuated by endothelial dysfunction, with the strongest effect on HDL followed by glucose, waist, BP and TG (decrease in beta was 29.3, 21.7,15.6, 13.2 and 12.5%, respectively) and hardly by inflammation (decrease in beta was 14.7 to 2.1%, respectively). Total attenuation with insulin resistance, endothelial dysfunction *and* inflammation added to the model was strongest for glucose followed by HDL, waist, TG and BP (decrease in beta was 92.3, 82.4, 73.7, 58.3, 47.4%).
Conclusion: The associations between fatty liver, when measured as plasma ALT, and all components of the metabolic syndrome were for a very large part explained by insulin resistance with a small additional effect of endothelial dysfunction but, unexpectedly, not by systemic inflammation.
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651

Prevalence of features of the metabolic syndrome in healthy white-collar employees with or without fatty liver disease: role of plasma glucose levels of ≥ 100mg/dl versus ≥ 110mg/dl
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Background and aims: Non alcoholic fatty liver (NAFL) is the most common chronically liver disease and often associated with the features of the metabolic syndrome. But, there is little knowledge about the prevalence of single components of the metabolic syndrome in healthy individuals with or without fatty liver disease.
Materials and methods: In this study data of more than 1500 healthy, male white-collar employees (30 to 60 year old) participating in a regional prevention program were collected.

Results: Sonographic analysis indicated a fatty liver in 32% of all cases investigated. Approximately 50% of these individuals showed triglycerides levels >150 mg/dl and in 12% of cases had HDL-levels below 40 mg/dl. More than 67% of probands showed hypertension with values above 130/85 mmHg and fasting blood glucose levels exceeding 110 mg/dl were detected in 13% of individuals investigated. These data indicated a more than twofold increase of pathologically levels for triglycerides, HDL-cholesterol and hypertension in patients with fatty liver disease. Interestingly, pathologically increased fasting blood glucose values were detected in only 2.5% of individuals without fatty liver, but occurred with a 7-fold higher prevalence in patients with fatty liver disease. Focusing on the three prominent parameters crucial for the metabolic syndrome i.e. increased triglyceride levels, hypertension and increased fasting blood glucose >110 mg/dl, the criteria were fulfilled in 14% of individuals with fatty livers, but only in 2% of the non fatty liver group. Reducing the critical value for impaired fasting glucose from >110 mg/dl to >100 mg/dl even doubles the prevalence of this risk factor pattern in the fatty liver group investigated, indicating the importance of this parameter in respect to the metabolic syndrome.
Conclusion: The data indicate that reducing the pathological value of impaired fasting glucose to >100 mg/dl might be valuable for screening of patients also in respect for other components of the metabolic syndrome. Furthermore all individuals with fatty livers should also be routinely screened for criteria of the metabolic syndrome.
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652

Cerebrovascular effects of endocannabinoids
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Background and aims: Antagonists and inverse agonists of the CB1 cannabinoid receptors have been recently developed by several pharmaceutical companies as adjuvants to lifestyle modification for weight reduction, glycemic control and dyslipidemia in obese and type 2 diabetes patients. Since endocannabinoids have been implicated as important mediators in the cardiovascular system pharmacological manipulation of the CB1 mediated signaling pathway may have marked circulatory consequences. Obesity and diabetes are often associated with cerebrovascular dysfunction which can be influenced by inhibition of the CB1 pathway. In the present study we aimed to investigate the effects of endocannabinoids on the resting blood flow and on hypoxia/hypercapnia (H/H)-induced hyperemia of the cerebral cortex.
Materials and methods: Adult male Wistar rats were anesthetized with intraperitoneal injection of 1.3 g/kg Urethane. Cerebrocortical blood flow (CBF) was measured simultaneously in the parietal cortex of both hemispheres by laser-Doppler (LD) flowmetry. H/H was induced by inhalation of different gas mixtures. In the first step, during mild H/H, animals inhaled a gas mixture of $10\%O_2$-$10\%CO_2$-$80\%N_2$, which produced arterial pO_2 of 75-85 mmHg and pCO_2 of 50-60 mmHg. In the second step, during moderate H/H, animals inhaled a gas mixture of $5\%O_2$-$20\%CO_2$-$75\%N_2$, which produced arterial pO_2 of 55-65 mmHg and pCO_2 of 80-90 mmHg. In the third step, during severe H/H, animals inhaled a gas mixture of $0\%O_2$-$20\%CO_2$-$80\%N_2$, which produced arterial pO_2 of 45-50 mmHg and pCO_2 of 90-100 mmHg.
Results: Inhibition of endocannabinoid reuptake by AM-404 (10 mg/kg iv.) resulted in a transient increase of the resting CBF, which had a peak (+65±16 %) at 5.2±0.6 minutes and disappeared within 10 minutes. The CBF changes were accompanied by reduction of the ventilation rate as well as by increased arterial pCO_2 and decreased pO_2. Application of the CB1 receptor antagonist AM-251 (10 mg/kg iv.) did not alter baseline CBF but was able to prevent the effects of AM-404. The VR1 vanilloid receptor antagonist capsazepine (20 mg/kg iv.) influenced neither the resting CBF nor the effects of AM-404. Furthermore, AM-251 significantly increased the H/H-induced hyperemia: by 30 % during mild H/H, by 22 % during moderate H/H, and by 14 % during severe H/H. In contrast, the vehicle of AM-251 had no significant effect. H/H did not change mean arterial pressure in untreated or vehicle-treated animals, although following AM-251 treatment it induced slight hypertension (+11 ± 6 mmHg).
Conclusion: Our results indicate that endocannabinoids may increase the resting CBF either by a CB1-mediated direct vasodilator mechanism or indirectly via respiratory depression and the consequent changes of the blood gas tensions. Interestingly, activation of CB1 receptors attenuates the H/H induced cerebrocortical hyperemia by a yet undefined mechanism. Therefore, the cerebrovascular and systemic circulatory actions of CB1 antagonists have to be considered as potential side effects during management of obesity and diabetes.
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653

Phthalate metabolites are associated with insulin resistance in 3T3-L1 cells *in vitro*
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Background and aims: The metabolites of di-(2-ethylhexyl)-phthalate (DEHP), a ubiquitous environmental contaminant, might influence glucose homeostasis and insulin sensitivity of adipose tissue. We therefore investigated the effects of DEHP treatment on insulin stimulated glucose uptake, palmitate uptake into 3T3L1 adipocytes as well as on mRNA expression of key adipocyte genes *in vitro*.
Materials and methods: Mouse 3T3-L1 preadipocytes (American Type Culture Collection, Rockville, MD) were cultured with 0.01% DEHP for two days and differentiated into mature adipocytes. In fully differentiated 3T3L1 adipocytes, basal and insulin-stimulated glucose transport was measured using 2-deoxyglucose. In addition, uptake of palmitic acid into 3T3-L1 cells was estimated by a [1-^{14}C] palmitic acid based assay (Perkin Elmer, Massachusetts, USA). Triglyceride content was determined using the LabAssay Triglyceride (WAKO Pure Chemicals). The mRNA expression of key genes in adipose tissue function was performed using RT-PCR.
Results: Treatment with DEHP resulted both in increased basal and insulin stimulated glucose uptake. Palmitic acid uptake was not affected by DEHP treatment *in vitro*. DEHP treatment of 3T3L1 adipocytes significantly increased adipocyte proliferation rate. In parallel to that, expression of *IGF1R, PI3K, VAMP4* mRNA was up-regulated, whereas adiponectin mRNA expression was down-regulated in response to DEHP treatment.
Conclusion: The environmental contaminant DEHP has significant effects on adipocyte proliferation, gene expression and *in vitro* glucose uptake into adipocytes which may contribute to adipose tissue dysfunction in obesity.
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PS 48 Liver pathophysiology and metabolism

654

Identification and characterisation of candidate genes in diet-induced non-alcoholic fatty liver disease in inbred mice

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is associated with the metabolic syndrome, which comprises several disorders including obesity and type 2 diabetes mellitus, and is influenced by both genes and environmental factors. Mouse models are important tools in the dissection of the genetics of these complex diseases. The aim of this research is to identify susceptibility genes for obesity and NAFLD in inbred mice susceptible (129S6) and resistant (BALB/c) to high-fat diet induced obesity and NAFLD.
Materials and methods: 129S6 and BALB/c mice were intercrossed to generate a cohort of 180 F2 hybrids. Male F2 mice were fed high-fat diet (40% fat) for 15 weeks and scored for body weight, adiposity (obesity), NAFLD and liver inflammation phenotypes. Genome-wide scans were performed with 400 Single Nucleotide Polymorphism markers showing evidence of allele variation between 129S6 and BALB/c. Genetic maps were constructed using JoinMap. Linkage analyses between genotypes and quantitative phenotypes were performed using R/qtl.
Results: Statistically significant Quantitative Trait Loci (QTLs) linked to body weight were identified on chromosomes 3 (logarithm of the odds (LOD) 4.7) and 4 (LOD 5.1). The chromosome 4 QTL was also strongly linked to adiposity (LOD 5.6) and NAFLD (LOD 3.8). A region of chromosome 18 was significantly linked to NAFLD phenotypes including macrovesicular and microvesicular steatosis (LOD 5.1 and 8.4 respectively) and inflammation (LOD 4.2), but did not show evidence of linkage to adiposity. At the QTLs identified, 129S6 alleles contribute to increased body weight and adiposity, and increased incidence of NAFLD and inflammation phenotypes.
Conclusion: These data demonstrate the polygenic control of both obesity and NAFLD induced by high fat diet feeding. QTL data suggest that NAFLD and obesity underlie the effect of the same gene(s) on chromosome 4, which does not cause inflammation. In contrast, NAFLD can also develop through a different mechanism controlled by a gene on chromosome 18 which leads to liver inflammation without influencing adiposity. Genome annotations of NAFLD QTLs and sequence analysis have provided information for strong positional and functional NAFLD candidate genes which we are currently investigating.

655

Hepatocyte-specific inflammation enhances VLDL production in mice

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Background and aims: The low-grade inflammation that is associated with Type 2 Diabetes (DM2) and Cardiovascular Diseases (CVD) plays an important role in their pathophysiology. Shoelson et al have shown that activation of the inflammatory Nf-κB pathway in the hepatocyte induces severe metabolic changes. We questioned whether hepatocyte-specific activation of Nf-κB affects triglyceride and cholesterol metabolism directly.
Materials and methods: The LIKK-mouse has a transgenic hepatocyte expression of constitutive active IkB kinase, IKKβ. We crossbred this LIKK-mouse with the APOE*3Leiden mouse for its human-like lipid metabolism and investigated the effects of this continuous activation of liver Nf-κB on fasted plasma cholesterol, triglycerides and lipoproteins in 12 week old male LIKK.APOE*3Leiden and APOE*3Leiden mouse fed a chow diet (n=6). Livers were collected to determine hepatic steatosis. Furthermore, hepatic VLDL production was measured using Triton WR1339 to block peripheral VLDL lipolysis, in overnight fasted mice (n=8).
Results: Constitutive active IKKβ expression in the hepatocyte induced an increase in basal triglycerides ($p<0.05$) in APOE*3Leiden mice, which was confirmed by elevated levels of VLDL-triglycerides (0.576 vs 0.382 mM). Liver lipid content was not different between both mice. By blocking the VLDL lipolysis with Triton1339, VLDL-triglycerides were accumulating faster in LIKK.APOE*3Leiden mice than APOE*3Leiden mice (0.065±0.017 vs 0.044±0.013 mM min^{-1}, $p<0.05$), thereby showing an increased hepatic VLDL production.
Conclusion: These data show that hepatocyte-specific low grade inflammation directly increased VLDL-TG levels in plasma by upregulation of hepatic VLDL production. We can conclude that chronic low-grade inflammation plays an important role in the pathophysiology of dyslipidemia associated with Cardiometabolic risk.
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656

Influence of lipodystropy on gene expression in liver

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Background and aims: Sterol regulatory element binding protein (SREBP)-1c is a key transcription factor that mediates the gene regulatory effects of hormones, cytokines, metabolites as lipids or glucose as well as cholesterol. The systemic impact of this central regulator of lipid metabolism in vivo was analyzed by tissue specific overexeression of SREBP-1c in adipose tissue and liver.
Materials and methods: We generated transgenic mouse models that express the N-terminal transcriptional active domain of SREBP-1c adipose tissue specific under control of the aP2 promoter. The second model SREBP-1c is expressed liver specific under control of the albumin promoter and a liver specific enhancer. Animals were kept under standardized conditions with unrestricted access to water and chow until the age of 24 or 48 weeks, respectively. Following liver resection histological and gene expression studies (Mouse 430 2.0, (Affymetrix)) were performed. RNA-data were analyzed on statistical relevance and significant expression variation of at least 2-fold ($p=0.01$) over the complete experimental series (Genespring, Agilent).
Results: Overexpression of SREBP1c in adipose tissue results in a complete loss of adipose tissue i.e. features of lipodstropy with the parallel development of massive fatty livers. In contrast, the liverspecific overexpression of SREBP-1c results in hepatic lipid accumulation, featuring a fatty liver with massively increased of visceral adipose tissue. The direct comparison of the different gene regulation in the livers shows in the lipodystrophic mouse model the differential regulation of approximal 1000 transcripts. Here genes for lipid peroxidation, transport and degradation of oxidized lipids being essential in neutralization of oxidative metabolic products i.e. ATP-transporter as ABCA1 but also key signaling molecules as PPAR-gamma or apolipopoteines are differentially regulated. The increased expression of genes from stress- and apoptosis signaling pathways as heat shock proteins or caspases might indicate the declining compensation of hepatic lipid intoxication. In the fatty liver model approximately 2500 genes, including genes for lipid and energy- metabolism like mitochondrial NADH-dehydrogenase, aldehyd-dehydrogenases, various sterol carrier proteins or cytochrome subunits next to intra- and extra- cellular transporters, were regulated.
Conclusion: These mouse models allow the elucidation of the pathogenetic role of the transcription factor SREBP-1c, but also the investigation of a pathophysiology of primary lipid accumulation in liver as well as the long term effects of chronic fatty liver.

657

Liver metabolic fluxes in response to high fat diet

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Aim: To determine adaptations of metabolic fluxes in liver of adult rats fed with a high fat (HF) diet.

Methods: Hepatocytes isolated from rats fed either a standard (carbohydrate diet: CHO) or a HF diet were perifused with increasing concentrations of glycerol in the absence or presence of octanoate (0.4mM).Glucose, lactate, pyruvate, hydroxybutyric acid and oxaloacetic acid production were measured at each substrate concentration. Oxygen consumption and major enzymatic activities were measured in isolated hepatocytes and isolated liver mitochondria. Analysis of gene expression involved in major metabolic pathways was assayed in liver using RT-PCR.
Results: Gluconeogenesis from glycerol is increased in HF group in the presence of octanoate (+30%) but is decreased in the absence of fatty acid. This effect was associated with an increase in glycerol metabolism without effect on glycolysis. In both conditions, cytosol was more oxidized whereas mitochondrial compartment was more reduced. Cellular and mitochondrial oxidative capacities were reduced by HF diet (-40%). As glycerol metabolism requires a stoechiometric utilization of ATP and NAD+, control of this pathway is either at the dehydrogenase level, (at low rate of glycerol metabolism), or at the phosphorylation level (at high rate of metabolism) depending on the redox condition. Hence, the lower glycerol metabolism observed with HF at high flux is due to diminished oxidative phosphorylation capacity and to an inability to maintain ATP content in the cytosol. By contrast, when flux through the pathway is reduced by high redox pressure generated by fatty acid metabolism, the ability of HF rats to maintain an oxidized cytosolic compartment allow then to metabolize more glycerol. In addition, RT-PCR revealed an enhanced expression of glycerol kinase mRNA and phosphoenolpyruvate carboxykinase mRNA in HF rats supporting an elevation of gluconeogenesis pathway in presence of fatty acid.
Conclusion: Metabolic fluxes were highly altered by HF diet. These changes were attributed to oxidative phosphorylation adaptation and to genomic regulation of key enzymes of carbohydrate and fatty acid metabolic pathways.
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658

Prominent role of liver in elevated plasma palmitoleate levels in response to a low-dose rosiglitazone

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Background and aims: Thiazolidinediones, such as rosiglitazone, are insulin-sensitizing drugs, which exert their benefits as agonists of peroxisome proliferator-activated receptor-γ. Recently, palmitoleate (C16:1n-7) has been identified as adipose tissue-derived lipokine that stimulates muscle insulin action. In this study, tissue expression of stearoyl-CoA desaturase-1 (SCD-1), an enzyme driving the formation of palmitoleate, as well as complex changes of fatty acid (FA) composition induced by a low-dose rosiglitazone were analyzed in mice fed a high-fat diet.
Materials and methods: C57BL/6 mice were fed a corn oil-based high fat (lipids ~35% weight/weight) diet or high-fat diet supplemented with rosiglitazone (10 mg/kg diet) for 8 weeks. Insulin sensitivity was assessed by hyperinsulinemic-euglycemic clamps, while FA composition in various lipid fractions in plasma, liver, and adipose tissue was analyzed using gas chromatography and evaluated by principal component analysis (PCA). Gene expression was assessed by real-time quantitative RT-PCR.
Results: Low-dose rosiglitazone increased whole-body glucose turnover during clamp (41 ± 3 vs. 30 ± 5 mg/kg/min, rosiglitazone vs. high-fat, respectively; $p<0.05$) without significantly affecting hepatic glucose production, suggesting improved muscle glucose metabolism. After rosiglitazone treatment, *Scd-1* expression increased ~2-fold in adipose tissue and skeletal muscle, however in the liver it increased >5-fold. In general, rosiglitazone induced a shift towards monounsaturated FAs in the liver, adipose tissue and plasma, with palmitoleate being the most up-regulated FA (3-, 1.5-, and 2-fold, respectively). Specifically in the liver, enrichment of cholesteryl esters and phosphatidyl cholines with palmitoleate and vaccenate was found, and strong correlations between the changes of various lipid fractions in the liver and total plasma lipids were observed ($r = 0.74$-0.88).
Conclusion: Changes in plasma lipid profile favouring monounsaturated FAs, mainly palmitoleate due to induction of *Scd-1* in the liver, could be involved in the stimulatory effect of rosiglitazone on muscle insulin sensitivity in mice fed a high-fat diet.
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659

IL-1β in conditioned medium from TNFα pre-treated 3T3-L1 adipocytes mediates insulin resistance in Fao hepatoma cells

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Introduction: Central obesity is frequently associated with adipose tissue inflammation and hepatic insulin resistance. We hypothesized that an *in-vitro* system to assess if insulin resistance in liver cells could be induced by secreted products from adipocytes pre-exposed to an inflammatory stimulus could assist in identifying individual mediators in this process.
Methods: 3T3-L1 adipocytes were exposed to TNFα for 18 hours in the absence and presence of Rosiglitazone or YVAD (caspase I inhibitor II). Cells were washed thoroughly, and conditioned medium from control adipocytes (CM) or from TNF-pre-treated adipocytes (TNF-CM) was collected during the subsequent 24h. CM and TNF-CM were used to treat Fao hepatoma cells in the absence or presence of interleukin 1 receptor antagonist (IL-1Ra), after which insulin action was studied. Cytokines levels in CM or CM-TNF were analyzed by ELISA and LUMINEX technology.
Results: As compared to CM -treated Fao cells, TNF-CM induced a decrease in insulin-stimulated IR ($p<0.05$) and IRSs tyrosine phosphorylation ($p<0.05$), and in PKB ($p<0.01$) and GSK3 serine phosphorylation ($p=0.01$), with no effect on the total amount of either of these proteins. Moreover insulin stimulated glycogen synthesis by 2-fold in CM treated Fao, an effect nearly fully blunted in TNF-CM treated cells. Rosiglitazone added prior to and during TNFα treatment of adipocytes partly prevented ($p<0.05$) insulin resistance in Fao cells induced by TNF-CM. Screening of possible inflammatory cytokines elevated in TNF-CM showed a 10-fold increase in IL-1β ($p<0.01$) and 4-fold in IL-6 ($p<0.05$), whereas TNFα levels were without change and IL-1α decreased compared to CM. Direct exposure of Fao to IL-1β or to IL-6 impaired insulin signaling, which in the case of IL-1β could be prevented by IL1-Ra. Similarly, IL-1Ra prevented the insulin resistance induced in Fao cells by TNF-CM. Moreover, treatment of adipocytes with YVAD (during preparation of TNF-CM) decreased IL-1β levels in TNF-CM by 70% ($p<0.05$), and prevented insulin resistance in the Fao cells. Intriguingly, IL-6 levels in TNF-CM were also diminished by YVAD (an inhibitor of the production of IL-1β and IL-18).
Conclusion: We conclude that IL-1β is a central mediator of disturbed crosstalk between adipocytes and Fao cells that is induced by exposure of adipocytes to the prototypic pro-inflammatory cytokine TNFα. This effect can be attenuated using rosiglitazone and YVAD (on the adipocytes), or IL-1Ra (on the liver cells). Although other cytokines may also contribute to the disturbed adipocyte-hepatocyte crosstalk, it seems that in this system IL-1β plays a central role, potentially also acting in an autocrine fashion to induce IL-6 secretion from adipocytes.
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660

Association of fatty liver increases small, dense LDL in metabolic syndrome

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Background and aims: Small,dense LDL (sLDL)is known as an atherogenic lipoprotein and is often associated with metabolic syndrome (MS). High frequency of sLDL is found in hypertriglyceridemic subjects. Also, fatty liver (FL) is often associated with MS. Therefore,the present study was conducted to examine if association of FL increases sLDL-cholesterol (sLDL-C) in the subjects with MS.
Materials and methods: Two hundred and seven patients were enrolled in this study. Fatty liver was estimated by echogram. Presense of MS was diagnosed according to the Japanese Guidelines for the Definition of Metabolic Syndrome. Plasma sLDL-C was measured by the 2-step (precipitation and homogeneous) assay system established by Hirano et al. Plasma triglyceride and LDL-C were measured by a standard autoanalizer method. One- and two-way analyses of variance (ANOVA) were used to compare mean values among groups. All values were expressed as mean ± SD. A significant difference was defined as $p<0.05$.

Results: sLDL-C of MS group was higher than that of non-MS group(45.4±24.2 vs. 36.5±18.9). sLDL/LDL-C of MS group was also higher than that of non-MS group(0.34±0.15 vs. 0.28±0.12). Also sLDL-C and sLDL/LDL of FL groups were both higher than those of non-FL groups(46.5 ±24.1 vs. 35.9±19.1 , 0.35±0.14 vs. 0.28±0.12). Correlation coefficients between plasma triglyceride and sLDL-C and between plasma triglyceride and sLDL/LDL were 0.36 and 0.51, respectively. Furthermore, in MS group, those correlation coefficients were 0.32 and 0.52, respectively. While in non-MS group, they were 0.36 and 0.40, respectively. In FL group, they were 0.32 and 0.52, and in non-FL group, 0.32 and 0.38, respectively. Two-way ANOVA revealed that FL was more powerful determinant of plasma sLDL-C (p=0.010) or sLDL/LDL (p=0.008) , but not MS. When we divided all subjects into four groups such as MS(-)FL(-), MS(-)FL(+), MS(+)FL(-) and MS(+)FL(+) groups, sLDL/LDL of MS(+)FL(+) group was significantly higher than all other groups.
Conclusion: In this study, we found that if both MS and FL are present, sLDL/LDL is significantly higher than that in all other groups. The liver may produce triglyceride from exess circulating glucose, free fatty acid and triglyceride,which may result in development of fatty liver. In order to avoid further triglyceride deposition, fatty liver may secrete triglyceride-enriched VLDL particles, which is recognized as a precursor of sLDL particles. In the present study, we found a significant relationship between sLDL-C and plasma trigryceride. Furthermore, this became stronger in fatty liver group, indicating that fatty liver may produce triglyceride-rich VLDL, thereby contributing to an appearance of sLDL particles in plasma of MS patients with fatty liver. Together with the result that sLDL/LDL ratio was the highest in MS(+)FL(+) group, our present findings may support above hypothesis.

661

Independent relationship between decrease of liver triglyceride content and increase of plasma adiponectin levels after pioglitazone treatment in patients with type 2 diabetes

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Background and aims: Pioglitazone, an insulin-sensitising thiazolidinedione, has multiple clinical effects including improvement of insulin sensitivity, decrease of liver fat content, increase of plasma adiponectin levels, and improvement of serum lipid profiles. However, a correlation and causal relationship between these effects are not fully understood clinically. Therefore, we analyzed these in patients with type 2 diabetes treated with pioglitazone.
Materials and methods: Fifteen Japanese patients with type 2 diabetes were treated with piopglitazone (15 mg/day) for 24 weeks. Before and after the pioglitazone treatment, BMI (kg/m^2), liver triglyceride (TG) content (% out of water), fasting plasma glucose (mg/dl), fasting plasma insulin (U/ml), HbA1c (%), plasma adiponectin (μg/ml), plasma NEFA (mEq/l), serum lipids [total cholesterol (mg/dl), TG (mg/dl), LDL-cholesterol (mg/dl), HDL-cholesterol (mg/dl), RLP-cholesterol (mg/dl), and midband (%)], AST (IU/l), ALT (IU/l), and gamma-GTP (IU/l) were measured. Liver TG content was measured by magnetic resonance spectroscopy (MRS) at 3.0 tesla.
Results: Liver TG content (26.1±25.2 vs. 7.3±4.5, p<0.05), HOMA-R (5.1±3.6 vs. 2.6±1.5, p=0.07), HbA1c (8.1±1.5 vs. 7.1±1.6, p<0.01), plasma adiponectin (4.8 vs. 10.9, p<0.01), and serum levels of TG (181 vs. 118, p<0.01), HDL-cholesterol (60 vs. 67, p=0.07), RLP-cholesterol (5.9 vs. 4.9, p<0.01), midband (15 vs. 10, p<0.01), AST (32±15 vs. 21±5, p<0.05), ALT (45±38 vs. 25±9, p<0.05), and gamma-GTP (66±42 vs. 43±19, p=0.06) were significantly (or tendency of significance) decreased or increased, whereas BMI (26.9±4.7 vs. 27.0±4.3), and serum total cholesterol (223 vs. 216), LDL-cholesterol (137 vs. 128), and NEFA (0.42 vs. 0.39) were not significantly changed. There was a significant correlation between decrease of liver TG content and increase of plasma adiponectin levels after the pioglitazone treatment (R=-0.7, p<0.05). Stepwise multiple regression analysis adjusted for the all variables described above showed that decrease of liver TG content was independently and significantly associated with increase of serum adiponectin levels (β=-0.98, R^2=0.95, P<0.001).
Conclusion: These results indicate that the pioglitazone treatment increased plasma adiponectin levels and resulted in decrease of the liver TG content via activation of AMP-activated protein kinase (AMPK), because it has already been reported that adiponectin activates AMPK, which stimulates fatty acid oxidation in the liver.

662

Glucose intolerance in patients with chronic hepatitis B or chronic hepatitis C is related with different factors

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Background and aims: The prevalence of glucose intolerance in patients with chronic hepatitis B (CHB) or C (CHC) is higher than in the general population and moreover it is higher in CHC than CHB. Aim of the present study was to explore possible associations of glucose intolerance with different factors in CHB or CHC.
Materials and methods: We studied 83 consecutive CHB patients and 113 CHC patients examined at the outpatient liver clinic. Blood samples were obtained for laboratory investigations and OGTT was performed in all (except in known diabetics). A liver biopsy was performed in 80% of patients according to clinical indications and evaluated blindly. As glucose intolerance (GI) we considered abnormal OGTT or known diabetes. We compared biochemical and histological abnormalities and the use of interferon between CHB patients with GI (N=24) and CHC with GI (N=40). All comparisons were done after adjustment for age and BMI.
Results: CHB-GI patients, compared with those with CHC-GI had more frequent family history of diabetes (58.3% vs 27.5%, P=0.019), while CHC-GI compared with CHB-GI had higher systolic blood pressure (127.7 ± 2.2 mm Hg vs 115.6 ± 3.0 mm Hg, P=0.002), higher insulin resistance, by HOMA model (5.1 ± 0.6 vs 3.8 ± 0.3, P=0.044), c-peptide (1.2 ± 0.2 ng/ml vs 0.6 ± 0.2 ng/ml, P=0.039), Apo A (135.5 ± 6.6 mg% vs 102.1 ± 9.4 mg%, P=0.007) and lower HDL (42.0 ± 2.6 mg% vs 53.5 ± 3.6 mg%, P=0.015). The prevalence of cryoglobulinemia was higher in CHC-GI group (58.3% vs 10.5%, P=0.001). Insulin/c-peptide molar ratio (index of insulin uptake by the liver), and aminotrasferase level were not different. The majority of patients in both groups had mild histological alterations of fibrosis, but the grade of necroinflamatory activity, the stage of fibrosis, the presence of cirrhosis, the grade of steatosis and the use of interferon were not different between CHB-GI and CHC-GI patients.
Conclusion: a) The type and the grade of histological alterations of the liver are similar in patients with chronic hepatitis B or C and cannot explain the higher prevalence of Glucose Intolerance in C, while b) glucose intolerance is related with genetic factors (family history of diabetes) in B and epigenetic factors in C (insulin resistance, cryoglobulinemia), which may explain its higher prevalence in C.

663

Adipocytokines, insulin resistance and hepatitis C

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Background and aims: The aim of this study was to analysis if adipocytokines levels correlate with parameters exploring insulin resistance (IR) and indices of chronic liver disease.
Materials and methods: We selected 110 patients who are divided in 2 groups: group A - 65 patients with chronic hepatitis C (CHC); and group B - 45 healthy volunteers (without CHC or diabetes - control group). IR was determinated used Homeostasis model assessment (HOMA-IR). All patients with CHC underwent liver biopsy and the liver specimens were evaluated with the Ishak scoring system for viral liver disease. Body weight, BMI, lipid profile, alanin aminotransferase, aspartat aminotransferase, gamma glutamil transpeptidase, proinflammatory state and adipocyokines were measured.
Results: The average age was 55.45±11.63 in group A and 54.27±15 years in group B. HOMA-IR (3.35 versus 1.65), adiponectin (6.21 versus 3.12 μg/ml), TNF alpha (1.99 versus 0.54 pg/ml), IL-6 (3.45 versus 1.33 pg/ml) was significantly higher in CHC patients (all p < 0.05). By multiple linear regression, independent predictors of HOMA-IR included the body mass index, apparent liver disease duration, and the serum levels of leptin, TNF alpha, IL-6 (positive correlation) and adiponectin (negative correlation). TNF alpha and IL-6 levels was correlated with the extent of histological injury (portal/periportal inflammation, p = 0.001).
Conclusion: Several pathogenic mechanisms may be involved in the effect of CHC on insulin resistance and adipocytokines and inflammatory cytokines play an important part.
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PS 49 Fat depots and body composition

664

Search for low-frequency variants associated with overall and central adiposity

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Background: Recent genome-wide association studies have identified more than 20 common variants that are associated robustly to overall and central adiposity. However, with these associations we can explain only ~2% of the variance of adiposity. Available sample-sizes and traditional statistical association analysis techniques are well-powered for searches for common variants but are likely to be under-powered to identify low frequency variants (LFV) with moderate effect sizes.
Methods: We have developed a method for detecting association of quantitative phenotypes with accumulations of minor alleles at LFV within gene coding regions (± 50kb to incorporate additional functional elements and the promoter region). With our approach, the phenotype is modelled in a regression framework as a function of the proportion of LFV at which an individual carries minor alleles, heterozygous or homozygous, and can be adjusted for necessary covariates. Using this method we have screened 6,686 samples from the Type 2 Diabetes(T2D)-WTCCC cohort (genotyped using the Affymetrix GeneChip Human Mapping 500K Array) and the Northern Finnish Birth cohort (NFBC) 1966 (genotyped using the Illumina HumanHap 370 BeadChip). We tested for association of accumulations of variants with a minor allele frequency <5% to overall (body mass index, BMI) and central adiposity (waist-circumference [WC] and waist-hip ratio [WHR], both adjusted for BMI). All three phenotypes were log transformed, with analyses adjusted for age and gender. Analyses were performed separately, within each cohort, and combined through meta-analysis for each gene containing rare variants on both genotyping platforms.
Results: The strongest signal of association with BMI was observed through meta-analysis on chromosome 4p13 ($p=9.3x10^{-6}$, effect size (beta)=0.033, standard error (se)=0.0075), with consistent effects in both cohorts, despite differences in the SNPs genotyped in each. Strong signals of association with WC were observed in the T2D-WTCCC cohort on chromosomes 17q12 ($p=4.4x10^{-5}$, beta=-0.014, se=0.0034) and 12p13 ($p=6.2x10^{-5}$, beta=-0.020, se=0.0051), and with WHR in the NFBC on chromosome 9q34 ($p=8.7x10^{-5}$, beta=-0.010, se=0.0026).
Conclusion: None of these loci have been previously associated with adiposity in studies of common variants but at least two of them are plausible biological candidates (i.e. one putative phospholipase and one neuronal sodium channel). We are currently following up these regions, together with a few weaker signals of association with strong candidacy for adiposity, with deep re-sequencing of samples in the tails of the adiposity distribution.
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665

Regulatory mechanisms for adipose tissue M1 and M2 macrophages in diet-induced obese mice

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Background and aims: Obesity and insulin resistance are closely associated with a state of low-grade inflammation in adipose tissue. Adipose tissue macrophages (ATMs) consist of at least two different phenotypes, i.e., classically activated M1 macrophages secreting pro-inflammatory cytokines and alternatively activated M2 macrophages that secrete anti-inflammatory cytokines, including IL-10.
Materials and methods: After the mice had been subjected to a high-fat diet and pioglitazone (an insulin sensitizer) treatment (Pio), ATMs were analyzed by flow cytometry using CD206 as an M2 marker and CD11c as an M1 marker.
Results: Not only the numbers of M1 ATMs and the expression of M1 marker genes, such as TNFa and MCP-1, but also the M1/M2 ratio were increased by a high-fat diet (HFD) and decreased by subsequent pioglitazone treatment. We also found that the increased number of M2 ATMs after an HFD was associated with the increased expression of IL-10, an anti-inflammatory cytokine, in the adipocyte fraction as well as in adipose tissue. The systemic overexpression of IL-10 by an adenovirus vector increased the expression of M2 markers in adipose tissue. In addition, overexpression of IL-10 improved insulin sensitivity and insulin signaling both in lean and high fat-induced obese mice. Interestingly, the expression of genes involved in mitochondrial OXPHOS and b-oxidation was increased in the skeletal muscle, which may be mediated by IL-10's effect on hypothalamus. These results suggest that IL-10 improved whole body insulin sensitivity by suppression of hepatic gluconeogenesis and an increase in genes involved in fatty acid oxidation and mitochondrial OXPHOS in the skeletal muscle.
Conclusion: M1 and M2 ATMs constitute different subsets of macrophages. Insulin resistance is associated with both the number of M1 macrophages and the M1/M2 ratio. IL-10 secreted from M2 ATMs may contribute to the improvement of insulin resistance.

666

Fat depot-specific differences in inflammatory markers in lean and obese insulin resistant pregnant rats

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Background and aims: In healthy pregnancy, several components of the metabolic syndrome, such as insulin resistance, hypertrygliceridemia and up-regulation of inflammatory markers, are present. Furthermore, obesity before pregnancy is becoming a common issue in obstetric management. We have shown that lumbar adipose tissue (AT) exhibits a low grade inflammation during pregnancy that correlates with insulin resistance in the mother. Fat depots vary in size, function, and their potential contribution to disease. It is not known, however, whether in pregnancy there are regional differences in AT inflammation. Thus, the present study was aimed at characterizing the contribution of different fat depots to the inflammatory state associated with late pregnancy, and at determining whether obesity before pregnancy impairs this inflammatory condition of the mother.
Materials and methods: 2 months old rats were fed ad libitum a standard chow diet (control) or a palatable cafeteria diet (CF-diet). After 30 days, all animals were mated and the respective control or CF-diet was maintained during pregnancy. On day 20 of gestation (late pregnancy), animals were sacrificed, and lumbar and mesenteric AT were removed. BMI, insulin sensitivity indexes (HOMA and FGIR), and biochemical parameters were estimated before and at late pregnancy. We also assessed plasma and tissular markers of inflammation (including TNF-alpha/IL-6 ratio as an index of Th1/Th2 response).
Results: Animals fed on CF-diet showed a significant increase of body weight. In fact, 70% of the CF-diet rats were obese (BMI>95th). These animals had a significant increase in plasma triglycerides (TG), insulin, leptin, and IL-1. CF-diet also provoked a deterioration of insulin sensitivity as compared to controls. Pregnancy was followed by an elevation of both TG and insulin in the control group but not in the obese animals. Furthermore, insulin resistance associated with late pregnancy was not further enhanced in the CF rats. Circulating levels of MCP-1 and PAI-1 were increased by pregnancy in both control and CF animals. Analysis of cytokines in various fat depots revealed that leptin, IL-1, TNF-alpha, and MCP-1 were higher in lumbar than in mesenteric AT, whereas PAI-1 was increased in the mesenteric AT. In both fat tissues, no change of IL-6 was observed. Thus, lumbar AT of pregnant animals had a significantly higher index of type 1/type 2 inflammatory response as compared to mesenteric AT. Leptin was the only adipokine that was increased in both fat depots of animals that were obese before pregnancy. Finally, adiponectin, an adipokine associated with insulin sensitivity and vascular relaxation, was significantly higher in mesenteric than in lumbar AT, and was significantly decreased in the mesenteric fat of the obese pregnant rats.
Conclusion: Our results show that insulin resistance during late gestation is an inflammatory state irrespective of body fat mass and that this inflammatory state could have a fat depot-specific physiological function. At one hand, lumbar AT exhibits a predominantly Th1 response, with increasing pro-inflamatory markers, such as TNF-alpha and IL-1, which may be implicated in the insulin resistance state of adipose tissue during pregnancy. At the other

hand, mesenteric adipose tissue shows an increase of PAI-1 and adiponectin, which may be involved in the changes of vascular function associated with pregnancy.
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667

The gene expression of the main lipogenic enzymes is down-regulated in visceral adipose tissue of obese subjects

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Background and aims: Contradictory findings regarding the gene expression of the main lipogenic enzymes in human adipose tissue depots have been reported. In this cross-sectional study, we aimed to evaluate the mRNA expression of fatty acid synthase (FAS) and acetyl-CoA carboxilase (ACC) in omental fat depots from subjects who varied widely in terms of body fat mass.
Materials and methods: Fatty acid synthase and acetyl-CoA carboxilase gene expression were evaluated by RT-PCR in 188 samples of visceral adipose tissue which were obtained during elective surgical procedures in 119 women and 69 men.
Results: Decreased sex-adjusted FAS (-59%) and ACC (-49%) mRNA were found in visceral adipose tissue from obese subjects, with and without DM-2, compared with lean subjects (both $p<0.0001$). FAS mRNA was also decreased (-40%) in fat depots from overweight subjects ($p<0.05$). Indeed, FAS mRNA was significantly and positively associated with ACC gene expression ($r=0.316$, $p<0.0001$) and negatively with BMI ($r=-0.274$), waist circumference ($r=-0.437$), systolic blood pressure ($r=-0.310$), serum glucose ($r=-0.277$) and fasting triglycerides ($r=-0.226$), within others (all $p<0.0001$). Similar associations were observed for ACC gene expression levels. In a representative subgroup of non-obese (n=4) and obese women (n=6), relative FAS gene expression levels significantly correlated ($r=0.657$, $p=0.034$; n=10) with FAS protein presence. FAS protein levels were also inversely correlated with blood glucose ($r=-0.640$, $p=0.046$) and fasting triglycerides ($r=-0.832$, $p=0.010$).
Conclusion: The gene expression of the main lipogenic enzymes is down-regulated in visceral adipose tissue from obese subjects.
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668

Insulin sensitivity correlates with visceral adipose tissue heme oxygenase-1 expression that is determined by hip to waist ratio

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Background and aims: Insulin resistance in visceral obesity is substantially driven by adipose tissue inflammation, in particular macrophages accumulating in obese adipose tissue. On the other hand, adipose tissue macrophages express the hemoglobin scavenger receptor (CD163) and heme oxygenase-1 (gene: *HMOX1*) that together protect from oxidative stress. Aim of this study was to evaluate the expression of *CD163* and *HMOX1* in intra-abdominal visceral (omental) and subcutaneous adipose tissue as well as circulating soluble CD163 concentrations in human obesity and its association with adipose tissue inflammation, body fat distribution, and insulin resistance.
Materials and methods: *CD163, HMOX1* and *CD68* mRNA expression in visceral and subcutaneous adipose tissue, serum concentration of soluble CD163 in morbidly obese patients (BMI>40 kg/m^2) who underwent laparoscopic surgery for gastric banding (n=20), matched for age and sex to lean control subjects (BMI<30 kg/m^2; n=20) was analyzed.
Results: *CD163* expression was highly upregulated in human adipose tissue and soluble CD163 serum concentration was elevated in obese vs. lean subjects. *HMOX1* was upregulated in adipose tissue by obesity as well and expressed predominantly in macrophages. While *CD163* expression strictly correlated with macrophage abundance, *HMOX1* was additionally upregulated within macrophages, and this upregulation was significantly lower in visceral compared to subcutaneous adipose tissue. Strikingly, relative visceral adipose tissue expression of *HMOX1* negatively correlated with waist to hip ratio and HOMA-IR (both $p=0.024$).
Conclusion: Our data suggest visceral obesity to promote insulin resistance by defective upregulation of heme oxygenase-1 in visceral adipose tissue thereby abrogating its anti-oxidative and anti-inflammatory action.
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669

Gene expression of glucagon-like peptide 1 receptor in visceral and subcutaneous adipose tissue. Decreased expression in obesity

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Background and aims: It has been observed a dual effect of Glucagon-like peptide 1 (GLP1) in human adipocytes stimulating lipogenesis and lipolysis. These effects are exerted through a GLP1 specific receptor (GLP1R). It has been observed a reduced lipogenic action of GLP1 in subcutaneous adipose tissue (SAT) from morbidly obese patients compared with normal weight. A down regulation in the expression of GLP1R in adipose tissue of obese patients could be the mechanism implicated in this modulation. The aim of the study was to test the hypothesis that GLP-1R gene expression levels in adipose tissue may be affected by the presence of obesity.
Materials and methods: SAT and Visceral adipose tissue (VAT) was obtained from 61 non-diabetic subjects (aged 55,90±14,61 years; 37 men and 24 women) who underwent an elective abdominal surgery. There were 19 lean, 27 overweight, and 15 non-severely obese subjects. Anthropometrics, biochemical and metabolic parameters including glucose, lipidic profile, HbA1c and high-sensitivity C-reactive protein (hsCRP) were recorded or quantified by routine biochemical techniques. Gene expression of GLP1R and a profile of enzymes and adipokines involved in adipogenesis and inflammation were measured by Real-time quantitative PCR on a 7900HT Fast Real-Time PCR System using commercial Taqman Assays.
Results: In VAT: Lean patients showed significantly higher GLP1R expression levels compared with overweight and obese patients ($p<0,05$) and a negative correlation was observed between GLP1R expression and BMI ($r=-0.273$; $p<0,05$). GLP1R was negatively correlated with the lipogenic enzyme Acetyl-CoA-sythetase (ACoAS) (r: -0,349; p=0,006) and Visfatin (r: -0.350; p=0,006) gene expression. A positive correlation was observed with serum triglycerids levels (r:0,266; p=0,045). To select which of the variables explain better GLP1R expression levels a stepwise linear regression model was developed including sex, BMI, triglycerids, ACoAS and visfatin as independent variables. ACoAS (B: -0.519; $p<0.0001$) and Visfatin (B: -0.414; $p<0.0001$) were the unique variables included in the model. In SAT: No differences were observed in GLP1R mRNA expression according to the degree of obesity. However, a negative correlation was observed with abdominal circumference (AC) (r: -0,462; p=0.000) and HOMA index (r: -0,340; p=0.021). Likewise, a negative correlation was observed with GLUT1 expression levels (r:-0.376; p=0,003) and a positive one with Omentin (0,309; p=0,015) and Retinoid orphan receptor (ROR) expression levels (0,356; p=0,015). In order to strengthen the independence of these associations in SAT, a stepwise linear regression analysis was developed including sex, HOMA index, AC, GLUT1, Omentin and ROR expression levels. AC was the only parameter independently associated with GLP1R expression in SAT (B: -0.015; p=0,003).
Conclusion: GLP1R gene is expressed both in SAT and VAT depots and seems to be down regulated in obesity. Expression in SAT seems to be inversely determined by parameters of visceral fat content. In VAT, expression of GLP1R is inversely associated with gene expression of the insulin-mimetic adipokine, Visfatin and possibly with a decrease in fatty acid synthesis.
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670

Neck fat area is strongly associated with insulin resistance

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Background and aims: Obesity is known to be strongly associated with insulin sensitivity (IS) and type 2 diabetes. The distribution of the deposited fat harbors unique information about the individual metabolic impact of obesity. Subcutaneous abdominal adipose tissue (SCAT), visceral adipose tissue (VAT) and fat in the neck area (NF) have previously been described to be metabolically relevant fat depots of the upper body. Whole body magnetic resonance imaging (MRI) offers a unique approach to quantify these different fat depots in lager cohorts. The present analysis was designed to study i) the impact of the three afore mentioned fat depots of the upper body on insulin sensitivity in a cross sectional design and ii) changes in these fat depots and their effect on changes in IS during lifestyle intervention (LI).
Materials and methods: A total of 187 subjects at an increased risk for type 2 diabetes were included in the cross-sectional study. Follow-up data after participation in the LI program were available in 174 subjects. Body fat depots were quantified in single reference slices at the umbical level (SCAT, VAT) and at the level of the shoulder joint (NF) using whole body magnetic resonance imaging.
Results: Cross-sectionally SCAT, VAT and NF were all negatively associated with IS (all p<0.0001). In multivariate linear regression analyses, insulin sensitivity adjusted for gender and age was significantly associated with NF (p<0.01) and VAT (p<0.001) but not with SCAT (p=0.97). The change in IS during LI adjusted for gender, age and, all fat depots at baseline was associated with change in NF (p<0.01) but not with change in SCAT (p=0.3) or VAT (p=0.3).
Conclusion: We conclude that NF represents a subcutaneous fat depot of particular importance for whole body IS, both cross sectionally and during LI. Further studies are needed to clarify the metabolic properties of NF.
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671

Diabetes mellitus type 2 is associated with a redistribution of fat mass in postmenopausal women

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Background and aims: Diabetes mellitus type 2 is characterized by impaired glucose metabolism mainly due to obesity and insulin resistance. The aim of the study was to investigate any alterations caused by diabetes mellitus type 2 either in the distribution of fat mass in the whole body or in the correlation between whole body fat mass and the whole body bone mineral density.
Materials and methods: Eighty-six diabetic and 96 healthy postmenopausal women, matched for age and BMI, were studied [age 60.1±.4.7 and 59.5±4.4 yrs (mean±1SD), BMI 32.1±4.2 and 31.7±3.8 kg/m2 respectively, HbA1c 7.9±1.1% for the diabetic women]. None of the women had ever received either any medication known to affect bone metabolism (except from sulphonylureas when appropriate) or insulin treatment. The estimations of either the distribution of fat mass in the extremities and the abdomen or the WBMD were performed by dual-energy X-ray absorptiometry (DXA). They included the average proportions of fat mass (PF, %) in the upper and lower extremities (ARMPF and LEGPF), the average PF of the four extremities (EXTPF), the PF in the abdomen (ABPF),as well as the whole body PF (WBPF) and whole body BMD (WBBMD).
Results: No significant differences were observed between the groups regarding age, WBBMD and WBPF. The diabetic women exhibited significantly higher ARMPF (p<0.005) and significantly lower LEGPF (p<0.01), while the EXTPFs and WBPF did not differ significantly between the groups. The ABPF was significantly higher in the diabetic women (p<0.05). No significant correlation was observed between WBBMD and any PF. In both groups the correlation of BMI to LEGPF was not significant (r=0.23 and 0.14, NS), in contrast to its correlations with any other PF. The ABPF was stronger correlated to ARMPF [r=0.73 in diabetic and 0.71 in healthy women (p<0.001)] than LEGPF [r=0.43 and 0.31(p<0.05), respectively). Age was significantly correlated to all PFs only in the healthy group.
Conclusion: Diabetes mellitus type 2 seems to be accompanied by a redistribution of fat mass which is shifted from the lower extremities to the upper ones and to the abdomen. Whole body bone mineral density does not seem to be affected by the diabetic status. In both diabetic and obese non-diabetic postmenopausal women bone mineral density does not seem to significantly correlate to the proportion of fat mass in total body mass.

672

Low relative skeletal muscle mass is independently associated with insulin resistance in Korean diabetic patients

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Low relative skeletal muscle mass (sarcopenia) is associated with an increased risk of adverse health outcomes. Insulin resistance could be involved in age-related muscle protein loss, progressively leading to sarcopenia. Only few studies has examined the association between skeletal muscle mass and insulin resistance in diabetic patients. In this study, we investigated whether skeletal muscle mass was associated with insulin resistance in type 2 diabetic subjects. We enrolled 3034 patients (mean age 57±10 yr, men 50%) with type 2 diabetes, who visited our Diabetes Center from 2004 to 2008. The skeletal muscle mass was estimated from bioimpedance analysis measurements and expressed as skeletal muscle mass index (SMI = skeletal muscle mass/body mass x 100). Insulin resistance was directly assessed by short insulin tolerance test as a rate constant for plasma glucose disappearance (*kitt*, %/min) after intravenous injection of regular insulin (0.1U/kg). The SMI was negatively correlated with total cholesterol (r=-0.084, p<0.01), triglyceride (-r=0.089, p<0.01), systolic blood pressure (r=-0.063, p<0.05) and diastolic blood pressure (r=-0.108, p<0.001), and positively correlated with HDL-cholesterol (r=0.074, p<0.01) and *kitt* (r=0.100, p<0.001). In multiple regression model, the SMI was independently associated with *kitt* after adjusting for age, glucose, triglyceride, HDL cholesterol, systolic blood pressure and fat mass in both sexes (men: β=0.083, p<0.01, women: β=0.050, p<0.05). The odds ratio for severe insulin resistance (those with lowest quartile of *kitt*) was 1.41 (95% CI: 1.03, 1.91) in men and 1.50 (95% CI: 1.04, 2.17) in women with lowest quartile of the SMI compared with men and women with highest quartile of the SMI.
In conclusion, the low relative skeletal mass indicative of sarcopenia is associated with insulin resistance in type 2 diabetes. Interventions leading to skeletal muscle hypertrophy such as resistance training and/or amino acid supplementation may need to be tested in diabetic patients with severe insulin resistance.

673

Body composition in type 1 and type 2 diabetes patients with similar body mass index

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Background and aims: Increased body fat is associated with type 2 diabetes while this association with type 1 diabetes is weak or nonsignificant. The objective of this study was to evaluate differences regarding body composition and fat distribution in type 1 and type 2 diabetic people with similar body weight.
Materials and methods: Anthropometric and body composition measurements [body fat mass (BFM), percent body fat (PBF), estimated visceral fat area (eVFA) and skeletal muscle mass (SMM)] were performed using a multifrequency bioelectric impedance analyzer (InBody 720) in diabetic patients with BMI<25 Kg/m^2 (Group A: 30 type 1 and 36 type 2 diabetes patients) or BMI>25 Kg/m^2 (Group B: 35 type 1 and 72 type 2 diabetes patients).
Results: In both groups, type 2 diabetes patients had significantly greater mean (SD) values for age [Group A: 65.5 (9.1) vs 45.6 (12.4) years; p<0.0001, Group B:59.0 (11.9) vs 40.4 (15.1) years; p< 0.0001] and eVFA [Group A: 114.0 (17.3) vs 84.0 (21.0) cm^2 ; p<0.0001, Group B: 145.6 (24.4) vs 114.9 (28.9) cm^2 ; p<0.0001]. No significant differences in BMI, waist circumfer-

ence (WC), BFM, PBF and SMM, between groups, were observed. In a larger group of T2DM patients,in simple linear regression analysis, BFM (r=-0.283; n=172; p<0.0002), SMM (r=-0.458; n=172; p<0.0001) and WC (r=-0.332; n=146; p<0.0001) were inversely correlated with age, while in T1DM patients WC (r= 0.656; n=51; p<0.0001) and eVFA (r= 0.520; n=62; p<0.0001) were directly correlated with age. We calculated the eVFA/BFM ratio to estimate the relationship between eVFA and BFM with increasing weight. As expected, the ratio eVFA/BFM decreased with increasing weight both in T1DM and T2DM patients, but remained comparatively greater, inside every group [Group A: 7.5 (2.8) vs 5.8 (2.1) cm^2/Kg; p<0.007, Group B: 5.6 (0.9) vs 4.5 (1.0) cm^2/Kg; p<0.0001] in T2DM patients. In 30 from 88 T2DM (34%) but only in one from 32 (3.1%; p<0.001) T1DM patients abdominal ultrasound examination disclosed liver steatosis. In logistic regression analysis, liver steatosis was significantly correlated to BFM and eVFA.
Conclusion: Our data suggest that, at similar levels of body fatness, T2DM (as compared to T1DM) patients have a different body composition with significantly more ectopic fat accumulation in visceral area and liver steatosis.

PS 50 Brain / CNS and metabolism

674

Insulin promotes fatty acid storage in white adipose tissue by a CNS-mediated and CD36-dependent mechanism
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Background and aims: Central insulin administration has been shown to increase mass and cell size of white adipose tissue (WAT). The aim of this study was to determine to what extent the effects of insulin on fatty acid (FA) metabolism in WAT are mediated through the central nervous system (CNS).
Materials and methods: The effects of central (intracerebroventricular administration) or peripheral (during hyperinsulinemic-euglycemic clamp) insulin on tissue-specific partitioning of plasma triglycerides (TG) and FA were assessed in male C57Bl/6 mice using continuous i.v. infusion of glycerol tri[^{3}H]oleate-labeled VLDL-like particles and albumin-bound [^{14}C]oleate as tracers.
Results: Intracerebroventricular (i.c.v.) administration of insulin does not affect plasma parameters (insulin, glucose, FA and TG) but selectively increased the retention of both TG-derived FA (+69%; p<0.05) and albumin-bound FA (+86%; p<0.05) in WAT. This effect is abolished in diet-induced insulin resistant mice and is also completely blocked by co-administration of the K_{ATP} channels inhibitor tolbutamide, indicating the involvement of central insulin signaling pathway(s). In addition, the i.c.v.-induced retention of FA in WAT was totally abrogated in CD36-/- mice, demonstrating that the long chain FA transporter CD36 plays a crucial role in this effect. In order to investigate its physiological relevance, we next studied to what extent insulin signaling in the CNS is involved in the tissue-specific partitioning of plasma TG and FA induced by circulating insulin. Very interestingly, the rise in peripheral insulin level during hyperinsulinemic-euglycemic clamp increased retention of both TG-derived FA (+72%; p<0.01) and albumin-bound FA (+265%; p<0.01) in WAT, an effect which is abrogated by concomitant i.c.v. administration of tolbutamide.
Conclusion: Insulin promotes FA retention in WAT by a CD36-dependent mechanism involving activation of K_{ATP} channels in the brain. These findings highlight a paradigm where circulating hormones can act directly on target tissues as well as indirectly through the CNS.
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675

Insulin in the brain promotes locomotor activity in lean
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Background and aims: The insulin signaling pathway coordinates multiple cellular responses in the periphery and the brain, and when insulin action fails a diabetic phenotype occurs. In the presence of cerebral insulin resistance, neuronal activity is diminished and followed by alterations in glucose and energy homeostasis, however, the impact of insulin in the brain with regard to behavioural aspects like locomotion is unknown.
Materials and methods: To address insulin action in the brain with regard to cortical activity in and its behavioural consequences, the insulin signaling pathway was followed from the receptor up to electrical activity, and locomotion. Western Blot analysis, electrocorticogram recordings with intracerebroventicular (icv) application of insulin, and measurements of locomotor activity were performed in lean and obese mice and correlated to magnetoencephalography measurements in humans.
Results: Here, we show that insulin application icv into mice was accompanied by a profound increase in locomotor activity, whereas a PI 3-kinase inhibitor or adiposity-related factors abolished insulin-induced locomotion. In diet-induced obesity, insulin delivery to the brain was not able to enhance both, cerebrocortical activity and locomotion. A potential candidate that links insulin signaling to locomotion is the insulin-sensitive potassium channel Kv1.3. In accordance with this concept, selective pharmacological inhibi-

tion of Kv1.3 channels promoted locomotor activity in lean and obese mice. In addition, human subjects carrying a single-nucleotide polymorphism in the promoter region of the Kv1.3 gene displayed significantly impaired aerobic capacity, and the insulin-mediated desire to move was tightly correlated with spontaneous cortical activity in human subjects that underwent a hyperinsulinemic-euglycemic clamp.
Conclusion: Thus, our data provide functional evidence for a direct effect of insulin on brain activation patterns associated with locomotor activity and show that adequate insulin action in the brain is the basis for the maintenance of locomotion, while in obese, insulin-mediated locomotor activity is blunted and further aggravates physical inactivity.
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676

IL-6 links exercise to hypothalamic insulin and leptin sensitivity through IKKβ and ER stress in DIO rats
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Background and aims: Recent studies provide an intriguing link between metabolic inflammation and dysfunction of the hypothalamic insulin and leptin signaling through activation of IKKβ and endoplasmatic reticulum (ER) stress. It has been proposed that physical exercise improves insulin and leptin signaling in the hypothalamus and induced appetite suppressive actions, but the mechanism by which exercise increases the insulin and leptin response in obese rats remains uncertain. Here we investigated the central role of interleukin-6 (IL-6) on hypothalamic IKKβ activation and ER stress in diet-induced obese (DIO) rats after physical exercise.
Materials and methods: Immunohistochemistry, Western blot, RT-PCR and ELISA assays were combined to evaluate the effects of acute swimming exercise in the hypothalamus of DIO rats and C3H/Hej mice.
Results: High-fat diet atypically activated the hypothalamic IKKβ and ER stress and induced central insulin and leptin resistance and orexigenic response in rats. A single bout of exercise was sufficient to reduce IKKβ phosphorylation and ER stress, restored central insulin and leptin sensitivity and reduced food intake in DIO rats. Interestingly, the pretreatment with intracerebroventricular (icv) infusion of IL-6 antibody blocked these effects of exercise. The icv infusion of recombinant IL-6 reduced IKKβ phosphorylation and ER stress and restored central insulin and leptin signaling in DIO rats at rest. In addition, exercise and the icv infusion of recombinant IL-6 reduced ER stress induced by icv infusion of Tapsigargin in lean animals. However, in contrast to control mice, in IL-6-deficient mice (C3H/Hej) injected with icv Tapsigargin, exercise failed to reduced ER stress.
Conclusion: Our results show that exercise reduced the aberrant IKKβ activation and ER stress in the hypothalamus and prevented hyperphagic response in an IL-6 dependent-manner in rodents. Hence, IL-6 is an important physiological contributor to the central insulin and leptin action mediated by exercise, linking it to hypothalamic ER stress and inflammation.
Supported by: FAPESP

677

Changes in cerebrospinal fluid insulin during non-neurological surgery
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Background and aims: Recently, insulin has been found to play an important role in central nervous system (CNS) signalling, and is thought to influence cognition, the regulation of body weight and other metabolic parameters. The CNS is dependent on an active uptake of insulin over the blood-brain barrier (BBB) as no insulin seems to be synthesized de novo in the CNS. However, very few studies regarding the different factors affecting insulin uptake in the CNS have been published, especially in humans. In this study, the Surgical Stress Study, the aim was to study how the levels of different substances and their metabolites in cerebrospinal fluid (CSF) were affected by major stress in the form of non-neurological surgery.
Materials and methods: Thirty-five patients undergoing non-neurological major surgery (knee arthroplastics) had serum and CSF samples drawn before, three hours after and on the morning after surgery (point A, B and C). Serum and CSF samples were analyzed for concentrations of insulin and albumin. Statistical calculations were performed in order to assess correlations between the different outcome measures at the three different points, and with body mass index (BMI).
Results: The CSF insulin concentration was compared with the serum concentration as well as with albumin and other CSF proteins. Preliminary results demonstrate that the concentrations of insulin in CSF and serum were strongly correlated at all three points. The changes in CSF insulin were considerably smaller than the changes in serum insulin at all time points. The albumin levels in CSF and serum and the CSF/ serum albumin ratios showed no correlation with CSF insulin before surgery, but a strong correlation was seen between CSF albumin and insulin at both points after the operation. BMI was negatively correlated with the CSF/serum insulin quota before and directly after surgery.
Conclusion: In this study, we replicated in humans animal findings showing that serum and CSF insulin levels are correlated and that the changes in CSF insulin mirror those in serum insulin under stress, although at a much lower absolute level. It seems like the central nervous system is protected from strong fluctuations in peripheral insulin levels. CSF insulin levels decreased (though not significantly) during the operation, but increased again, although not to the level at point A, after the following night. An interesting observation is that CSF insulin and albumin are correlated during the operation. This could be a sign of BBB permeability being influenced by the operative stress, so that at least a subgroup of subjects experience a drastic decrease in permeability and transport of both proteins. A high BMI impaired the transport of insulin into the CNS. The effect of insulin in the CNS is opposite that of peripheral insulin, decreasing food intake and promoting weight loss, so this could indicate a possible mechanism by which stress promotes obesity.
Supported by: SMRC, FAS, VG region, SVLS, GLS

678

Human primary astrocytes represent a novel insulin-responsive cell type
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Background and aims: After systemic or intranasal application of insulin, we recently showed modulation of the activity of very specific brain regions using MEG and fMRI techniques.
Since insulin receptors are thought to be nearly ubiquitously expressed throughout the brain, insulin's effects on specific regions may be mediated by additional processes. One hypothesis that could explain this observation is a modulation of neuronal activity by other insulin-sensitive cell types. Due to the fact that astrocytes constitute the predominant cell type in the brain and can influence neurons in several ways, they are therefore potential candidates. Astrocytes can (i) store glycogen and supply neurons with lactate as energy source; (ii) take up and release neurotransmitters, and (iii) form the blood-brain barrier in cooperation with endothelial cells and pericytes. In this study, we asked whether primary human astrocytes are insulin-responsive at the molecular level.
Materials and methods: Commercially available Normal Human Astrocytes (Lonza) were grown in the recommended media. Before each experiment, they were starved in media containing 0.5 % FCS for 48h. After insulin stimulation, cells were lysed and the protein content of the lysates was determined using the Bradford method. Proteins were separated by SDS-PAGE. Major players in the insulin signaling pathway were detected by Western blotting using specific antibodies. Protein phosphorylation levels were investigated with phospho-specific antibodies. Glucose uptake was determined using 3H-Deoxy-D-Glucose. Lactate levels were measured enzymatically.
Results: We could demonstrate relevant expression of key proteins of the insulin signal cascade, such as insulin receptor β-subunit, insulin receptor substrat-1, Akt / protein kinase B and glycogen synthase kinase 3, in Normal Human Astrocytes. After stimulation with increasing concentrations of insulin (0 - 50 nM) for 15 minutes, activating phosphorylation of these proteins was detected. We could neither detect significantly increased glucose uptake after insulin treatment for 15 minutes (0-100 nM) nor lactate secretion after insulin stimulation for 6-8 hours (0-100 nM).
Conclusion: This study demonstrated the expression of key signaling molecules of insulin signal transduction in primary human astrocytes. Detection

of phosphorylation proved the functional activity of this signaling pathway. Nevertheless, we could not detect the ultimate endpoint of insulin signaling in astrocytes, yet. While insulin increases glucose uptake in many cell, it does not so in commercially available Normal Human Astrocytes. Another important function of astrocytes, the secretion of lactate, is also unchanged by insulin in these cells. Thus, astrocytes are a candidate insulin-responsive cell type for the modulation of neuronal activity after insulin stimulation, but the interaction pathway is still unknown.

679

Feeding modulates human brain responses to food cues

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Background and aims: The central responses to food ingestion are important in maintaining normal energy balance and dysregulation may result in overeating , leading to obesity and Type 2 diabetes. We studied modulation by food ingestion of regional brain activation at rest and in response to food cues in health using functional Magnetic Resonance Imaging (fMRI).
Materials and methods: Ten healthy volunteers were studied using fMRI (1.5T MRI scanner) in a random order after an eight hour fast or a 400Kcal mixed meal. Changes in regional brain perfusion were measured during rest using continous arterial spin labelling imaging (CASL) and during food and non food imaging viewing in a block design paradigm using blood oxygen level dependant (BOLD) imaging. Time series were extracted from brain regions which showed statistically significant differences between food and non food, fed and fasted conditions .Satiety responses were recorded using a visual analogue scale. Analysis for the BOLD data was made using XBAM, and for CASL using SPM-5 FIL London.
Results: Food ingestion increased satiety scores ($p<0.0001$), while hunger and desire to eat scored more highly in the fasted state($p<0.001$ $p<0.0004$) Preliminary analysis of CASL analysis showed increase perfusion in the fed state in thalamus (sensory relay betweeen subthalamic areas andsesory cortex), hypothalamus (energy sensing) , precuneus (responsible for self processing), cerebellum, gustatory cortex and the visual and auditory cortices. We also observed significant reduction in perfusion in the orbito-frontal cortex. Food ingestion enhanced BOLD responses to food image viewing in dorsolateral prefrontal cortex, insula, anterior cingulate, while responses in visual cortex were not different between food and non food images in either the fed or fasted state.
Conclusion: Food ingestion alters neuronal activation at rest and in response to furtherfood cues in classicalcue response network and brain regions known known to be involved in appetite control and satiety sensing.The modulation of cerebral perfusion and food cue-induced response by feeding provides a measure of the normal response to feedingwhich may be exploited in the study of groups at risk of overeating, obesity and Type 2 diabetes and possible treatments that work through modulating appetite and food ingestion.
Supported by: Wellcome Foundation

680

Evidence of a regulation in peripheral glucose metabolism by central nervous system in humans: from Parkinson to diabetes

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Background and aims: Recent animal studies have demonstrated an important link between central sensing of substrates and endogenous glucose production, suggesting that specific neurons in the hypothalamus and second order neurons might control peripheral glucose metabolism. Subthalamic nucleus-deep brain stimulation (STN-DBS) is an alternative treatment for patients with uncontrolled symptoms of Parkinson disease. As subthalamic nucleus are located close to hypothalamus, we hypothesized that DBS may induce changes in the hypothalamus activity and modify hepatic glucose production in humans.
Materials and methods: To this aim, endogenous glucose production (EGP) was measured during 5h infusion of D-[6,6-$^2H_2$]-Glucose in 8 STN-DBS treated patients after DBS interruption (Stim OFF) and under DBS (Stim ON) conditions. EGP was then compared to EPG of matched healthy subjects (control group) as well as plasma insulin, glucagon and free fatty acids.
Results: In Stim OFF conditions, EGP was higher in patients than in the control group (2.62 ± 0.09 vs 2,27 ± 0.10 mg/kg/min, $p<0.01$). Despite no significant change in plasma glucose (p=NS), a significant and consistent decrease of EGP was observed in the patients between Stim OFF and Stim ON (2.04± 0.07 mg/kg.min, $P<0.05$), and there was no more difference between control group EPG (2.09 ± 0.03 mg/kg.min) and Parkinson patients in Stim ON (p=NS). No difference in insulin, glucagon or FFA levels was observed between the patients in Stim OFF and Stim ON and with the control group.
Conclusion: These results demonstrate for the first time an effect of deep brain stimulation on endogenous glucose production and suggest a role of central nervous system on peripheral glucose regulation in human. In addition, these data indicate that non-diabetic patients with Parkinson disease may have impaired glucose metabolism likely secondary to dysfunction of autonomous nervous system.

681

Alfa linolenic (w-3) and oleic (w-9) fatty acids revert inflammation and apoptosis in the hypothalamus of diet-induced obese rats

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Background and aims: In obesity, the adequate hypothalamic function is disrupted by local inflammation and apoptosis of neurons involved in the control of feeding and thermogenesis. Recent studies have revealed that long-chain saturated fatty acids present in western diets are involved in the induction of the pro-inflammatory and apoptotic response. Here, we evaluate the properties of w-3 and w-9 fatty acids as candidate protective factors against diet-induced inflammation and apoptosis of the hypothalamus.
Materials and methods: Obese and diabetic male Wistar rats were separated into three groups and submitted to stereotaxic implantation of a cannula to receive either w-3, w-9 or BSA via icv administration into the 3rd ventricule, for 5 days. Feeding behavior was measured daily. Molecular, histological and biochemical parameters were analyzed at the end of the experimental period. The pro- and anti-inflammatory proteins IL6, IL10, TNFa, iNOS, and JNK; the insulin and leptin pathways proteins IR, IRS1/2, AKT, FOXO, JAK, and STAT; and the pro- and anti-apoptotic proteins BAX and Bcl were evaluated by Western Blot and RT-PCR. For the immunohistochemical (IH) analysis, the specific antibody F4/80 was utilized to detect activated microglia.
Results: Control group consumed 15±2 g/d diet as compared with w-3 and w-9 groups, which consumed 3±1 and 4±1 g/d, respectively. Blood glucose levels were lower in w-3 and w-9 groups (113 mg/dL±5 and 125 mg/dL±7 vs. 198±3mg/dL, Control). The IH images of the hypothalamus showed a distinct reduction in the pro-inflammatory process mediated by macrophages in both FA groups, in comparison with Control. The molecular analysis revealed the potential activity of w-3 and w-9 to decrease pro-inflammatory proteins, as well as the pro-apoptotic protein. The most important proteins involved in the insulin and leptin pathways showed an increase in their activity on the hypothalamus. In both groups, w-3 and w-9, an important increase in IL-10 and Bcl-2 expression was observed in the respective anti-inflammatory and anti-apoptotic responses.
Conclusion: In conclusion, w-3 and w-9 re-established the correct hypothalamic signaling of te insulin and leptin. The hypothalamic cell survival was increased by w-3 and w-9, and all the effects together resulted in the control of the hyperphagic state, reducing the body weight and glycemic levels.
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682

Combination of mitochondrial targeting nutrients for the management of brain failures associated with type 1 diabetes

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Background and aims: The brain is known to be abnormally susceptible to oxidative damage from high ambient oxidant levels associated with diabetes. Much attention has been recently focused on the important role of mitochondria in the interplay between energetic failure and oxidative stress within nerve cells. If such a mechanism is indeed relevant to the pathogenesis of diabetes-associated brain disorders, the agents that can both attenuate mitochondrial dysfunction and directly or non-directly scavenge free radicals might prove to be efficacious. The study was performed to evaluate neuro-

protective efficacy of a combined supplementation of mitochondria-specific antioxidants (acetyl-L-carnitine (ALC) and alpha-lipoic acid, ALA) and nicotinamide (NAm), as a multiple-action drug.

Materials and methods: All studies were carried out after 4 weeks of STZ-induced diabetes (60 mg/kg of body weight, i.p.) in rats treated for 14 days with or without combination of ALC, ALA and NAm (respectively 100, 50 and 100 mg/kg b.w., i.p.). Reactive oxygen species (ROS) were analysed using 2',7'-dichlorodihydrofluorescein diacetate, converted to fluorescent 2',7'-dichlorofluorescein (DCF) upon oxidation. Mitochondrial membrane potential ($_{\Delta}\Psi_{m}$) was assayed spectrofluorimetrically with rhodamine123 (Rh123). NAD^{+} and sorbitol levels were assayed enzymatically.

Results: Diabetes was shown to be associated with 23.0±1.7% elevation of Rh123 fluorescence under self-quenching conditions and decreased response to protonophore FCCP that is indicative of mitochondrial membrane depolarization. Research into brain energetic revealed that compared with the ATP and NAD^{+} contents in control, those in diabetic brain were respectively 28.3±1.9 and 30.4±2.0% decreased, p<0.05. Combined treatment of diabetic rats with ALC, ALA and NAm countered loss of $_{\Delta}\Psi_{m}$, restored NAD^{+} and partially reversed ATP towards control levels most likely due to normalization of the efficiency of mitochondrial function. Dysfunctional mitochondria appear to account for increased formation of ROS, since DCF fluorescence level in diabetic mitochondria was 37.2±2.8% higher than in control, p<0.05. Combined therapy diminished the diabetes-associated DCF-sensitive mitochondrial ROS production by 29.3±2.0%, p<0.05. In keeping with cellular respiration defects and oxidative stress Na^{+},K^{+}-ATPase activity was found to be reduced to 153±10 in diabetes vs. 249±15 nmol Pi/min per mg protein in control, p<0.05. The enzyme activity was significantly increased (by 19.5±1.2 % vs. diabetes, p<0.05) after the treatment. Following administration of the drugs to diabetic rats, hyperglycaemia was reduced to 15.2±1.0 vs. 20.3±1.2 mmol/l in diabetes and two-fold elevated brain sorbitol level was markedly normalized, p<0.05. It is also notably that the combination of therapies in this study can ameliorate brain lesions induced by diabetes more effectively than each nutrient alone as was previously shown.

Conclusion: The findings suggest that neuroprotective therapy in diabetes should proceed towards the simultaneous protections of nerve cells against impairments of mitochondrial energy transformations and enhanced endogenous oxidant burden by using combination of multiple naturally occurring compounds with various mechanisms of action. Beneficial effects of combined supplement of ALC, ALA and NAm offer a non-toxic, well tolerated and rational option for diabetes-associated brain disorders.

683

Alterations of PED/PEA-15 gene at the interface of diabetes and Parkinson disease

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Background and aims: Recent studies show that an increased risk of neurodegenerative disorders including Parkinson's disease (PD) is associated with type 2 diabetes. PD results from the loss of dopaminergic cells in the substantia nigra. Studies in animal models have shown also that chronic hyperglycemia impairs striatal dopaminergic transmission. However the molecular mechanisms underlying the abnormal dopaminergic function in individuals with T2D are still unknown. PED/PEA-15 is a 15 kDa cytosolic protein overexpressed in peripheral tissues of T2D patients. Transgenic mice ubiquitously overexpressing PED/PEA15 (PED-TG) feature insulin resistance and impaired pancreatic beta-cell function. Moreover, PED/PEA-15 is highly expressed in brain, in particular in the prefrontal cortex and substantia nigra. We have therefore investigated whether PED/PEA-15 may affect dopaminergic function.

Materials and methods: Motor-behavioural, immuhistochemical and biochemical studies were performed.

Results: Motor function in PED-TG mice was investigated by measuring the time to climb an inclined platform and the time to rotate 180 degrees after placed nose down on an incline (negative geotaxis). The experiments showed a two-fold increased time of climbing (P<0.02) and impaired negative geotaxis (P<0.05) of PED-TG, compared to their wild-type littermates. Moreover, grip strength testing, hanging wire test and pole test excluded significant differences in muscular force. Footprint analysis excluded locomotor ataxia, whereas the presence of air righting reflex excluded abnormalities in vestibular functions. Learning rate for motor ability tasks, measured with four consecutive trials on a rotarod, and for spatial tasks, measured with Barnes Maze (ability to find and remember the shortest path to an exit on a round board) was also significantly slower (P<0.02). Moreover, PED-TG mice suspended by the tail present foot-clasping reflex; an aberrant behaviour linked to deranged dopaminergic function. No impairment of cerebral structures, including striatum size, was noticed by morphological examination. Nevertheless, tyrosine hydroxylase (TH) content was 60% lower in caudate-putamen protein extracts from PED-TG compared to controls (P<0.02), as assessed by Western blot analysis. Immunostaining of brain slides from PED-TG mice also revealed a reduced content of TH and of dopamine, compared to controls.

Overexpression of PED/PEA-15 in PC12 cells leads to 40% reduction of TH levels. In particular insulin increase TH expression in parental PC12, while not in those overexpressing PED/PEA-15.

Conclusion: Our data support the hypothesis that PED/PEA-15 overexpression in mice reduces dopamine levels inducing a Parkinson-like syndrome and modifies neuronal response to insulin.

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PS 51 Linking exercise to metabolic effects

684

Subjects with early-onset type 2 diabetes show defective activation of the skeletal muscle PGC-1α/mitofusin-2 regulatory pathway in response to physical activity

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Background and aims: Type 2 diabetes is associated with insulin resistance and skeletal muscle mitochondrial dysfunction. Recent studies have indicated that subjects with early-onset type 2 diabetes show an incapacity to increase VO2max in response to chronic exercise. This suggests a defect in muscle mitochondrial response to exercise. Here, we have explored the nature of the mechanisms involved.
Material and methods: Muscle biopsies were collected from young type 2 diabetes subjects and obese controls before and after acute or chronic exercise protocols and the expression of genes and/or proteins relevant to mitochondrial function was measured. In particular, the regulatory pathway PGC-1a/mitofusin-2 (Mfn2) was analyzed.
Results: At baseline, subjects with diabetes showed reduced expression (by 26%) of the mitochondrial fusion protein, Mfn2 and a 39% reduction of the a-subunit of ATP synthase. Porin expression was unchanged consistent with normal mitochondrial mass. Chronic exercise led to a 2.8-fold increase in Mfn2, as well as increases in porin, and the a-subunit of ATP synthase in muscle from control subjects. However, Mfn2 was unchanged following chronic exercise in those with diabetes while porin and a subunit of ATP synthase were increased. Acute exercise caused a 4-fold increase in PGC-1a expression in muscle from control subjects but not in those with diabetes.
Conclusion: Our results demonstrate alterations in the regulatory pathway that controls PGC-1a expression, and induction of Mfn2 in muscle from patients with early onset type 2 diabetes. Patients with early-onset type 2 diabetes display abnormalities in the exercise-dependent pathway that regulates the expression of PGC-1a and Mfn2.

685

The effects of suppression of endogenous insulin on hepatic and quadriceps glycogen stores after 60 min of submaximal exercise in healthy adult males

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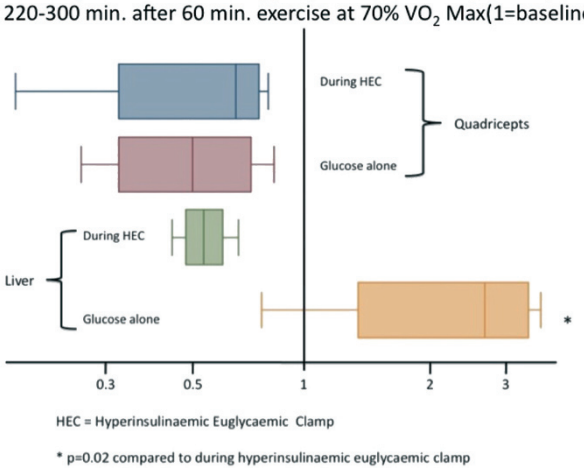
Background and aims: Hypoglycaemic episodes are more common and more severe within 24 hours of exercise in patients with type 1 diabetes. This may in part be due to depleted hepatic glycogen stores. Physiologically at least 50% of insulin is subject to hepatic first-pass metabolism, exposing the liver to higher concentrations of insulin than the peripheral circulation. Management of type 1 diabetes using peripherally administered insulin is associated with a relative hepatic hypoinsulinaemia. We aimed to explore whether relative hepatic hypoinsulinaemia is associated with impaired hepatic glycogen replenishment after exercise.
Materials and methods: Four men, aged 18-25, attended after an overnight fast and cycled for 60 min at 70% of their VO2 max, after which they underwent a hyperinsulinaemic euglycaemic clamp (HEC) for 300 min, in order to suppress endogenous insulin secretion. Measures of liver and quadriceps glycogen stores were taken pre-exercise, 0-80 mins (early) post-exercise and 220-300 min (late) post-exercise using 13C magnetic resonance spectroscopy (MRS - 40 minute protocol per tissue). The protocol was repeated after no less than 1 month without endogenous insulin suppression, by administration of glucose alone in the same quantities as received during the HEC.
Results: Exercise approximately halved the glycogen stores in the quadriceps in both the HEC and glucose alone studies (Proportion of baseline glycogen present ± SD = 0.54±0.16 and 0.44±0.36 respectively). There was no difference in the recovery of glycogen in the quadriceps in the late phase between the groups (Figure 1). In the early post exercise phase there was a trend towards a difference in hepatic glycogen store in the two groups with lower values in the HEC group compared to the endogenous Insulin group (Proportion of baseline ± SD = 0.81±0.2 and 2.0 ± 1.2 respectively). In the late post exercise phase (figure 1) hepatic glycogen stores remained depleted in those on the HEC whereas those treated with glucose alone had replenished their hepatic glycogen stores (p for difference = 0.02).
Conclusion: A HEC, simulating the effects of a deficiency in endogenous insulin replaced by peripherally administered insulin, in healthy males after 60 min of exercise at 70% VO2 max impaired glycogen replenishment in the liver over at least 6 hours. If this is representative of patients with type 1 diabetes, it may account for the increased risk and severity of delayed hypoglycaemia observed after exercise.

Figure 1: Proportion of baseline glycogen stores in the quadriceps and liver 220-300 min. after 60 min. exercise at 70% VO_2 Max(1=baseline)



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686

The effect of exercise on intrahepatocellular (IHCL) and intramyocellular lipids (IMCL)

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Background and aims: Intramyocellular (IMCL) and intrahepatocellular lipids (IHCL) are both related to insulin sensitivity and thus of crucial importance for the epidemic "metabolic syndrome". While it is well known that IMCL are metabolically active and the concentration in skeletal muscle can be changed within several hours with diet and exercise, short-term variations of IHCL in liver tissue are much less recognized. 1H-MR spectroscopy allows to assess changes of IMCL and IHCL repeatedly. This study aims at short-term variations of IMCL and in particular IHCL due to exercise.
Materials and methods: Nine healthy volunteers (age 35 +/- 12 years; BMI 22.6 +/- 2.7 kg/m2; VO2peak 56.1 +/- 10.7 ml/kg/min) were enrolled in this sub-study. Three days prior to the study, the volunteers did not perform exercise and increased the daily fat intake by additional fat snacks (0.75 g/kg/d). Following a first MR examination with determination of IMCL and IHCL, the volunteers exercised on a treadmill at 50-55% of VO2peak for two hours, followed by a second MR examination.
Results: IMCL decreased significantly during exercise (reduction in %, mean±SD: -25±5, p =0.006), which contrasts to a significant increase of IHCL (increase in % mean±SD:32±11, p = 0.026). During exercise, serum free fatty acids (FFA) increased significantly (p << 0.001, two-way ANOVA).
Conclusion: The reduction of IMCL during exercise has been expected from several reports in literature. However, an increase of IHCL as a consequence of 2 hours exercise has not been reported to the best of our knowledge. Obviously, during exercise the skeletal muscle uses IMCL; in parallel, adipose tissue releases FFA, which may result in an increase of IHCL.
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687

Effects of six months of moderate resistance-versus endurance-training on muscle ATP synthesis in first-degree relatives of patients with type 2 diabetes

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Insulin resistant first-degree relatives of type 2 diabetic patients (REL) feature reduced flux through myocellular ATP synthase (fATPase) and increased myocellular (IMCL) and hepatocellular (HCL) lipids. As exercise training can improve insulin sensitivity, we compared effects of moderate resistance (RE) vs. endurance (EN) training on fATPase. Glucose tolerant REL (RE: 3f/5m, age: 37±13a, BMI: 25±5kg/m2; EN 5f/3m, age: 43±11a, BMI: 26±2kg/m2) with comparable O2 uptake at the respiratory compensation point (VO2rcp) underwent OGTT to measure insulin sensitivity (OGIS) and 1H/31P magnetic resonance spectroscopy to assess fATPase, IMCL in soleus muscle and HCL before and after 26 weeks of exercise training (twice weekly at 90% of VO2rcp, duration was individually adjusted and increased). OGIS was similar at baseline (RE: 380±73 vs. EN: 381±70 ml.min-1.m-2) and after exercise training (389±40 vs.382±90 ml.min-1.m-2). fATPase was also comparable at baseline (12±5 vs.12± 3 µmol.ml-1.min-1) and after exercise training (13±3 vs. 11±2 µmol.ml-1.min-1). Likewise, IMCL were comparable at baseline and did not change by exercise training (basal: RE: 1.08±0.20 vs EN: 1.13± 0.46, after training RE: 1.06± 0.53, EN: 1.11±0.08) and HCL were similar at baseline (5.0±6.1 vs. 6.7±9.8% water) and tended to decrease in both groups.
VO2rcp remained unchanged in RE, but rose by 30% in EN (RE: 24.3±6.4 vs. 24.8±6.9 ml.kg-1.min-1, ns; EN: 20.3±4.7 vs. 26.4±6.4 ml.kg-1.min-1, p=0.001). Evaluating maximal strength of knee extensors and flexors using isokinetic dynamometer (30°.s-1 and 60°.s-1) torque (Nm) (as sum of both knees and both measures) increased in RE (flexors: 294±88 vs. 412±132; extensors: 586±171 vs. 720±203) and did not significantly change in EN (flexors: 319±135 vs. 337±12,1, ns; extensors: 606±250 vs. 637±231, ns). Moderate exercise training does not affect insulin sensitivity and mitochondrial ATP turnover in nonobese, glucose tolerant and insulin sensitive relatives of type-2 diabetic patients.

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688

Change in expression levels of lipid droplet coat protein in human skeletal muscle during strength training

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Background and aims: Depositions of fat in non-adipose tissues like skeletal muscle and liver are associated with insulin resistance and type 2 diabetes. Excess fat is stored as triacylglycerol in intracellular lipid droplets. The perilipin family of lipid droplet coat proteins (perilipin, adipophilin, S3-12, TIP47 and LSDP5/OxPAT/MLDP) are essential for this cellular lipid storage. The aim of the present study was to study the effect of physical exercise on gene expression of these lipid droplet coat proteins.
Materials and methods: Ten healthy men (26.7 ± 4.6 years of age) with no regular strength training during the last year participated in an eleven weeks strength training study with three workouts per week. Each workout included leg and upper-body exercises. Skeletal muscle biopsies from a thigh muscle (*m. vasus lateralis*) were obtained at baseline and after two and eleven weeks of training. mRNA expression was analyzed with TaqMan® Gene Expression Assays.
Results: In eight of ten subjects, the expression of markers of muscle fiber type I (MYH7) and IIx (MYH1) decreased from baseline to two weeks and was increased again after further nine weeks of exercise training. The expression of LSDP5, S3-12 and adipophilin followed a similar pattern, and the expression at week two was reduced as compared to baseline for LSDP5 (-0.011, 95%CI: -0.019, -0.0036), S3-12 (-0.030, 95%CI: -0.044, -0.016) and adipophilin (-0.016, 95%CI: -0.032, 0.00). At week eleven the expression of the same genes were increased again (LSDP5: 0.011, 95%CI: 0.00, 0.021; S3-12: 0.021, 95%CI: 0.00, 0.040; adipophilin: 0.021, 95%CI: 0.010, 0.036), and did not differ from baseline. The expression of TIP47 increased marginally during the intervention whereas perilipin expression did not change.
Conclusion: These are the first results indicating a possible important role of exercise in the regulation of the perilipin family of lipid droplet coat proteins in skeletal muscle. Two weeks of strength training changed gene expression of several genes, including the lipid droplet coat proteins LSDP5, S3-12 and adipophilin. After further nine weeks of exercise the muscles seem to be adapted to the intervention and the expression levels are comparable to baseline levels.

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689

Effect of resistance exercise on muscular subfascial adipose tissue and retinol-binding protein-4 concentration in individuals with diabetes

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Introduction: Mounting evidence indicates that elevated muscular lipid accumulation is associated with diminished insulin sensitivity in skeletal muscle. It is reasonable to hypothesize that resistance exercise decreases muscular adipose tissue and have favorable effects on retinol-binding protein-4 (RBP4), which is linked to adipose tissue, and insulin sensitivity in women with diabetes.
Methods: Forty four overweight women with type 2 diabetes (55.7±6.5 yr) were randomly assigned to control (C, N=16), resistance exercise (RG, N=13), using

Table 1

		RG			AG			C	
	baseline	12w	p	baseline	12w	p	baseline	12w	p
BMI (kg/m2)	27.1±2.2	26.7±2.2	.008	27.1±2.4	26.3±2.1	.000	27.4±2.8	27.1±2.8	NS
ASAT (g)	24402.4±7903.2	22731.8±7264.2	.005	23896.5±6035.3	21851.3±5766.6	.000	24685.8±5617.4	24057.4±5541.3	.027
AVAT (g)	15657.8±4753.6	14677.8±3455.9	NS	15889.5±4592.9	15037.5±3369.1	NS	17529.8±4746.6	17362.4±4727.7	NS
SCAT (g)	6697.0±2674.2	7660.0±2759.5	.011	7186.9±2960.3	7848.7±2510.4	.019	7370.5±2620.3	7312.6±2479.0	NS
SFAT (g)	2205.0±848.6	1884.8±616.6	NS	2013.9±487.5	2016.2±638.8	NS	2248.4±760.4	2562.9±760.1	NS
IMAT (g)	411.6±160.4	416.2±158.5	NS	509.4±178.1	477.5±183.9	NS	564.1±221.6	532.2±215.0	NS
RBP4 (ul/mL)	98.5±28.8	82.1±27.1	.007	87.0±25.4	84.7±15.3	NS	95.0±20.5	96.2±28.7	NS
Kitt	1.9±1.0	2.1±.8	NS	2.8±1.0	2.5±.6	NS	2.0±.8	2.1±.6	NS

elastic band three times per week at about 40-50% of maximal exercise capacity under supervision and aerobic exercise group by walking (AG, N=15), which was monitored with accelerometer. We assessed anthropometric parameters, subcutaneous (ASAT) and visceral adipose tissue (AVAT) at abdomen, subcutaneous (SCAT), subfascial (SFAT), and intramuscular adipose tissue (IMAT) at mid-thigh level using computed tomography, plasma level of RBP4 and insulin sensitivity by insulin tolerance test (KITT), before and after 12-week exercise program.
Results: After 12-wk training program, changes of SCAT, SFAT, IMAT, RBP4, and KITT were not different across three groups, although change of BMI and ASAT in AG was greater than that in C. Within group analysis using paired T test, BMI, ASAT, and SCAT was decreased from baseline in both exercise groups, and plasma RBP4 concentration was significantly decreased only in RG. The change of RBP4 concentration had positive correlation with that of SFAT (r=0.664, p=0.013) in RG, but not in AG (r=-0.405, p=0.151).
Conclusion: Resistance exercise didn't alter intramuscular adipose tissue but reduced plasma RBP4 level without improvement of insulin sensitivity.

690

Severity of obstructive sleep apnea syndrome (OSAS) is highly correlated with a decreased ability to oxidize lipids during exercise
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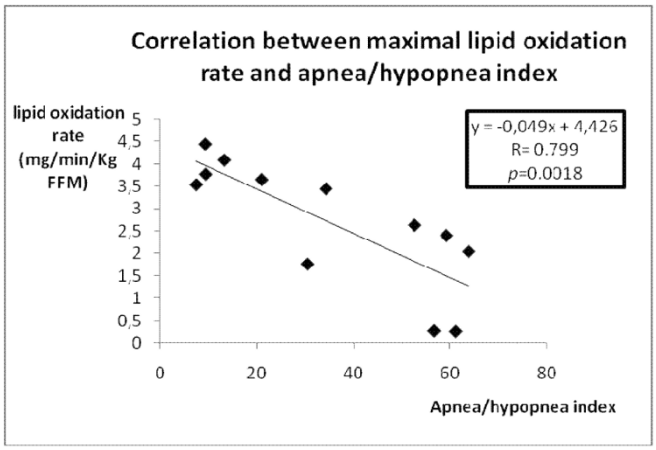
Background and aims: To determine if OSAS in the metabolic syndrome is associated with an impaired skeletal muscle ability to oxidize lipids during exercise.
Materials and methods: Thirteen patients with metabolic syndrome (3 women/10men; age: 55.5 ± 9.6 years; BMI: 37.5 ± 6.22 $kg.m^{-2}$; Waist Circumference: 125.4 ± 21.8 cm; body fat %: 40.8 ± 11.5 %, free fat mass (FFM): 65.3 ±15.8 kg; mean ± SD) were evaluated by overnight polygraphy to assess OSAS status (airflow, thoracoabdominal effort and oxymetry), which was quantified with the apnea/hypoapnea index (AHI). The balance of substrates oxidized during exercise was studied with exercise calorimetry, with determination of the maximal lipid oxidation rate (lipoxmax).
Results: During polygraphy, the AHI was 35.31 ± 21.7 with mild (AHI 5-15), moderate (AHI 15-30), and severe (AHI >30) OSAS in respectively 4, 1, and 8 patients. The lipoxmax occurred at 23.1 ± 13.7 % of the theoretical maximal power. At this level 2.7 ± 1.39 $mg.kg^{-1}.min^{-1}$ of lipids were oxidized. The lipoxmax was highly correlated with AHI (R=0.799; p=0.0018) and body fat% (R=0.794; p= 0.0021), while no correlation was found between AHI and Body fat% (R=0.48, p=0.35). A partial regression analysis showed that if the variable Body fat% is kept constant, the correlation between AHI and lipoxmax remains highly significant (R=0.778; p=0.002). Maximal lipid oxidation at exercise was significantly decreased in severe compared to mild and/or moderate OSAS (1.85 ±0.533 vs 3.91 ± 0.164; p= 0.0081).
Conclusion: The severity of OSAS is an independant determinant of the ability to oxidize lipids during exercise, regardless % body fat. Although the mechanism of this relationship remains to be determined, it could be a mechanism of resistance to weight reduction. This suggests to investigate the efficiency of exercise training targeted at the LIPOXmax in OSAS patients.



Supported by: LVL Medical group

691

Exercise during pregnancy reduces high fat induced macrosomia
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Background and aims: Dietary insults on the intrauterine environment are associated with altered fetal development and subsequent progressive metabolic disorders in adulthood. Evidence of the fetal origins of adult diseases in humans has been identified in epidemiological studies linking birth weight to subsequent risk of type 2 diabetes, obesity and cardiovascular disease. We have previously reported elevated abdominal adiposity together with reduced hepatic insulin signalling proteins in 15 week old offspring of dams fed a high fat diet during gestation. Physical activity is recognised as a positive intervention for the metabolic related disorders of high fat feeding. Potentially, specific exercise training during pregnancy could present as a method for obesity and diabetes prevention in offspring of high fat fed mothers. We aim to investigate the effect of exercise training during pregnancy on offspring development of high fat fed pregnant dams.
Materials and methods: 8 female (160g) and 8 male Wistar rats (215g) were fed a high fat diet (34% omega-6 PUFA, 26% Protein, 22.3 kJ/g). An oral glucose tolerance test (OGTT) (3g glucose / kg) was performed on day 24 with blood collected at fasting as well as 15, 30, 60 and 120 minutes post gavage. Glucose and insulin response was assessed as the total area under the curve (TAUC) for the 2 hour period. On day 28, mating pairs were established. Dietary intervention proceeded for a further 3 weeks. After 7 days of co-habitation, mating pairs were separated. Four females were randomly allocated to a training intervention. Training consisted of 30min of treadmill running at a moderate intensity for 10 days of the 16 between separation from males and spontaneous delivery. An OGTT was performed on all females on the 12th day following separation. Following delivery, all pups were weighed and litter sizes standardised to 8 (4 male, 4 female) per dam. During lactation all mothers were fed standard laboratory chow (11% Fat, 23% Protein, 13.9kJ/g).
Results: Exercise training did not significantly affect total body weight of mothers prior to delivery (Sedentary 312.2 ± 28.5g versus Trained 326.6 ± 23.8g). While insulin and glucose TAUC were similar between groups, plasma glucose 15 minutes following gavage was significantly lower in trained mothers (Trained 4.4 ± 0.7 versus Sedentary 5.8 ± 0.7 mM p = 0.05). Fasting plasma Leptin was lower following training but this was not significant. Six of eight mothers successfully delivered, 2 sedentary litters (31 pups, 6.4 ± 0.6g) and 4 trained litters (56 pups, 6.1 ± 0.9g). Pup weights showed significantly reduced birth weight in litters from trained mothers (p = 0.03). Pup blood glucose at day 1, was similar between groups (Trained 5.2 ± 0.9 versus Sedentary 5.0 ± 0.9 mM). Pups are currently being weaned for tissue collection at 15 weeks of age.
Conclusion: Exercise training during pregnancy reduced pup birth weight compared to pups from sedentary mothers. This was independent of changes in glucose TAUC, however improved insulin sensitivity may be inferred from the reduced glucose levels observed acutely following oral gavage for comparative insulin levels. This data suggests that exercise during pregnancy reduces if not prevents macrosomia observed in offspring of high fat fed mothers. The overarching effect this reduced birth weight may have on offspring metabolic development is yet to be determined.
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692

A single bout of low-intensity exercise strongly reduces the prevalence of hyperglycaemia throughout the day in type 2 diabetes patients
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Introduction: Glycemic instability is a severely underestimated problem in type 2 diabetes treatment. Therapeutic targets should aim to reduce postprandial blood glucose excursions. Exercise prescription can effectively improve blood glucose homeostasis and reduce the risk of cardiovascular complications.
Aim: To asses the impact of a single bout of either low- vs high-intensity exercise on the prevalence of hyperglycemia throughout the subsequent 24 h period in longstanding type 2 diabetes patients.
Methods: Nine sedentary, male type 2 diabetes patients (age: 57±2 y, BMI: 29.0±1.0 kg/m^2, Wmax: 2.2±0.2 W/kg body weight) were selected to partici-

pate in a randomized cross-over study. Subjects performed either an equicaloric bout of exercise for 60 min at 35%Wmax (LI) or 30 min at 70%Wmax (HI) or no exercise at all (NE). Thereafter, blood glucose homeostasis was assessed over the subsequent 24 h post-exercise period by continuous glucose monitoring under strict dietary standardization but otherwise free-living conditions.
Results: Average 24 h glucose concentrations were reduced following the low-intensity exercise bout (7.8±0.9 mmol/L) when compared with the control experiment (9.4±0.8 mmol/L; P<0.05). The high-intensity exercise bout did not significantly lower mean glucose concentrations (8.7±0.7 mmol/L; P=0.14). Hyperglycemia was prevalent for as much as 35±9% (8:17±2:04 h) throughout the day (NE). A single bout of exercise reduced the prevalence of hyperglycemia with 49.7±4.4% (P<0.05) and 18.6±8.8% (P=0.13) in the LI and HI exercise experiment, respectively.
Conclusion: A single bout of low-intensity exercise substantially reduces the prevalence of hyperglycemia throughout the subsequent 24 h period in long-standing, type 2 diabetes patients.
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693

Dynamic response of growth hormone secretion to aerobic exercise in subjects with type 1 diabetes mellitus under eu- and hyperglycaemic clamp conditions
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Background and aims: Individuals with type 1 diabetes mellitus (T1DM) exhibit an exaggerated growth hormone (GH) response to a variety of stimuli including exercise. While physical exercise is known to stimulate GH secretion, GH is alleviated by hyperglycaemia both at rest and during exercise in healthy individuals. Data on the impact of exercise under differing glycaemic levels in T1DM is scarce and controversial. We aimed, therefore, at comparing exercise stimulated GH secretion under eu- and hyperglycaemia in individuals with T1DM.
Materials and methods: In this randomized single-blinded cross-over trial 7 men with T1DM were examined twice in the fasting state after overnight euglycaemia. Blood glucose clamp was started at 08:15 a.m. to a target of either 5 mmol/l or 11 mmol/l for eu- and hyperglycaemia, respectively. Cycling over 120 min at 55-60% VO_{2peak} was initiated at 10:00 a.m. Serum GH was measured before clamp and before exercise (in duplicate), then every 15 min throughout exercise and 120 min thereafter. Areas under the curve (AUC) over 120 min of exercise were calculated. Log-transformed GH data were analysed data using paired Student's t-test and geometrical means with 95% confidence intervals (CI) are presented.
Results: The subjects' (mean±standard error) age was 33.5±2.4 years, diabetes duration 20.1±3.6 years, HbA_{1c} 6.7±0.2% and IGF-1 103.4±7.8 ng/ml. Until the start of exercise GH had modestly increased from baseline under both euglycaemia (from 0.60 ng/ml, CI 0.46-0.76, to 1.06 ng/ml, CI 0.58-1.92) and hyperglycaemia (from 0.70 ng/ml, CI 0.53-0.92, to 1.14 ng/ml, CI 0.68-1.91). Exercise led to a more than 10 fold increase in both groups. But 41% higher GH peaks were reached in euglycaemia with 18.9 ng/ml, CI 9.6-37.2, compared to 13.2 ng/ml, CI 7.2-24.5, in hyperglycaemia (p<0.01). AUC_{GH} during exercise were 1430 ng/ml*min, CI 703-2910, and 1061 ng/ml*min, CI 538-2091, in eu- and hyperglycaemia, respectively (p=0.02). In both groups GH returned to baseline two hours after exercise.
Conclusion: Departing from similar basal levels before exercise, GH response to prolonged exercise diverged according to glycaemic levels with lower values observed in hyperglycaemia. These data are consistent with a glycaemic regulation of GH secretion under exercise conditions in T1DM which is comparable to healthy individuals.
Supported by: Swiss Diabetes Foundation, Oetliker-Stiftung für Physiologie, NovoNordisk and Swiss National Science Foundation

PS 52 Clinical and mechanistic aspects of therapeutics

694

Metabolic and adipogenic properties of insulin detemir in human subcutaneous and visceral adipocytes differentiated *in vitro* from precursor stromal cells
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Background and aims: In clinical trials, insulin Detemir (D) is associated with no or lesser body weight gain as compared to human insulin (HI), particularly in individuals with high BMI. The objective of this study was to compare the metabolic and adipogenic properties of D and HI in human subcutaneous (SC) and visceral (V) adipocytes differentiated in vitro from precursor stromal cells.
Materials and methods: Human fat precursors were isolated from paired SC and V fat biopsies of 7 subjects (3F/4M, age 63±1 yrs, BMI 25.9±1.4) with normal glucose tolerance. Preadipocytes were isolated from the stromal fraction of the SC and V biopsies, respectively, and differentiated in vitro with dexamethasone, IBMX, insulin and rosiglitazone. Terminal differentiation of the adipocyte cultures was verified through morphological evaluation, Oil-O-Red staining, and assessment of gene expression of fat-specific molecules (PPARgamma2, adiponectin, GLUT4, and FAS) by real-time RT-PCR. Glucose uptake was assessed through evaluation of [^{3}H]1,2-deoxy-D-glucose uptake rates. Erk-1/2 expression and phosphorylation were evaluated by immunoblotting of total cell lysates with specific antibodies.
Results: In SC adipocytes, 100 nM D and 100 nM HI stimulated [^{3}H]2-deoxy-D-glucose uptake by 1.8- and 1.5-fold, respectively (p<0.05 vs. basal). In contrast, in V adipocytes, 100 nM HI induced a 3-fold increase in glucose transport (p<0.05), whereas 100 nM D appeared to be much more active, leading to 5- and 8-fold increases in glucose uptake at 10 and 100 nM, respectively (p<0.05 vs basal and HI). However, D and HI induced activation of Erk-1/2 proteins to a similar extent. Regression analysis showed an inverse correlation between D-stimulated glucose uptake in V adipocytes and BMI (β =-20.7, R^2 =0.87, p<0.05) and HOMA-IR (β =-70.7, R^2 0.92, p<0.05) of the donor subjects, whereas no correlation was found for HI. Then, precursors were differentiated in the presence of 500 nM D or HI. mRNA levels of PPARgamma2, GLUT4, adiponectin and FAS, which were undetectable in SC and V precursors, were increased by 10- to 50-fold in adipocytes differentated with HI (p<0.005). By contrast, they were lower by 10-fold in SC and V adipocytes differentiated with D (p<0.005 vs HI). In addition, triglyceride accumulated as large lipid droplets in about 90% of cells differentiated with HI and as minute lipid droplets in only 40% of adipocytes exposed to D.
Conclusion: In comparison with HI, D stimulates glucose uptake more efficiently in V adipocytes, and induces adipogenesis to a lesser extent in both SC and V precursors. Moreover, the metabolic effect of D is profoundly influenced by the degrees of adiposity and insulin resistance of the donor subject. The differential properties of D compared to HI may contribute to the weight gain-sparing effect of this insulin analog.
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695

6-year baros of 1890 primary bariatric operations. Decrease in mortality and similar resolution of comorbidities, especially type 2 diabetes independent of primary bariatric operation used
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Background: Comparative long-term results with high follow-up rates after primary bariatric operations are scarce.
Methods: We analyzed and compared our 6 years BAROS after 57 vertical gastroplasties (VBG), 152 biliopancreatic diversions (BPD), 1245 adjustable gastric bandings (AGB) and 441 standard RY gastric bypasses (RY). Standard techniques were used for VBG, AGB and RY. BPDs are heterogeneous, but have a common channel of 100cm or less. VBGs and BPDs were operated mainly through laparotomy, RYs and AGBs were mostly laparoscopic operations.
Eye-to-eye follow up was more than 91 %. All data were recorded prospectively. Reoperations were done for technical problems, weight regain, late

dumping as clinically necessary and defined for gastric banding by an algorhytm (Surgery 2005). An intention to treat analyses was performed.

Results:

Group	VBG	BPD	AGB	RY
N	57	152	1245	441
BMI 0 Years	48 +/-7	53 +/- 8 *	43 +/- 6	46 +/- 6
BMI 6 Years	33 +/- 7	34 +/-6	32 +/- 6	31 +/- 6
%EWLoss at 6 years	61 +/- 23	64 +/- 17	55 +/- 23*	63 +/- 19
BAROS points EWL	1.90 +/- 0.74	2.08 +/- 0.71	1.74 +/- 0.91	2.03 +/- 0.77
BAROS points QoL total	1.21 +/- 0.82	1.13 +/- 0.88	1.15 +/- 0.84	1.21 +/- 0.88
-physical	0.29 +/- 0.19	0.29 +/- 0.20	0.24 +/- 0.20	0.23 +/- 0.20
-social	0.24 +/- 0.23	0.25 +/- 0.22	0.17 +/- 0.19	0.18 +/- 0.19
-labor	0.25 +/- 0.21	0.21 +/- 0.26	0.19 +/- 0.21	0.20 +/- 0.23
-sexual	0.03 +/- 0.18	0.03 +/- 0.24	0.06 +/- 0.20	0.05 +/- 0.21
-self esteam	0.20 +/- 0.19	0.26 +/- 0.20	0.24 +/- 0.19	0.27 +/- 0.20
-eating behaviour	0.11 +/- 0.15**	0.25 +/- 0.20	0.12 +/- 0.12**	0.27 +/- 0.20
BAROS, Complications major	0.31 +/- 0.64	0.18 +/- 0.41	0.09 +/- 0.32	0.11 +/- 0.43
BAROS, Reoperations major	1.11 +/- 1.16	1.14 +/- 0.94	0.38 +/- 0.71***	0.32 +/- 0.67***
BAROS, Comorbidities	1.50 +/- 0.97	1.50 +/- 0.97	1.59 +/- 0.90	1.44 +/- 0.73
TOTAL BAROS	2.85 +/- 2.48*	3.69 +/- 2.03	3.60 +/- 2.14	3.75 +/- 1.81
%Failures	21****	10	10.5	7
%Good or better	42****	71	68	63

* p<0.05 using ANOVA vs all other groups; **p<0.05: ANOVA vs BPD and RY. *** p <0.05: ANOVA GB or RY vs VBG or BDP. ****p<0.05 using non-parametric comparisons by Kruskall-Wallis.

EWL was lowest in GB patients. Total QoL was similar in all groups, but eating behaviour was significantly better in BPD and RY compared to VBG and GB- patients. Moreover rate of reoperation during the 6 years of follow up was similar in GB and RY patients, but more than twice as high in VBG and BPD subjects. Interestingly improvement in comorbidities was similar in all groups. According to total BAROS, overall 10.1% were considered as failures. Failure rate was highest in VBG and lowest in RY. In contrast rating of good or better by BAROS was highest in BPD patients. Overall procedural and nonprocedural mortality was 19 per 10000 personyears as was resolution of Type 2 Diabetes. In conclusion 6 years after bariatric surgery only 10% of subjects were considered as failures, whereas about two third of patients were considered as good or better with the exception of VBG. Overall mortality was 3 times lower as that reported for non operated morbid obese subjects (>50 per 10'000 person years)

696

Weight loss induced by bariatric surgery improves the ability to oxidize lipids at exercise but shifts the power of maximal lipid oxidation (LIPOXmax) to the left

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Background and aims: It is attractive to develop combined strategies in which exercise will be employed to improve the effects of bariatric surgery and provide to patient the possibility to become less sedentary on the long term, as has been successfully done in other chronic diseases. In the case of obesity after bariatric surgery, the question arises of which kind of exercise should be proposed. Recent studies propose in metabolic diseases (obesity, diabetes mellitus) to target exercise training at the intensity level where lipid oxidation is maximal (LIPOXmax). Thus, the purpose of this study was to determine LIPOXmax in obese patients that had lost weight after bariatric surgery, compared to obese subjects matched for body mass index (BMI) before and after surgery, and nonobese subjects.

Materials and methods: Evaluation by indirect exercise calorimetry of the LIPOXmax, as well as the Crossover point (exercise level at which carbohydrates become the predominant fuel) in 28 obese patients after bariatric surgery (17 by sleeve gastrectomy, 7 by Roux-en-Y gastric by-pass and 4 by gastric ring; BMI before surgery: 45,5 ± 1,3 Kg/m²; BMI after surgery: 32,7 ± 1,2 Kg/m²; average weight loss 41.7 ± 4.3 kg; age: 39 ± 2 years; average ± SEM), compared to two groups of obese, 33 matched with the preoperative BMI (45,2 ± 0,8 Kg/m²) and 87 matched for the actual BMI (32.4 ± 0.14 Kg/m²), and one group of 322 nonobese subjects (BMI: 24,8 ± 0,2 Kg/m²), all being matched for age and gender.

Results: The LIPOXmax occurs at a lower exercise intensity in obese patients treated by bariatric surgery compared to all the other groups, although the maximal lipid oxidation rate is quite the same in all groups.

Table 1: * p<0.05 Vs A; ** p<0.01 Vs A; *** p<0.001 Vs A;

		A Post surgery obese subjects	B Matched on post slimming BMI	C Matched on initial BMI	D Non obese controls
Crossover point	Watts	51 ± 4	62 ± 4	51,2 ± 7	60 ± 2,4
	%Pmax	27 ± 2	34.6 ± 0.02	36 ± 6	37,7 ± 1,2
LIPOXmax	Watts	43 ± 3	56 ± 3 *	52 ± 3 *	55 ± 2 *
	%Pmax	23 ± 1,7	30 ± 0.01 **	34 ± 2***	33 ± 0.01***
maximal lipid oxidation point (Mg/min)		216 ± 13	213 ± 12	186 ± 15	207,5 ± 7

Conclusion: Compared to matched obese and nonobese subjects, the patients of group A, who have lost an average of 42 kg after bariatric surgery, exhibit a peak oxidation of lipids shifted to lower exercise intensities. This mechanism may contribute to resistance to slimming, suggesting that targeted training with the aim of increasing lipid oxidation at exercise may be interesting to evaluate in this context.

697

Electronic health technology for assessment of physical activity and eating habits in children and adolescents with overweight and obesity

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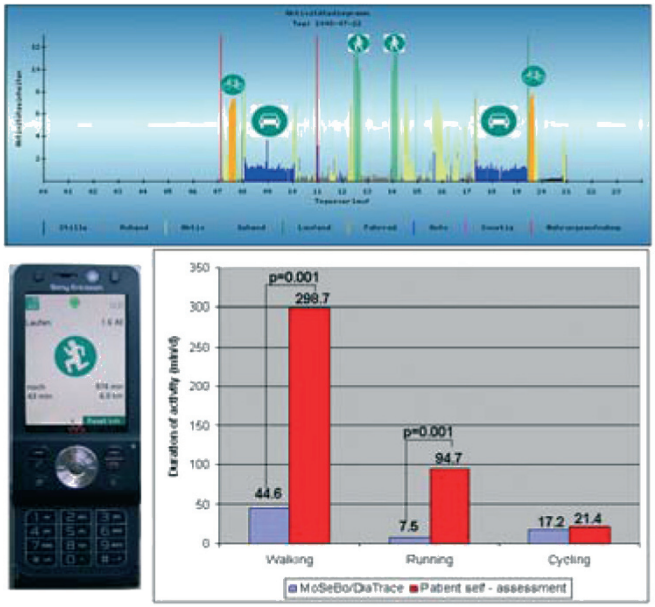
Background and aims: During the last decades overweight/obesity has increased markedly. They are associated with a high risk for diabetes and death. Effective intervention is mandatory. Following participation in treatment programmes children/adolescents often fail to reach sufficient long-term weight reduction. The present trial aims to integrate telemedical support in therapy to improve long term outcome.

Materials and methods: All children/adolescents with overweight/obesity were included (n=66, age 13.9±2.6 years, BMI 31.2±5.4 kg/m2, BMI-SDS 2.41±0.6) admitted to our hospital 04-03/2009. To asses physical activity and eating habits electronic health technology was used (Fraunhofer-Institute, IGD, Rostock, Germany). The system consists in a mobile motion sensor board (MoSeBo) or a motion sensor integrated in a mobile phone (DiaTrace). The system analyses kind, intensity and duration of physical activity and eating habits.

Results: Follow-up period was 182 days. Total physical activity measured with MoSeBo/DiaTrace was 310.4±;122.3 min/d (15.6±;12.3 units/d). Patients spent 44.6 (3-173.3) min/d with walking, 7.5 (0-42.3) min/d with running, 17.2 (0-52.0) min/d with cycling and 24.7 (0-88.7) min/d with car driving. In contrast, in most domains activity documented by patients was significantly higher: Walking 298.7 min/d (p=0.001 vs MoSeBo/DiaTrace)and running 94.7 min/d (p=0.001). In respect of cycling patients' estimation (21.4 min/d) and objective documentation were comparable.Similar differences were seen concerning eating habits. Multivariate analysis: There was an association between weight reduction (R-square=0.590) and intrinsic motivation (ß=0.732, p< 0.001), intrafamilial conflicts (ß=0.461, p=0.002), duration of physical activity measured with MoSeBo/DiaTrace (ß=-0.438, p=0.002) and body fat mass (ß=-0.393, p=0.005).

Conclusion: 1. There are differences between patients´ self-assessment and objective perception of physical activity and eating habits. Discrepancies in perception are maybe important determinats of poor long-term outcome. **2.** Using modern electronic health technology allows the objective assessment of kind, intensity and quantity of physical activity and eating habits. This could be an important new advanced method to improve the therapy of obesity and diabetes.



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698

Diabetic patients' response to clopidogrel is independently related to fibrinogen plasma level but not to glycaemic control

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Background and aims: There is a higher prevalence of stent thrombosis and of inadequate platelet response to clopidogrel in diabetics. However, whether or not glycemic control is implicated in this suboptimal response remains unknown. The aim of this study was to determine the clinical and biological factors implicated in clopidogrel platelet response in diabetic patients.
Materials and methods: ADP-induced platelet aggregation (ADP-Ag) and Platelet Reactivity Index of Vasodilatator-Stimulated Phosphoprotein (PRI VASP) were analysed in 124 diabetic patients treated with clopidogrel. Platelet tests were repeated for 15 poorly controlled patients after glycemic optimization with the use of continuous subcutaneous insulin infusion.
Results: No correlation between platelet tests and glycemic control (fasting blood glucose, HbA1c) was observed. ADP-Ag and PRI VASP did not differ before and after glycemic optimization (53.5 ± 25 versus 52.8 ± 21 % and 64.5 ± 18 versus 66.9 ± 18 %). No relationship was found between ADP-Ag or PRI VASP and gender, duration of the disease, BMI, hypertension, lipid profile, creatinine, microalbuminuria, high sensitive CRP, or adiponectin. However, type 2 diabetic patients had higher ADP-Ag and PRI VASP than type 1 diabetic patients (49±19 vs 33±19 %, p= 0.008; 62±16 vs 48±27 %, p= 0.02, respectively). After adjustment for potential confounders (age, time since diagnosis, BMI, triglycerides, fibrinogen), only ADP-Ag remained statistically different between the two types of diabetes (p= 0.007; p=0.11 respectively). Remarkably, a positive independent correlation was found between ADP-Ag and fibrinogen (r=0.38, p= 0.0005). Fibrinogen was an independent predictor of ADP-Ag, and clopidogrel non responders had elevated plasma fibrinogen, compared with clopidogrel responders: 8.2 ± 0.5 versus 3.8 ± 0.1 g/L ($p<10^{-4}$). ROC curve analysis showed that a threshold value of 3.57 g/L gave a negative predictive value of 94.7%. The addition of purified fibrinogen to platelet rich plasma obtained from diabetic patients with an adequate response to clopidogrel (final concentration 11g/L) did not lead to a significant increase in ADP-Ag (41 ± 19 vs 40 ± 18 %, p = 0.69), eliminating a direct effect of fibrinogen on ADP-Ag.
Conclusion: High post treatment platelet reactivity assessed by ADP-Ag is not related to glycemic control but to the type of diabetes. Fibrinogen is a strong predicting factor of ADP-Ag in diabetics treated with clopidogrel. Evaluation of inflammation and treatment of infection in diabetics could improve clopidogrel platelet response and reduce the risk of stent thrombosis.

699

The inhibitory effect of metformin on nitric oxide production via MyD88-independent pathway in macrophages

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Background and aims: The United Kingdom Prospective Diabetes Study 34 (UKPDS 34) demonstrated that administration of metformin reduced all-cause mortality, myocardial infarction, and stroke more than insulin or sulfonylureas, although its glucose-lowering effect was similar to them. UKPDS 34 suggested that metformin confers vascular benefits beyond its hypoglycemic action. Recent clinical studies further suggested that metformin may alter inflammation as determined by decreased inflammatory markers in plasma. Nitric oxide (NO) is a free radical molecule known to play an important role in the pathogenesis of inflammation. Recent studies suggested that plasma levels of NO metabolites correlate with body mass index and body fat mass, that plasma nitrite levels are elevated modestly in diabetic patients, and that inducible nitric oxide synthase may be involved in the pathogenesis of metabolic disorders associated with a low-grade chronic inflammatory state, such as atherosclerosis and obesity-linked Type 2 diabetes. However, the effects of metformin on nitric oxide production are not known yet. In the present study we investigated the effects of metformin on nitric oxide production and the molecular mechanism of its effect in macrophages to evaluate the anti-inflammatory effects of metformin.
Materials and methods: The murine macrophage cell line, RAW264.7 cells were maintained in RPMI 1640 medium containing 5% heat-inactivated fetal calf serum. RAW264.7 cells were stimulated by lipopolysaccharide (LPS) from Escherichia coli O55:B5 or poly(I:C). Nitric oxide was measured as its end product, nitrite, using Griess reagent. TNF-α or interferon-β (IFN-β) was measured by ELISA.
Results: We examined the effects of metformin on LPS or poly(I:C)-induced nitric oxide production, LPS-induced IFN-β production, and LPS-induced TNF-α production. Previous studies indicate that NO production by LPS is induced via Toll-like receptor 4 (TLR4) and NO production by poly(I:C) is via Toll-like receptor 3 (TLR3). LPS/TLR4 signaling is divided into MyD88-dependent and MyD88-independent pathways, but poly(I:C)/TLR3 signaling has only MyD88-independent pathway. NO production by LPS is induced via MyD88-independent pathway which mediates IFN-β production. TNF-α production by LPS is induced via MyD88-dependent pathway. Metformin inhibited LPS-induced nitric oxide production in RAW264.7 cells (6.6±0.2 μM vs. 3.4±0.3 μM, p<0.005). Metformin inhibited poly(I:C)-induced nitric oxide production (8.8±1.2 μM vs. 2.9±0.4 μM, p<0.005). Metformin inhibited LPS-induced IFN-β production (349.5±7.7 pg/mL vs. 178.9±1.8 pg/mL, p<0.05). Metformin did not inhibit LPS-induced TNF-α production.
Conclusion: Metformin inhibited nitric oxide production via MyD88-independent pathway in macrophages. Our data indicates that metformin has beneficial effects on diabetic complications through its anti-inflammatory effects via MyD88-independent pathway.

700

Miglitol decreases gene expressions of inflammatory cytokines/cytokine-like factors in peripheral leukocytes in parallel with the reduction in blood glucose fluctuations in type 2 diabetes

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Background and aims: Many patients with type 2 diabetes are treated with α-glucosidase inhibitors, which have beneficial effects in improving postprandial hyperglycemia. Miglitol, an α-glucosidase inhibitors, is more potent in reducing early postprandial blood glucose than other α-glucosidase inhibitor, such as acarbose and voglibose, and delays the peak of postprandial blood glucose. In this study, we focused the daily blood glucose fluctuations and gene expressions of inflammatory cytokines/cytokine-like factors in patients with type 2 diabetes and examined the effects of the switch from acarbose or voglibose to miglitol on them.

Materials and methods: The subjects comprised 43 patients with type 2 diabetes (22 men and 21 women) aged 26-81 years with HbA_{1c} levels from 6.5% to 7.9%. They had received combination therapy with acarbose (100 mg/meal) or voglibose (0.3 mg/meal) and insulin or sulfonylurea for more than six months. At the start of the study, all α-glucosidase inhibitors were switched to miglitol (50 mg/meal) and the new treatments were continued for three months. At each end of the study period, basic clinical parameters, such as HbA_{1c}, fasting glucose, triglyceride, total-cholesterol and C-reactive protein, were measured, and the severity of adverse events, such as hypoglycemia, fecal disturbance (diarrhea/constipation), flatulence and abdominal distention, were monitored. Blood glucose was determined just before and one hour after meals by self-monitoring. Gene expressions of inflammatory cytokines/cytokine-like factors in peripheral leukocytes were determined by real-time RT-PCR.
Results: The levels of HbA_{1c} and fasting blood glucose were maintained even after acarbose or voglibose was switched over to miglitol. Meanwhile, hypoglycemic events were significantly reduced by 58% ($p<0.001$) after the change of treatments. Blood glucose fluctuations were ameliorated and the M-value was decreased by 24% ($p<0.001$). In peripheral leukocytes, the gene expression levels of IL-1β, TNF-α, S100a4, S100a6, S100a9, S100a10, S100a11 and S100a12 were significantly decreased by 25% ($p<0.01$), 24% ($p<0.001$), 17% ($p<0.05$), 20% ($p<0.05$), 23% ($p<0.05$), 19% ($p<0.01$), 25% ($p<0.01$) and 24% ($p<0.05$), respectively.
Conclusion: After the switching from other α-glucosidase inhibitors, miglitol improved blood glucose fluctuations without changes in both HbA_{1c} and fasting blood glucose in patients with type 2 diabetes. On the other hand, miglitol also decreased the gene expressions of inflammatory cytokines/cytokine-like factors in peripheral leukocytes. These results suggest that the blood glucose fluctuation may affect the gene expressions of inflammatory cytokines/cytokine-like factors in peripheral leukocytes in type 2 diabetes.

701

The effects of decreased gastric secretion on treatment of obesity
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Background and aims: Over many years of our medical practice we noticed that patients with gastric troubles who were treated with ranitidine hydrochloride (selective H2 receptor antagonists) have been maintaining their restricted diets more easily and successfully. The aim of this study was to examine the effects of using ranitidine in the treatment of obesity.
Materials and methods: We examined 26 healthy obese persons (BMI >30 kg/m^2), mean age 42.1 ± 10.1 years. All patients came to endocrinologist only because of their problem with obesity. Three months before this study all patients have already been on therapy with the low calories diet and physical activity, but they didn't succeed to reduce their body weight. In this study the patients continued with the same diet and physical activity and in addition they were given to take daily two tablets of 150 mg ranitidine (morning and evening) for the next eight weeks. At baseline and after 8 weeks period we determined body weight, waist circumference (WC) and BMI (kg/m^2), lipid status (total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol). Also a 2 hours 75-g oral glucose tolerance test (oGTT) was done during which we followed plasma insulin concentration (by radioimmunoassay) at the beginning and after one hour of the test (60').
Results: At the end this study has shown that all patients have significantly reduced their body weight from 2 to 23 kg, mean 7.2±4.6 kg (103.1±14.1 vs 95.9±13.5 kg, $p<0.01$), BMI (36.8±4.9 vs 34.2±5.1 kg/ m^2, $p<0.01$) and WC (108.4±9.3 vs 99.6±10.2 cm, $p<0.01$). Also, they had better lipid status with significantly reduced triglycerides (2.2±0.9 vs 1.7±0.7mmol/l, $p<0.05$) and increased HDL cholesterol (1.1±0.3 vs 1.3±0.3 mmol/l, $p<0.01$). Fasting plasma insulin was improved (10.1±5.2 vs 10.3±4.9, NS) and insulin secretion after 60'of oGTT was significantly reduced (76.2±41.5 vs 58.1±37.6, $p<0.05$).
Conclusion: Potential explanation for our findings is that ranitidine has effects like gastric bypass surgery on gut hormones that control insulin secretion (GLP-1, YYP, Ghrelin). All patients also affirmed that they were feeling good during this therapy and they could maintain the advised dietary programme with pleasure and without being hungry, nervous or depressed.

702

Effects of immunosuppressive agents on insulin stimulated glucose transport in primary rat adipocytes
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Background and aims: Glucocorticoids (GC) and immunosuppressive agents (IA) used in transplant recipients and patients with autoimmune diseases can cause insulin resistance. However the mechanism behind the development of insulin resistance needs to be further investigated. One hypothesis is that GC and IA cause metabolic changes in adipocytes leading to impaired insulin sensitivity. If IA induce changes in whole body glucose and lipid metabolism leading to abnormal insulin signaling and the accumulation of lipid in skeletal muscle, this is likely to contribute to the development of whole-body insulin resistance, glucose intolerance and fasting hyperglycemia. The aim of this study was to elucidate the direct effects of IA and GC on insulin stimulated glucose uptake in rat adipocytes.
Materials and methods: Rat adipocytes isolated from epidydimal fat pads, where incubated with different IA and GC: Cyclosporine A (CsA: 0.5-30 μM), n=9; Tacrolimus (FK 1-50 μM), n=9; Sirolimus (Sir: 5-250 μM), n=9, Dexamethasone (D 0.01-10 μM), n=15; and Prednilosone (P: 0.01-10 μM), n=12, for an initial 5 min. Thereafter adipocytes were further incubated with or without insulin (10 nM) for 10 min before glucose uptake was measured by adding 14C-glucose (0.86 μM) for 30 min. After incubation the reaction was stopped by centrifugation in silicon oil. The cellular uptake of labeled glucose was analyzed by scintillation measurement.
Results: Insulin stimulated glucose uptake was reduced after incubation with the different IA; CsA (0.5-30 μM) by 27-39% ($p<0.05$), with FK (10-50 μM) by 30% ($p<0.05$) and Sir (5-250 μM) up to 67% ($p<0.05$). D (0.01-10 μM) caused a reduction in insulin stimulated glucose uptake by 26% ($p<0.05$), whereas P had no statistically significant effect on basal or insulin stimulated glucose uptake. Basal glucose uptake was left intact with all IAs and GCs.
Conclusion: These results demonstrate that the IAs, Cyclosporine A, Tacrolimus, and Sirolimus, as well as, the GC Dexamethasone inhibit insulin stimulated glucose uptake in rat adipocytes after short-time incubations. Further elucidations of the mechanisms underlying adipose tissue insulin resistance caused by IA and GC, will help to optimize treatments, in order to prevent post-transplant diabetes.
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703

Acute rhabdomyolysis after fucidic acid and atorvastatin therapy
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We report the case of a 58-year-old patient who was admitted with progressive and generalised muscle pains and weakness. The patient had a history of type 2 diabetes mellitus combined with hypertension, lower limb arteriopathy and recent myocardial infarction. He had been taking atorvastatin (80 mg daily) for 6 months. He was also treated for rheumatoid arthritis. In October 2008, he underwent surgery for osteoarthritis of the foot and oral antimicrobial therapy (fucidic acid 1500 mg per day, and cotrimoxazole 1600 mg per day) was prescribed. Three weeks later, he developed symptoms consistent with severe rhabdomyolysis, confirmed by high serum creatine kinase level (56000 UI/L) (normal limit < 350 UI/L). He could not move his legs and arms, and rapidly developed dysarthria, swallowing disorders and dyspnea. Extensive investigations ruled out other known causes of polymyositis : absence of muscle antibodies and histological analysis of muscle biopsy confirm toxicity mechanism. Atorvastatin and fucidic acid were discontinued. Eight days after discontinuation, his serum creatine kinase concentration fell to 320 UI/L and the patient was again able to walk.
Less than ten cases involving an interaction between an HMG CoA-reductase inhibitor and fusidic acid have been reported. We presume that the most likely cause was hepatic inhibition of the CYP3A4 isoenzyme by fucidic acid, resulting in a dramatic rise in plasma statin levels and then increase the risk of rhabdomyolysis.
Conclusion: Clinical should be aware of this potential adverse effect between acid fucid and statin therapy. The extensive use of statin therapy in patients with vascular disease and the frequency of MRSA infections may increase the incidence of this presumed drug interaction.

PS 53 Lipid effects and metabolism

704

Opposite changes in enzyme expression of "the sphingolipid rheostat" by palmitate and oleate

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Background and aims: Development of obesity-induced type 2 diabetes mellitus is characterized by rising blood glucose and lipid levels. Long-term exposure of β-cells to free fatty acids has negative effects on both function and mass especially when combined with elevated levels of glucose. In this context, saturated fatty acid palmitate (PA) causes more severe effects and rates of apoptosis compared to mono-unsaturated fatty acid oleate (OA). When OA is administered together with PA, attenuation or even reversal of the negative effects of PA on b-cell function has been observed. One of the proposed mechanisms for the negative effects of PA in b-cells exposed to elevated levels of glucose is inhibition of fatty acid oxidation causing elevated generation of ceramide. Ceramide is a sphingolipid formed either by de novo synthesis from palmitate and serine, cleavage of the sphingolipid metabolite sphingomyelin or conversion from sphingosine, which is another sphingolipid metabolite. The contribution of ceramide from conversion from sphingosine is the result of the dynamic balance between sphingosine-1-phosphate (S1P) and sphingosine. Indeed, the balance between anti-apoptotic S1P and pro-apoptotic ceramide/sphingosine ("the sphingolipid rheostat") is an important factor determining cell fate. To define how these three different ceramide generation pathways are affected in b-cells exposed to PA, OA or the combination of the two, the expression levels of key enzymes of sphingolipid pathways were measured.
Materials and methods: Insulin-secreting mouse insulinoma (MIN6) cells were cultured for 48 hours in the absence or presence of 0.25 mM palmitate (PA), oleate (OA) or PA plus OA (0.25mM each). After culture, expression levels of enzymes of sphingolipid metabolism were measured by RT-qPCR.
Results: Prolonged exposure of β-cells to PA resulted in limited rise in expression levels of some enzymes of ceramide de novo synthesis. With regard to expression levels of sphingomyelin cleavage enzymes they were not significantly affected in comparison with those measured in control cells. When PA was replaced by OA, expression levels of enzymes of ceramide de novo synthesis were slightly lower than in control cells. Such reversal of metabolic flux towards reduced ceramide production was also observed in the sphingomyelin cleavage pathway of cells exposed to OA. In cells treated with the combination of PA and OA, ceramide de novo synthesis was decreased as compared to cells exposed to PA alone. Synthesis was still slightly increased in comparison with control cells, however. Enzymes catalyzing sphingomyelin cleavage were not altered in cells exposed to PA and OA in comparison with cells exposed to PA alone. With regard to "the sphingolipid rheostat" PA altered the expression of enzymes consistent with shifting the equilibrium towards synthesis of pro-apoptotic sphingosine and ceramide. In contrast, treatment with OA enhanced expression of enzymes promoting formation of anti-apoptotic S1P. After treatment with PA and OA, the equilibrium was shifted towards sphingosine formation rather than ceramide as observed in the presence of PA alone.
Conclusion: Shifts induced by PA, OA or the combination of the two fatty acids primarily in "the sphingolipid rheostat" and to a lesser extent ceramide de novo synthesis and ceramide formation from sphingomyelin cleavage are proposed to contribute to explain the different effects of the fatty acids on the b-cell.
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705

TBC1D1-dependent regulation of glucose and lipid metabolism in cultured C2C12 myotubes

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Background: We have recently identified *Tbc1d1* as a novel susceptibility gene for high-fat diet-induced obesity and diabetes in mice. TBC1D1, a Rab-GTPase-activating protein (GAP) is homologous to the insulin signalling protein AS160 (TBC1D4) and has been linked to familial forms of human obesity. Recombinant congenic mice lacking *Tbc1d1* show reduced body weight, decreased respiratory quotient, increased fatty acid oxidation and reduced glucose uptake in isolated skeletal muscle. Our results indicate that TBC1D1 controls whole body energy homeostasis by reciprocally regulating fatty acid oxidation and glucose use in skeletal muscle. However, the molecular mechanism by which TBC1D1 controls fuel metabolism in muscle remains unknown. We have therefore investigated the role of TBC1D1 in glucose and lipid metabolism oxidation in cultured C2C12 muscle cells.
Methods: For expression experiments, C2C12 myotubes were either electroporated with siRNA or subjected to lentiviral gene transfer. Uptake and oxidation of glucose and palmitate was determined by tracer techniques. Gene expression was analyzed by Western Blot and TaqMan-Realtime-PCR.
Results: *Tbc1d1* is predominantly expressed in skeletal muscle of mice and humans. Consistent with the tissue expression profile, differentiation of C2C12 myoblasts into myotubes induces TBC1D1 protein expression ~3-fold. SiRNA-mediated knockdown of *Tbc1d1* significantly increased palmitate uptake and oxidation in C2C12 myotubes by ~25%. Conversely, lentiviral overexpression of wildtype *Tbc1d1* in C2C12 myotubes led to a concomitant reduction of palmitate uptake and oxidation. Interestingly, knockdown of *Tbc1d1* also significantly increased 2-deoxyglucose uptake by ~40% whereas overexpression of *Tbc1d1* had the opposite effect. However, overexpression of the GAP-inactive mutant, R941K, had no effect on glucose uptake or palmitate uptake and oxidation, respectively, indicating that an intact GAP domain is required for the metabolic actions of *Tbc1d1*. Expression of GLUT1, the main glucose transporter in C2C12 cells, was not changed on both mRNA and protein levels after overexpression or knockdown of *Tbc1d1*. In contrast, the increased fatty acid combustion in *Tbc1d1*-depleted myotubes was accompanied by substantially increased levels of mRNA for fatty acid translocase (*Cd36*) and long-chain acyl-CoA dehydrogenase (*Acadl*), and elevated expression of genes involved in lipid metabolism including *Fabp3, Pgc1a*, *Pdk4, Ucp2 and Ucp3*. Importantly, additional knockdown of *Cd36* in *Tbc1d1*-depleted C2C12 myotubes completely abrogated the increase in palmitate uptake.
Conclusion: Our data demonstrate that CD36 is responsible for TBC1D1-mediated fatty acid uptake in C2C12 cells. Moreover, TBC1D1 exerts control on fuel selection and energy metabolism in skeletal muscle at least in part through regulating gene expression of key enzymes for lipid uptake and oxidation, including targets for peroxisome proliferator-activated receptor delta (*Ppard*). In contrast to skeletal muscle, lipid and glucose use are not reciprocally regulated by TBC1D1 in C2C12 cells. This implicates that TBC1D1 controls the flux of glucose and lipid through two independent pathways.
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706

Beneficial effects of phytosterols isolated from Aloe vera on obesity-associated metabolic disorders in Zucker diabetic fatty (ZDF) rats

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Introduction: We have confirmed the hypoglycemic effects of Aloe vera gel in db/db mice, an animal model of type II diabetes. We also identified five phytosterols (lophenol, 24-methyl-lophenol, 24-ethyl-lophenol, cycloartanol, and 24-methylene-cycloartanol) as anti-diabetic compounds from Aloe vera gel. In this study, we administered two kinds of the aloe phytosterols, lophenol (Lo) and cycloartanol (Cy) to an obese animal model of type II diabetes, Zucker diabetic fatty (ZDF) rats, in order to assess the effects of Aloe phytosterols on hyperglycemia and hyperlipidemia.
Methods: Male ZDF rats (9 weeks old) were orally administered Lo and Cy (25 μg/kg/day) once a day everyday for 6 weeks. During treatment, body weights, random and fasting blood glucose levels were monitored. After 6 weeks of treatment, fasted rats were sacrificed, and blood was collected by cardiac puncture. Abdominal adipose tissues (epididymal fat, mesenteric fat and retroperitoneal fat) were excised and weighed. The biochemical parameters of serum were also investigated. Additionally, we analyzed the mRNA levels of genes involved in lipid and glucose metabolisms in liver by real-time RT-PCR.
Results: ZDF rats exhibit marked obesity, hyperglycemia, hyperlipidemia and hyperinsulinemia compared with their lean littermates. In the Lo and Cy treatment groups, random and fasting blood glucose levels were lower

than the ZDF-control. Consistent with the blood glucose level, hemoglobin A1c (HbA1c) percentages were also reduced in aloe phytosterol-treated rats. These findings indicated that administration of aloe phytosterols suppressed hyperglycemia in ZDF rats. Interestingly, body weights were not affected by aloe phytosterol treatments, however the weights of total abdominal fat tissues were significantly lower in ZDF rats with Cy (26.3%) and Lo (27.7%) administration compared to those in controls. Additionally, the serum triglyceride level was significantly reduced and adiponectin concentration was elevated in aloe phytosterol-treated ZDF rats compared with those in ZDF controls. Furthermore, the expression levels of genes involved in lipid and glucose metabolisms in liver were changed in the aloe phytosterol-treated group compared with those in ZDF controls. Especially, the expression levels of lipogenic enzymes (Fasn, SCD1) and gluconeogenic enzymes (PEPCK and G6Pase) were significantly decreased, and the expression of β-oxidation enzyme (ACOX) was increased in the aloe phytosterol-treated groups compared with those in ZDF control groups.
Conclusion: Our study showed that aloe phytosterols ameliorated hyperglycemia, hyperlipidemia and visceral fat obesity in ZDF rats. In addition, the quantitative gene expression analysis of ZDF rats indicated that aloe phytosterols might improve lipid and glucose metabolism in the liver. These findings suggested oral ingestion of aloe phytosterols could be beneficial in preventing and improving metabolic disorders associated with obesity.

707

Acyl CoA-profiling in biological tissues using online SPE-LC-FTMS (Orbitrap)

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Background and aims: Coenzyme A (CoA) activated compounds (e.g. malonyl CoA, medium- and long chain acyl CoAs) play a pivotal role in energy and lipid metabolism. The aim was to develop a robust online-SPE-LC/FTMS-based method for the quantification of acyl CoAs in a wide range of biological tissues. The method should require minimal sample preparation and should enable structural analysis to be performed by accurate mass determination in MS and MSn.
Materials and methods: All experiments were carried out on an Ultimate 3000 (Dionex) comprising an autosampler with cooled tray and a column oven with switching unit coupled to an Orbitrap (ThermoScientific). The sample extraction procedure comprises three steps: (1) addition of buffer and internal standards, (2) homogenization and (3) centrifugation. The supernatant is injected directly into the SPE-LC-FTMS system (Online-SPE-cartridge: Oasis HLB, analytical column: Zorbax C18 Extend). Samples were loaded onto the SPE-column using 0.1% aqueous formic acid. For washing, the acetonitrile content of the SPE-eluent was increased. After online-SPE, separation was performed on the analytical column using acetonitrile and water containing 100 mM ammonium hydroxide as solvents. Data acquisition was performed in full scan mode using a resolution of 60.000 FWHM.
Results: A combination of online SPE/LC and high resolution mass spectrometry (LTQ Orbitrap XL) was used to identify and to determine the relative levels of multiple CoA-activated compounds in samples of mouse liver and muscle without prior sample extraction and solvent evaporation steps. Reproducible ionization efficiency for quantification issues was still achieved despite this minimal sample pre-treatment. The selectivity and sensitivity provided by the Orbitrap system in full MS-scan mode was moreover comparable to that obtained using triple quadropol SRM-Scans and enabled the generation of profiles of CoA-activated compounds from less than 10mg of tissue. The use of non-targeted, full MS data acquisition and automated peak detection, integration, alignment and grouping using the open source software XCMS provided data about every detectable metabolite eluted from the LC-system: hundreds of features in the LC-MS data including the CoA-activated compounds were detected in this manner. Identifications were confirmed by accurate mass data, data dependent MS/MS scans and retention times. As well as the above, nine CoA activated compounds (malonyl-, acetyl-, C6:0-, C16:0-, C16:1-, C18:0-, C18:1-, C18:2, C20-4) were absolutely quantified using calibration standards. Accuracies between 85 and 115% for quality control samples (CoAs spiked in tissue homogenates) were achieved using internal standards (isotopically labelled for malonyl-CoA and acetyl CoA and C17-0 CoA for medium and long chain acyl CoAs).
Conclusion: The developed online SPE/LC-FTMS method enables accurate quantification and profiling of acylCoAs in a broad range of different biological tissues.
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708

Muscular lipid droplet dynamics: ADRP and OXPAT protein expression upon lipid loading in cultured myocytes

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Background and aims: Type 2 diabetes is associated with elevated intramyocellular lipid levels. Intracellular lipids are stored in lipid droplets (LDs) covered with a protein coat comprising of a range of different proteins, dependent on the maturation and metabolic fate of the LD. These LD-coating proteins have been mainly studied in adipose tissue; less is known about their role in skeletal muscle. Here we aimed to examine the role of LD-coating proteins in muscular fat accumulation. To that end, we investigated the effect of fatty acid (FA) incubation on adipose differentiation-related protein (ADRP) and OXPAT protein content in skeletal myotubes. As it is known that different types of FAs differentially affect myocellular fat accumulation, we investigated the effects of FA-induced myocellular lipid accumulation on ADRP and OXPAT protein content.
Materials and methods: C2C12 mouse muscle cells were differentiated into myotubes and incubated for 24h with 200 uM oleate, palmitate, eicosapentaenoate, or octanoate, in the presence or absence of 1 mM carnitine. Lipids were extracted for triglyceride (TG) measurements and ADRP and OXPAT protein contents were measured using Western blotting.
Results: As shown in Figure 1, FA incubation led to marked accumulation of myocellular TG, which was more pronounced in the absence of carnitine. Octanoate did not increase TG and ADRP protein levels and the presence of carnitine did not influence TG accumulation and ADRP protein content upon eicosapentaenoate incubation. With most FAs, relative ADRP protein levels paralleled changes in myocellular TG content. However, oleate incubation in the absence of carnitine resulted in a two-fold larger increase in ADRP protein content compared to the increase in TG content. OXPAT protein content was not increased upon lipid loading, though a small increase was observed upon octanoate incubation.
Conclusion: The present study demonstrated parallel changes in TG and ADRP upon lipid loading in muscle cells, suggesting a role for ADRP in lipid storage in muscle. Only after oleate incubation the induction of ADRP protein content was discordant with TG storage. In contrast, OXPAT protein levels did not match changes in TG content. Our experiments suggest that the roles of ADRP and OXPAT in muscular lipid storage are complex. The discrepancies in TG, ADRP, and OXPAT levels might be explained by differences in the metabolic fate of the type of FA entering the cell. Experiments investigating the effects of siRNA-mediated ADRP knockdown on myocellular lipid accumulation and its metabolic consequences (insulin signaling) are in progress.

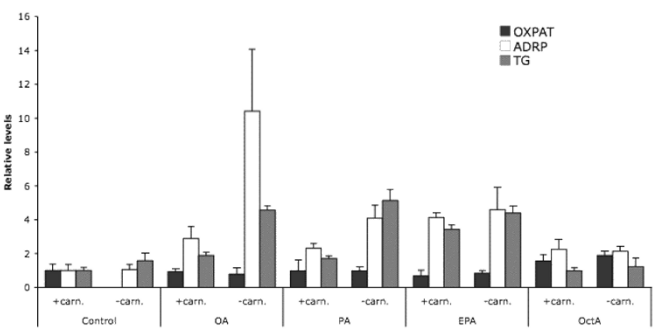

Figure 1: Relative triglyceride and ADRP levels upon incubation with 80 μM BSA complexed to 200 μM oleate (OA), palmitate (PA), eicosapentaenoate (EPA), octanoate (OctA), or control (solely 80 μM BSA) (n=3) in the presence or absence of 1 mM carnitine (carn.). ADRP and OXPAT protein contents were normalized to β-actin content and TG levels were normalized to total cellular protein content. The control treatment with carnitine addition was set at 1.0 (reference treatment). Data are expressed as mean +/- SEM.

709

***LPIN1* mRNA levels are more associated with lipogenic/lipolytic genes in subcutaneous than in visceral adipose tissue. *In vitro* lipogenic/lipolytic effects on human SGBS adipocytes**

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Background and aims: Adipose tissue dysfunction implies the appearance of insulin resistance, partly due to the increased plasma free fatty acids and to the lipid accumulation in not fat tissues. Human subcutaneous adipose tissue (SAT) *LPIN1* mRNA expression is positively associated with insulin sensitivity and a negative association with the body mass index (BMI) has been described. We have analysed the visceral (VAT) and SAT *LPIN1* transcription in the adipose tissue of a cohort of subjects that includes a wide range of obesity, and its relationship with genes involved in lipogenesis and lipolysis. We also aimed to analyse *LPIN1* mRNA expression in human adipocytes in response to lipogenic and lipolytic stimuli.
Materials and methods: Adipose tissue samples were collected from 62 apparently healthy subjects (38 men and 24 women; mean age 55.5±14.8). Anthropometric and metabolic parameters were recorded. Gene expression (*LPIN1, ACSS2, DGAT1, ACC1, CD36, FABP4, PCK1, ATGL, HSL, PLIN, PDE3B, PPIA*) was assessed by quantitative real time PCR. SGBS human preadipocytes were differentiated to adipocytes and stimulated with insulin/isoproterenol/TNFα and *LPIN1* mRNA levels were quantified.
Results: *LPIN1* levels were positively correlated between adipose depots (R=0.615, p<0.001) and no differences between SAT and VAT depots was observed. Overweight and obese subjects had significant reduced *LPIN1* levels in both adipose depots (p<0.01 in SAT and p<0.05 in VAT) and it was also decreased in subjects with metabolic syndrome with significant differences in SAT (p<0.001 in SAT and p=0.052 in VAT). *LPIN1* levels negatively correlated with waist circumference (p<0.001); BMI, fasting triglycerides and insulin levels, HOMA and SBP (p<0.01); and glucose (p<0.05) in SAT, whereas in VAT we found negative correlations only with waist (p<0.01), BMI and insulin (p<0.05). We found positive correlations between *LPIN1* mRNA levels lipogenic and lipolytic genes. In SGBS adipocites, *LPIN1* was up- regulated by insulin, whereas lipolytic stimuli (isoproterenol) had no effect on LPIN1 mRNA levels. On the contrary, TNFα (a pro-inflammatory stimuli that is known to induce lipolysis) significantly down-regulated *LPIN1* transcript levels.
Conclusion: Unlike other adipogenic genes that are higher epressed in SAT, *LPIN1* mRNA levels are comparable between SAT and VAT. Whereas SAT showed strong and positive associations with insulin sensitivity and with lipogenic/lipolytic genes, VAT only showed moderately association. *In vitro* experiments showed that *LPIN1* transcription levels are up-regulated by insulin and down-regulated by TNFα. Insulin resistance and the inflammatory milieu that characterize obesity could explain the decreased levels of LPIN1 mRNA in adipose tissue of obese patients.
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710

Effect of 24-hour lipid infusion on adipocyte fatty acid binding protein (A-FABP) in patients with type 2 diabetes and healthy subjects

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Background and aims: Intracellular transporter adipocyte fatty acid binding protein (A-FABP) is a) involved in a regulation of lipid metabolism, b) considered as a marker of the metabolic syndrome and c) thought be involved in the development if insulin resistance (IR). Our aim was to evaluate its plasma concentrations and expressions in abdominal subcutaneous adipose tissue during lipid infusion and consequent hyperlipidemia.
Materials and methods: 11 patients with type 2 diabetes (D) and 9 age-matched healthy control subjects (C) underwent 24-hour infusion of lipid emulsion (Intralipid 20%; 3 g of fat/kg/day; HTG). At 0, 4, and 24 hours A-FABP plasma concentrations and its mRNA expressions in abdominal subcutaneous adipose tissue were assessed. Adipose tissue sample was obtained by needle biopsy and mRNA measured by real-time PCR.
Results: In group D vs. C, both higher plasma concentrations (p<0.01) and expressions in abdominal subcutaneous adipose tissue (p<0.001) of A-FABP were found. During the lipid infusion plasma concentrations of A-FABP decreased significantly (p<0.001), with difference in between groups (p<0.01): significant decrease in C, only a trend to decrease in D. mRNA expressions in abdominal subcutaneous adipose tissue did not change in time in both groups.
Conclusion: In patients with type 2 diabetes, both higher plasma concentrations and adipose tissue mRNA expressions of A-FABP were found. During lipid infusion induced hyperlipidemia A-FABP plasma concentrations significantly decreased in healthy subjects, whereas only a non-significant trend was observed in patients with type 2 diabetes.

Time (h)		0	4	24
C	Plasma (μg/l)	**14.8** ± 6	**13,5** ± 4.8	**9.9** ± 4
	Expression	**922** ± 379	**1012** ± 439	**880** ± 297
D	Plasma (μg/l)	**27.1** ± 11.5	**23.9** ± 7.2	**24.8** ± 7.5
	Expression	**2630** ± 1117	**3143** ± 2221	**3186** ± 3623

Plasma concentrations and mRNA expressions in abdominal subcutaneous adipose tissue. Data shown as average ± SD, expressions presented as an A-FABP mRNA / cyclophylin mRNA ratio.

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711

Hyperlipidaemic conditions induce 'metabolic memory' within human abdominal subcutaneous adipose tissue and isolated adipocytes, *in vivo*

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Background and aims: Both chronic hyperlipidemia and hyperglycemia are established as key factors in the pathogenesis of obesity mediated diabetes. Chronic elevation of free fatty acids (FFAs) and glucose (Glc) appears to activate an inflammatory response in multiple cell types; which may be compounded by continual feeding. Restoration of physiological FFA and Glc levels may not attenuate the original insult on the cell; a finding that has been termed 'metabolic memory' in other systems. Therefore we investigated (1) the effect of FFAs and Glc on the inflammatory signaling pathway in adipose tissue (AT) and adipocytes (Ads) (2) whether Ads are subject to 'metabolic memory'.
Materials and methods: Abdominal (Abd) subcutaneous (Sc) AT was collected from patients undergoing elective liposuction surgery (age 45±3.3 yrs; BMI: 21.9±2.4 kg/m^2; n=6). AbdSc AT explants and Ads were treated with chronic low glucose (L-Glc): 5.6mM and high glucose (H-Glc): 7.5mM, with low (0.2 mM) and high (2 mM) doses of a palmitate:stearic tri-mix for 48 hrs. Further, AbdSc AT explants and Ads were also exposed to the aforementioned treatment regimen for 12hr periods, with alternating rest periods of 12hrs in L-Glc. Conditioned media were collected and pro-inflammatory cytokine secretions were assessed by ELISA. Western blot was utilized to examine components of the NFκB pathway.
Results: Chronic treatment with L-Glc and high FFAs, H-Glc and high FFAs up-regulated key factors of the NFκB pathway in both AbdSc AT explants and Ads (AbdSc AT explants TLR4, Control: 1.09±0.02, H-Glc & FFA (2 mM) 2.00±0.47*; Ads TLR4, Control: 1.04±0.02, H-Glc & FFA (2 mM) 1.32±0.13*; AbdSc AT explants NFκB, Control: 1.06±0.03, H-Glc & FFA (2 mM) 1.44±0.02*; Ads NFκB, Control: 1.04±0.03, H-Glc & FFA (2 mM) 1.95±0.01*; *p<0.05) whilst MyD88 protein levels remained unchanged. Similarly, intermittent treatment with Glc and FFAs increased TLR4 (p<0.05), NFκB (p<0.05) and IKKβ (p<0.05) in explants and Ads. However, in contrast to chronic treatment, MyD88 protein expression was significantly increased (AbdSc AT MyD88, Control: 1.03±0.01, H-Glc & FFA (2 mM) 1.30±0.13*; Ads MyD88, Control: 1.04±0.01, H-Glc & FFA (2 mM) 1.67±0.2*). No change in JNK2 or JNK1 protein expression was observed across all treatments. Downstream both TNF-α (p<0.05), resistin (p<0.05) and IL-6 (p<0.05) secretion were markedly increased in chronically treated AbdSc AT explants and Ads

whilst, in intermittently treated cells, a metabolic memory effect was noted in absence of treatment.
Conclusion: Hyperlipidemia upregulated the innate immune response in human AbdSc AT explants and Ads with chronic and intermittent exposure. AbdSc explants and Ads were subject to 'metabolic memory', differentially activated by the MyD88 dependent pathway, in contrast to the chronically treated cells. This study implicates elevated FFAs as a key instigator of the inflammatory response in both AT and Ads, via NFκB, which suggests that short-term exposure of cells to uncontrolled levels of FFAs and Glc lead to a longer-term inflammatory insult within the Ad. These findings indicate the importance of maintaining a low fat diet in order to reduce inflammatory risk in patients with obesity and type 2 diabetes.
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712

Lipid-altering efficacy of ezetimibe/simvastatin 10/20 mg compared to rosuvastatin 10 mg in high-risk patients with and without type 2 diabetes mellitus

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Background and aims: This post-hoc exploratory analysis compared the lipid-altering effects of switching to ezetimibe/simvastatin (EZE/SIMVA) 10/20 mg or rosuvastatin (ROSUVA) 10 mg in high-risk hypercholesterolemic patients (pts) with and without type 2 diabetes mellitus (T2DM).
Materials and methods: In this randomized, double-blind study, 618 high-risk pts with elevated LDL-C ≥2.59 and ≤4.92 mmol/L despite prior use of statins entered a 6-wk open-label stabilization/screening period during which they continued on the same statin dose prior to enrollment. Pts were randomized 1:1 to EZE/SIMVA 10/20 mg or ROSUVA 10 mg for 6 wk. Pts were classified as having T2DM based on ≥1 of the following: prior diagnosis of T2DM, use of antihyperglycemic medication or baseline fasting plasma glucose ≥7 mmol/L. The primary endpoint was % change from baseline in LDL-C at study endpoint in the overall study population. This post-hoc analysis evaluated % change from baseline in lipids and % of pts attaining LDL-C, non-HDL-C and apo B goals at study endpoint in pts with (n=182) and without T2DM (n=434). Consistency of the treatment effect across subgroups was evaluated by testing for treatment × subgroup interaction. No multiplicity adjustments were made.
Results: EZE/SIMVA 10/20 mg was more effective than ROSUVA 10 mg at lowering LDL-C, TC, non-HDL-C and apo B in the overall population and across the subgroups (Table 1). Numerically greater between-treatment reductions in LDL-C, TC, non-HDL-C and apo B were seen in pts with T2DM vs those without T2DM. A significant treatment × subgroup interaction was observed for LDL-C (p=0.015) suggesting that pts with T2DM achieved larger between-treatment reductions vs. those without T2DM. More pts with and without T2DM achieved LDL-C levels < 2.59, < 2.00 and < 1.81 mmol/L, non-HDL-C < 2.59 mmol/l and apoB < 0.8 g/L with EZE/SIMVA vs ROSUVA.
Conclusion: In this post-hoc analysis of high-risk patients with elevated LDL-C despite prior use of statin therapy, switching to EZE/SIMVA 10/20 mg vs ROSUVA 10 mg provided superior reductions in LDL-C, TC, non-HDL-C and apo B in pts with and without T2DM.

Between-treatment differences (EZE/SIMVA - ROSUVA; 95% CI) in least squares mean % change in efficacy parameters at study endpoint*

	Overall Population	P value†	Without T2DM	With T2DM
LDL-C (mmol/L)	-10.7 (-14.1, -7.3)	<.001	-8.4 (-12.3, -4.6)	-18.8 (-27.5, -10.1)
TC (mmol/L)	-7.2 (-9.5, -4.8)	<.001	-6.4 (-9.1, -3.7)	-12.0 (-17.8, -6.3)
HDL-C (mmol/L)	-0.9 (-3.2, 1.4)	.437	-1.9 (-4.8, 1.0)	-1.3 (-6.4, 3.8)
TG (mmol/L)‡	-5.2 (-9.7, -0.4)	.053	-4.7 (-10.3, 1.0)	-6.2 (-14.7, 2.3)
non-HDL-C (mmol/L)	-9.4 (-12.5, -6.3)	<.001	-8.0 (-11.6, -4.4)	-15.6 (-23.1, -8.1)
apo B (g/L)	-8.1 (-10.9, -5.3)	<.001	-7.8 (-11.0, -4.5)	-11.6 (-18.8, -4.5)
hs-CRP (mg/dL)‡	-6.5 (-16.7, 3.2)	.186	-4.8 (-16.8, 7.1)	-11.9 (-26.7, 5.2)

apo= apolipoprotein; CI= confidence interval; HDL-C= high-density lipoprotein cholesterol; hs-CRP=high sensitivity C-reactive protein; LDL-C= low-density lipoprotein cholesterol; T2DM=type 2 diabetes mellitus; TC= total cholesterol; TG= triglycerides
*Defined as the last available post-baseline value collected during the 6-wk active treatment period
†For treatment difference in overall population
‡Expressed as the difference in median % change from baseline obtained by Hodges-Lehman estimation

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PS 54 Adipokines - ”classical” and novel

713

Serum vaspin concentration is associated with metabolic syndrome in male subjects

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Background and aims: Vaspin (visceral adipose tissue derived serpin) is a newly indentified adipokine, serine protease inhibitor family with insulin sensitizing effect in animal study. It was expressed in visceral adipose depots in OLEF rats and the mRNA expression of vaspin was found in adipose tissue only in obese, insulin-resistant human. There were few studies to show the clinical impact of vaspin but some suggested its possible role in obesity and insulin sensitivity. Thus, we want to investigate which clinical conditions are related to serum vaspin concentrations in humans.
Methods: We finally analyzed 403 (male: 202, female: 201) subjects from NGT to DM and measured the correlation between various metabolic parameters and serum vaspin concentrations (by ELISA kit, Adipogen, Seoul, Korea). Multidetector coronary computed tomography (MDCT) was performed for estimating coronary atherosclerosis in our subjects.
Results: Serum vaspin concentrations is significantly elevated in female compared to male subjects (0.53 (0.04-5.45) vs. 0.44 (0.05-2.51), $p<0.05$). Over age of 50, this difference of serum Vaspin level disappeared between male and female. Comparing serum vaspin levels of subjects with NGT vs. IGT&DM showed trends of decrement in subjects with IGT&DM in male. Serum vaspin concentrations are significantly high in subjects with metabolic syndrome (MS) in male (0.40 (0.05-2.51) vs. 0.60 (0.12-2.44, $p<0.001$) but not in female. Serum vaspin concentration is significantly associated with the numbers of MS in male. Body mass index, waist circumference, triglyceride showed independent predictors for serum vaspin concentration in male, only HbA1c showed negative correlation to vaspin levels in female. Serum vaspin concentration did not show any association with the status of atherosclerosis (coronary calcium score, degree of coronary stenosis, plaque burdens by MDCT) in our subjects.
Conclusion: Serum vaspin concentration showed sexual difference, higher in female. BMI, waist circumference, triglyceride showed significant correlation to vaspin level. Subjects with MS showed higher vaspin concentration than subject without MS in male. Paradoxical increase of vaspin concentration is associated with MS and glucose intolerance in male subjects, suggests its possible role in obesity and insulin sensitivity in human.

714

Association of serum visfatin with insulin secretion and sensitivity among prediabetic and type 2 diabetic subjects

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Background and aims: Insulin resistance in IGT and T2DM subjects have been shown to be associated with Visfatin, a cytokine of visceral fat origin. The association, however, has been studied only in obese subjects. Since BMI is a major confounder of both subclinical chronic inflammation and insulin resistance we have explored the association of serum Visfatin with insulinemic status in normal to moderately overweight prediabetic and diabetic subjects.
Materials and methods: A group of 75 subjects with impaired glucose regulation (IGR) [mean (±) age 43±7 and BMI 25.3±4.5] and 43 T2DM [43±6 and 24.9±3.9 respectively] subjects were included in this study and 51 healthy subjects [42±5 and BMI 24.5±4.1 respectively] served as control. The three groups were matched for age and BMI. Anthropometric features and total body fat mass were determined following standard procedure. Glucose and lipids were measured by standard methods. Insulin and visfatin were determined by enzyme linked immunosorbent assay (ELISA). Insulin secretory capacity (HOMA%B) and insulin sensitivity (HOMA%S) were calculated by homeostasis model assessment (HOMA) using HOMA-Sigma Software. Data were analyzed by appropriate univariate as well as multivariate tools.
Results: Mean (±SD) age was, and in the Control, IGR and T2DM groups respectively and BMI was, and respectively. Fasting insulin (μU/ml) was significantly higher in the IGR and T2DM group compared to the Controls ($p=0.002$ and $p<0.001$ respectively) but between the IGR and T2DM group no significant difference was observed. HOMA%B was significantly lower in T2DM compared to the Control ($p=0.004$) and IGR ($p=0.008$) groups. HOMA%S was significantly lower in the IGR and T2DM groups compared to Controls ($p<0.001$). Fasting visfatin (ng/ml) was 3.1±3.0, 5.6±4.3 and 5.5±4.2 in the Control, IGR and T2DM respectively. Mean (±SD) visfatin in the IGR and T2DM was significantly higher compared to the Control ($p=0.002$ and $p=0.006$ respectively), but the value was almost similar in the two groups. Visfatin did not show any significant correlation with fasting glucose, insulin, HOMA%S and HOMA%B. But multinomial logistic regression revealed association of serum visfatin with both IGR and T2DM groups ($p=0.027$ and 0.033 respectively) when age, BMI and TG were adjusted. On multiple linear regression analysis Visfatin was found to be significantly associated with insulin when fasting glucose, age, BMI and TG were adjusted in T2DM ($p=0.035$) but the association was not significant in the Control and IGR groups. Visfatin, however, did not show any association with HOMA%S and HOMA%B in any of the three groups when the effect of age, BMI and TG was adjusted.
Conclusion: Visfatin may be associated with prediabetic and diabetic conditions, but it does not seem to be a direct mediator of B cell secretory capacity and insulin sensitivity.
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715

A novel quantitative flow cytometric method for the determination of a new adipocytokine, omentin, in human plasma

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Background and aims: Omentin is a recently described adipocytokine, selectively expressed and secreted from visceral, but not from subcutaneous adipose tissue. *In vitro* studies have shown that omentin increases insulin signal transduction by activating Akt/protein kinase B and enhancing insulin stimulated glucose transport. Preliminary studies have indicated a possible implication of omentin in metabolism. It is of major importance to determine whether there is any correlation between the plasma levels of circulating omentin and metabolic disorders such as obesity or type 2 diabetes and whether omentin exerts a protective action. The aim of this study was the development of a flow cytometric technique that would allow the quantitative determination of the circulating omentin.
Materials and methods: Blood (5ml) was withdrawn from 17 healthy women (BMI 22.2±4, age 34±9). The levels of circulating omentin in human plasma were detected by a novel technique based on the immunological transaction of a monoclonal anti-omentin (Ab) antibody (conjugated on the surface of capture beads) with omentin. A monoclonal biotin-conjugated Ab (detection Ab) was incubated with the capture beads, the test samples/standards and streptavidin-phycoeryhtrin reagent to form a sandwich complex. The samples were then analyzed by flow cytometry (FACS Calibur, BD Biosciences, SJ, California USA). The results were quantified by the usage of a standard curve. The Mean Fluorescence Intensity of the standards was plotted against the concentrations of the standards and the standard curve fitting was performed by the GraphPad Prism software (GraphPad Software Inc, San Diego, USA). Omentin plasma levels were interpolated from the standard curve. The omentin plasma levels versus waist circumference, HOMA, BMI, waist to hip ratio (w/h) were statistically tested for linear correlation (Pearson r) by GraphPad Instat.
Results: The introduced method has allowed the measurement of the plasma omentin in 17 women (average level 106±59ng/ml, range 20-250ng/ml). The omentin levels were negatively correlated with BMI ($r=-0.56$, $P=0.019$), HOMA ($r=-0.55$, $P=0.019$), waist circumference ($r=-0.55$, $P=0.021$) and w/h ratio ($r=-0.55$, $P=0.021$). These parameters are indicators of the metabolic status and reflect the distribution of the adipose tissue.
Conclusion: Our results lead to the conclusion that omentin plasma levels decline as deposition of fat in the waist line increases. This decrease may contribute to the insulin resistance observed in obesity, diabetes and generally in metabolic syndrome. Additionally, the measurements prove that the intro-

duced flow cytometric technique produces results similar to those produced by the classical method of quantitative western blotting assay. The advantages of the flow cytometric technique are the easiness of handling, the small amount of required blood, and the fact that the results are rapidly produced without compromising specificity and accuracy. This experimental approach permits the determination of this newly discovered adipokine in a large sample size of humans, in order to clarify the physiological role of omentin.

716

Association of plasma retinol binding protein 4 with insulin secretion and sensitivity among prediabetic subjects

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Background and aims: Retinol binding protein 4 (RBP4) is now thought to be an important molecule in the pathogenesis of insulin resistance in T2DM, but the causal association of the adepocytokine with the basic defects of T2DM (insulin secreatory defect and insulin resistance) are still not settled. An investigation of this molecule in prediabetic subjects can help in better understanding of this association. We have studied a group of IGR (IFG, IGT and IFG-IGT) cases for the relationship of RBP4 with glycemic and insulinemic status.
Materials and methods: A total 75 IGR subjects [17 IFG, 44 IGT and 14 IFG-IGT] were recruited purposively and 51 healthy subjects served as control. Anthropometric measures and total body fat mass were determined. Glucose and lipids were measured by standard biochemical methods. Insulin and RBP4 were estimated by using enzyme linked immunosorbent assay (ELISA). Insulin secretory capacity (HOMA%B) and insulin sensitivity (HOMA%S) were determined by homeostasis model assessment (HOMA) using HOMA-Sigma software. Data were analyzed by appropriate univariate as well as multivariate tools.
Results: The study subjects in the four groups were age- and BMI- matched. Waist to hip ratio and total body fat mass among the groups did not show statistical difference between groups. Fasting triglyceride was significantly higher in the IFG, IGT and IFG-IGT groups compared to Controls (p=0.002, <0.001 and 0.013 respectively). Mean HDL was significantly lower in IGT group compared to the Controls (p=0.009). Fasting insulin (μU/ml) in the IGT and IFG-IGT groups was significantly higher compared to the Controls (p=0.002 and 0.017 respectively). HOMA%B was 107±25, 76.5±18, 126±44 and 78.9±17 in Control, IFG, IGT and IFG-IGT- respectively; the value was significantly lower in IFG and IFG-IGT groups (p<0.001 for both) and higher in IGT (p=0.019) compared to the Control. HOMA%S was 81.9±18, 72.3±23, 67.7±18 and 62.3±17 in Control, IFG, IGT and IFG-IGT-respectively; which was significantly lower in the IGT and IFG-IGT groups compared to Controls (p<0.001 and p=0.001 respectively). Fasting RBP4 (μg/ml) was 30.4±6.1, 32.2±10.9, 36.7±9.0 and 35.5±7.6 in Control, IFG, IGT and IFG-IGT- respectively; it was significantly higher in IGT and IFG-IGT groups compared to the Controls (p<0.001 and p=0.011 respectively). RBP4 levels did not show any significant difference between the IFG and the Control groups. When the study subjects were categorized on the basis of 75th percentile of plasma RBP4, insulin level was found significantly higher (p=0.028) and HOMA%S lower (p=0.008) in the group with higher RBP4 compare to lower RBP4 group. HOMA%B, however, did not show any difference between the groups. In binary logistic regression analyses RBP4 showed association with IGR (IFG, IGT and IFG-IGT pooled together) group (p=0.001) after adjusting the confounding factors (age, BMI and WHR). Multiple linear regression analyses showed that RBP4 affected both HOMA%B (β=0.308, p=0.012) and HOMA%S ((β=-0.283, p=0.020) when the effect adjusted for age, BMI and WHR.
Conclusion: The present data indicate that RBP4 may have a causal association with type 2 diabetes mellitus.
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717

Relationship of Retinol binding protein-4 with insulin sensitivity and intima media thickness in women with untreated essential hypertension

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Background and aims: Retinol binding protein-4 (RBP4) is a novel adipokine able to modulate the action of insulin in several tissues. A variable degree of insulin resistance characterizes the vast majority of hypertensive patients. Aim of the present study was to evaluate the relationship between RBP4 and essential hypertension, exploring potential links between RBP4, other adipokines and insulin sensitivity with some proxies of early vascular damage in female, naïve for treatment, hypertensive patients.
Materials and methods: Serum RBP4, leptin, adiponectin and resistin levels were determined in 35 normotensive (CT - 46.9 ± 6.3 years; BMI 25.7 ± 1.4 kg/m^2) and in 35 hypertensive (HYP - 47.4 ± 5.0 years; BMI 25.0 ± 1.6 kg/m^2) women with normal glucose tolerance by OGTT. Carotid intima-media thickness (IMT) was measured using high-resolution B-mode ultrasound. Insulin sensitivity was estimated from the plasma glucose and insulin responses by the Oral Glucose Insulin Sensitivity (OGIS) index.
Results: All participants had normal glucose tolerance. Mean IMT was 0.499 ± 0.127 mm in CT and 0.543 ± 0.148 mm in HYP (p=ns). In HYP, RBP4 levels were markedly higher than in CT (37.1 ± 1.6 vs. 11.1 ± 0.77 μg/ml; p<0.0001), and adiponectin levels were slightly but significantly lower (5.6 ± 0.2 vs. 6.2 ± 0.2 μg/ml, p<0.05). No differences were observed in resistin and leptin concentrations. Fasting glucose was similar (4.7 ± 0.6 vs. 4.7 ± 0.4 mmol/l) as well 2-h glucose (6.2 ± 0.7 vs. 6.0 ± 0.8 mmol/l), insulin (108 ± 38 vs. 98 ± 40 pmol/l) and OGIS (492 ± 49 vs. 492 ± 45ml/min/m^2). In the whole study group, a strong linear relationship was observed between IMT value and both RBP4 (Rho 0.32, p=0.008) and resistin (Rho 0.34, p=0.005); there was a trend to correlate with OGIS (Rho 0.22, p=0.07), but not with fasting glucose and insulin or adiponectin. RBP4, resistin, mean blood pressure and triglycerides were, actually, the only variables independently related to IMT (r^2=0.30; p=0.0002) by multiple regression analysis.
Conclusion: In non-obese, non-diabetic naïve hypertensive women, RBP4 levels are increased and correlate with the degree of intima-media thickness, suggesting a participation of this adipocytokine in the modulation of the atherosclerotic process exerted by the adipose tissue as endocrine organ.

718

Apelin regulates luminal intestinal glucose absorption through AMPK dependent mechanisms

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Background and aims: Intestinal absorption of carbohydrate-digested products by the small intestine constitutes one highly regulated way to deliver glucose to the blood. Glucose absorption is mainly driven by sodium glucose transporter 1 (SGLT-1) in preprandial and interprandial states, and requires further assistance of GLUT2 transporter in brush-border membrane (BBM) upon ingestion of sugar-rich meal. This process is highly regulated by peptides and hormones. As other adipokines/gut peptides (ie leptin, resistin β) apelin is produced by proximal digestive cells and secreted in the lumen where it is believed to bind specific receptor APJ in apical membrane of enterocyte. Here we examined whether apelin can act from luminal side of intestine to regulate glucose absorption and which are the intracellular mechanisms involved in this regulation.
Materials and methods: Male C57Bl6J mice were used. Specimen of jejunal mucosa were prepared for immunohistochemical analysis of AJP. Proximal intestine from fasted mice was isolated in Ussing chamber and glucose absorption was measured after 10 mM glucose challenge as Na^+-induced short-circuit current (Isc) that sustains glucose entry through SGLT-1. Apelin (0.01 nM-1 μM) was added into luminal reservoir 3 min before glucose challenge. Jejunal loops were also prepared to quantify *in vitro* transepithelial transport of ^{14}C glucose in absence and presence of luminal apelin (1 nM). The GLUT2 and SGLT-1 proteins abundance in BBM and phosphorylation status of AMPK were studied by western blot after *in vivo* incubation of jejunal loops with apelin and/or 30 mM glucose.
Results: Mice proximal mucosa was immunohistochemically reactive for APJ. Apelin receptor was found expressed predominantly in villi. In Ussing

chamber, luminal apelin dose-dependently inhibited glucose-induced Isc (IC50 ~1 nM). This inhibition of SGLT-1 activity was maximal at 100 nM, and blocked by preincubation with a monoclonal antibody to the peptide. Accordingly, glucose-induced abundance of SGLT-1 in BBM was blunted after rapid incubation of jejunal loops with 1 nM apelin. At the same time, apelin enhanced glucose-induced abundance of GLUT2 in BBM that is a hallmark of AMPK stimulation. Thus we further examined the effect of apelin on AMPK. Total proteins from the same cells indicated this was associated with a rapid phosphorylation of AMPK. The result of this dual effect of apelin on transporters resulted in a 15% increased of the glucose transepithelial transport without change in mannitol permeability.
Conclusion: Apelin was previously shown to restore glucose tolerance and increase glucose utilization. Here we demonstrate that apelin, as a gut peptide, may bind APJ receptors at enterocyte BBM to rapidly balance glucose transport in response to alimentary sugars. As SGLT-1 cotransports water, apelin-induced regulation of glucose transport could ameliorate osmotic balance when luminal glucose increases. These data give new insight on apelin role, as a strong physiological regulator of glucose homeostasis in peripheral tissues.

719

The human adiponectin receptor R2 is expressed predominantly in adipose tissue and linked to the adipose tissue expression of MMIF-1

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Background and aims: Expression of adiponectin (ADN) receptors in human adipose tissue (AT) in relation to inflammatory cytokines is poorly understood. The aim of this study was to describe the regional AT-ADN and ADN receptor R1 and R2 expression and their relation with metabolic parameters, circulating and AT-derived cytokines and chemoattractants.
Materials and methods: Paired subcutaneous (SCAT) and visceral AT (VAT) was taken from 18 lean and 39 obese humans, mRNA expression of adipokines and cytokines analysed by rt-PCR and corresponding serum levels by ELISA. R1 and R2 adipose expression was compared with the distribution in 17 other human tissues.
Results: ADN gene expression was lower in VAT than SCAT (mean (SD) 1.54(1.1) vs 2.84(0.87); p<0.001), and lower in obese subjects (VAT:p=0.01;SCAT: p<0.001). SCAT-ADN correlated positively with serum ADN (r=0.33; p=0.036) but not VAT. There were inverse correlations between AT-expression of ADN and MMIF, IL18 and CD14 in both depots. R1 and R2 genes were expressed ubiquitously and R2 expression was highest in AT and this in adipocytes. Both receptors were expressed in VAT and SCAT, but R2 was the predominant receptor (X10); levels were similar in lean and obese subjects and unrelated to the metabolic syndrome, however, receptors correlated with expression of VAT-MMIF (R1:r=0.4;p=0.008; R2:r=0.35,p=0.02) and SCAT-MMIF (R2: r=0.43;p=0.004)and SCAT-R2 correlated with TNFα (r=0.30, p=0.05).
Conclusion: Unlike the protein adiponectin, its receptors R1 and R2 are ubiquitously expressed and R2 expression is highest in AT and not in the liver like in rodents. R2 expression in adipose tissue is associated with MMIF and TNF-alpha expression and may thus be under the influence of local inflammation in adipose tissue. This may potentially be mediated by obesity-induced hypoxia in AT which is known to reduce ADN and may also increase adiponectin receptor expression and which requires further investigation.
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720

HMW-adiponectin associates with triglyceride concentrations in type 1 diabetic patients

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Background and aims: Recent studies have observed higher adiponectin concentrations in patients with type 1 diabetes, compared with healthy subjects, but no correlation with plasma glycemic parameters.The objective of this study was to investigate relation between serum high molecular weight (HMW)-adiponectin concentration and fasting and postprandial blood glycemic and lipid parameters in type 1 diabetic patients.
Materials and methods: Type 1 diabetic patients treated with short-acting insulin analogs and healthy volunteers were recruited. The subjects were divided into two groups based on the mean HMW-adiponectin value of 12.2 mg/L: low HMW-adiponectin (men/women=7/2) and high HMW-adiponectin (men/women=3/8). Blood samples were collected after an overnight fast, and 30-180 min after consuming white bread (B) or white bread with butter (BB).The insulin dose injected for the BB meal should have been the same as that for the B meal.
Results: Mean HbA1c in patients with low and high HMW-adiponectin were 6.8 ± 0.8 % and 6.8 ± 0.9 %, respectively, and C-peptide concentrations also showed similar low levels in the two groups. Onset age and duration of diabetes did not differ between the two diabetic groups. BMI did not differ among the three groups. Type 1 diabetic patients with high HMW-adiponectin had lower triglyceride and remnant like particle (RLP)-triglyceride concentrations compared with those with low HMW-adiponectin (p<0.01), and had higher lipoprotein lipase mass (p<0.01) than healthy subjects (men/women =8/6). After the B and BB meals, type 1 diabetic patients with high HMW-adiponectin consistently had lower triglyceride and RLP-triglyceride concentrations up to 180 min than those with low HMW-adiponectin and healthy subjects (p<0.01) (Figure). However, apoB48 did not differ between these two groups. Fasting and postprandial plasma glucose were higher in both diabetic groups than in healthy subjects (p<0.001), with no significant difference between the patient groups.Multiple regression analysis resulted, log HMW-adiponectin was an independent negative predictor of fasting plasma glucose (beta coefficient -0.487, p=0.036) but not of fasting triglyceride and postprandial log areas under the curve (AUC) triglyceride in healthy subjects. However, in the diabetic patients, no independent predictor of any fasting glucose or AUC plasma glucose was observed, but log HMW-adiponectin was an independent negative predictor of log fasting triglyceride (beta coefficient -0.563, p=0.012), and log AUC triglyceride after both B (beta coefficient -0.584, p=0.009) and BB meal consumption (beta coefficient -0.666, p=0.002).
Conclusion: HMW-adiponectin is more strongly associated with very low density lipoprotein remnant metabolism than glucose utilization in type 1 diabetic patients receiving short-acting insulin analogs.

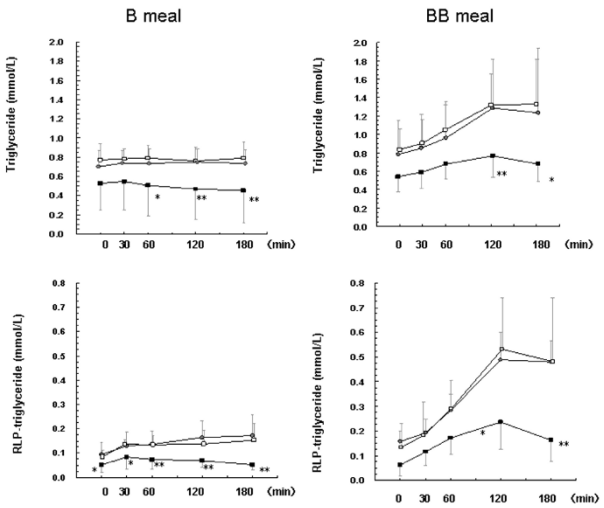

Figure. Postprandial serum triglyceride and RLP-triglyceride changes after B and BB meals in healthy subjects (●), patients with low HMW-adiponectin (□), and patients with high HMW-adiponectin (■). Data are mean ± SD. *: p<0.05, **: p<0.01; statistically significant differences compared with healthy subjects.

PS 55 Secretory function of adipose tissue

721

Profiling of adipokine secretion by adipose tissue in transgenic mice with homotopic overexpression of adiponectin

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Background and aims: We have generated a transgenic (Tg) mouse model allowing persistent and moderate overexpression of native adiponectin (ApN) targeted to white adipose tissue [the transgene being placed under control of aP2 (fatty-acid binding protein) promoter]. Adiposity was reduced in aging mice fed a high-sucrose diet and this anti-obesity effect favored enhanced insulin sensitivity and improved lipid profile. APN appears to be a potent regulator of other adipokines. We took advantage of this unique *in vivo* model to study the influence of APN on adipokine secretion profiling. In order to investigate the early and specific effects of APN, mice were studied before any significant decrease of fat mass or glycemia and circulating lipids occurred.
Materials and methods: 10-wk-old Tg mice receiving a high-sucrose-diet were compared to wild-type (WT) littermates. Subcutaneous adipose tissue was fractionated into adipocytes (A) and stromal-vascular (SV) cells (i.e. non-fat cells that contain macrophages), which were then cultured for 8 h. Medium was screened by medium-scale protein arrays allowing the simultaneous detection of 144 cytokines. Gene expression of some adipokines was measured by RTQ-PCR.
Results: As expected, ApN expression was much higher (30-fold) in A than in SV cells of WT mice, while leptin was virtually undetectable in the SV fraction. ApN was overexpressed in both cellular fractions of Tg mice, in line with the fact that aP2 is co-expressed in adipocytes and macrophages (but at a much lower extent in the latter cells). By using cytokine antibody arrays, we detected more than 40 cytokines secreted by each cellular fraction of WT and Tg mice. Profiling of adipocyte secretory products showed that 9 adipokines were less secreted by Tg than WT mice: one chemokine (regulated upon activation normal T-cell expressed and secreted, RANTES), three pro-inflammatory cytokines (IL-6, IL-17B, IL-21), two extracellular matrix remodeling-/growth arrest-factors and three hematopoietic growth factors (one for megakaryocytes, thrombopoietin; the other two for granulocytes and/or macrophages, GM-CSF/ G-CSF). Profiling of SV cell products showed that 13 peptides were differentially secreted between Tg and WT mice, with usually stronger genotype differences than those observed in adipocytes. Thus, secretion of 12 peptides was downregulated, while one was upregulated in Tg mice. Hyposecreted factors include five adipokines that were also reduced in adipocytes (RANTES, IL-6 and the 3 hematopoietic growth factors) together with another chemokine, four other pro-inflammatory cytokines/ factors (comprising two adhesion molecules), one receptor for an angiogenic factor (VEGF R1), and one factor involved in lipid metabolism (fatty acid translocase). We also found that the secretion of one anti-inflammatory factor (IL-R4, which is an IL1-receptor family member) was increased by SV cells of Tg mice.
Conclusion: At the onset of overexpression, ApN reduced the secretion of 9 to 12 inflammatory adipokines in each cellular fraction of adipose tissue in Tg mice, while upregulating the secretion of one anti-inflammatory factor. Thus, ApN induced a shift of the immune balance in both adipocytes and SV cells toward a less inflammatory phenotype, with a greater effect on SV cells. These adipokines, which are newly identified as downstream targets of ApN *ex vivo*, may have therapeutic potential for the management of the metabolic syndrome.

722

Regulation of the expression of angiopoietin-like protein 4 mRNA in diabetic mice

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Background and aims: Angiopoietin-like protein 4 (Angptl4) is mainly secreted from the liver and adipose tissue. It inhibits lipoprotein lipase activity, thereby causing an increase in the serum triglyceride level. The expression and plasma levels of Angptl4 are increased by fasting and decreased by feeding. We have recently shown that insulin negatively regulates Angptl4 mRNA expression in 3T3-L1 adipocytes, suggesting that insulin plays an important role in the regulation of Angptl4 expression. In this study, we aim to elucidate the regulation of Angptl4 expression in diabetic states.
Material and methods: Type 1 diabetes was induced in C57BL/6 mice by treating with streptozotocin (STZ). As a model of type 2 diabetes, the mice were fed with a high-fat diet (HFD) for 18 weeks. We determined Angptl4 mRNA expression by using a quantitative real-time PCR method and compared it with 36B4 mRNA expression.
Results: When compared with the normal mice, the STZ diabetic mice showed significantly higher serum glucose and triglyceride levels. The level of Angptl4 mRNA expression in the STZ diabetic mice had increased by 2.23 ± 0.47 fold in the liver ($p < 0.05$), 2.00 ± 0.37 fold in white adipose tissue (WAT) ($p < 0.05$), and 1.79 ± 0.26 fold in brown adipose tissue (BAT) ($p < 0.05$) when compared with the corresponding values in normal mice. Furthermore, this effect was attenuated by the administration of insulin. In the HFD diabetic mice, a significant increase in insulin resistance and triglyceride level was observed at 18 weeks. The level of Angptl4 mRNA expression in the HFD diabetic mice was increased by 2.15 ± 0.30 fold in the liver ($p < 0.005$), 1.68 ± 0.27 fold in WAT ($p < 0.05$), and 1.77 ± 0.31 fold in BAT ($p < 0.05$) when compared with the corresponding values in the normal chow-fed mice.
Conclusion: Thus, the level of Angptl4 mRNA expression was increased in both the insulin-deficient and insulin-resistant diabetic states, thus indicating that insulin negatively regulates Angptl4 mRNA expression in the liver and adipose tissue of diabetic mice.

723

N^{ε}-(Carboxymethyl)lysine trapping in adipose tissue in obesity: implications for obesity-associated changes in adipokine expression

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Background and aims: Obesity is linked to a wide variety of pathophysiological conditions. Dysregulated production of adipokines is associated with obesity and provides a link between obesity and obesity-related complications. Factors that lead to a dysregulated production of adipokines remain unknown. We hypothesize that accumulation of the advanced lipoxidation endproduct N^{ε}-(carboxymethyl)lysine (CML) in adipose tissue contributes to the altered expression of adipokines in obesity.
Materials and methods: Accumulation and localization of CML in adipose tissue of obese patients was assessed by immunohistochemistry using a CML specific antibody. Colocalization studies of CML with macrophages (CD68) and endothelial cells (CD31) were performed. Plasma levels of protein-bound CML were measured by UPLC-tandem MS in controls (n=77), familial combined hyperlipidaemia (FCHL, n=42) and obese patients (n=44). Blood clearance of fluorescently labeled CML-albumin was compared with unmodified-albumin in Db/Db mice at different time periods. Uptake of fluorescently labeled CML-albumin and unmodified albumin in adipose tissue was assessed with two-photon microscopy. To examine the biological effects of CML in adipose tissue, preadipocytes were incubated with CML-albumin, and changes in gene expression of plasminogen activator inhibitor-1 (PAI-1), IL-6 and the receptor for AGE (RAGE) were analyzed by Real Time PCR.
Results: Immunohistochemistry showed the presence of CML in adipocytes, macrophages and endothelial cells of adipose tissue of obese patients. CML staining was significantly higher in visceral (VAT) than in subcutaneous adipose tissue (SAT) (IH score 1.50±0.21 and 1.26±0.20, resp, $p<0.001$). Plasma CML levels were lower in obese (1.3±0.2 μM, $p<0.001$) and FCHL patients (1.3±0.3 μM, $p<0.001$) as compared with controls (1.9±0.5 μM). Weight reduction in obese patients led to an increase of plasma CML levels ($p=0.01$). CML plasma levels were negatively correlated with BMI (r=-0.475, $p<0.001$), and VAT (r=-0.548, $p<0.001$), but not with SAT (r=-0.148, $p=0.238$). This suggested an accumulation and trapping of plasma CML in VAT of obese patients. Experiments in Db/Db mice showed higher clearance of CML-albumin than unmodified albumin, and preliminary results of two-photon microscopy showed accumulation of CML-albumin, but not albumin, in VAT of Db/Db mice. Incubation of human preadipocytes with CML-albumin during 24h and 48h had no effect on gene expression of IL-6, PAI-1 and RAGE, while an incubation of 72h increased their expression by 2.4-, 1.8- and 4.9-fold, respectively.

Conclusion: We demonstrated the accumulation of CML in VAT of obese patients. Plasma CML levels are lower in obese patients as compared with controls. The inverse relation between CML plasma levels and VAT suggests trapping of CML in VAT, which was shown in Db/Db mice. The increased pro-inflammatory gene expression after incubation of preadipocytes with CML-albumin supports our hypothesis that CML has biological effects regarding the dysregulation of adipokines.
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724

AQP7 gene expression profile in context of obesity and type 2 diabetes mellitus. Relationship with lipogenic and lipolytic genes in subcutaneous and visceral fat

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Background: Adipose tissue is a dynamic organ in which a balanced activity between lipogenesis and lipolysis is crucial for adequate energy equilibrium. Lipolysis results in the hydrolysis of triglycerides to free fatty acids and glycerol, which is specifically transported outside the cell by the Aquaporin-7 (AQP7) channel. Modulation of *AQP7* expression has been suggested to play a role in the development of obesity and insulin resistance in rodents. No data about the relationship between lipogenic, lipolytic and inflammatory gene markers in the setting of obesity and tpe 2 diabetes has been reported in humans.
Aims: We have studied *AQP7* mRNA expression in adipose tissue from obese and type 2 diabetic patients and its relationship with lipogenic, lipolytic and adipokine gene expression, both in subcutaneous (SAT) and visceral (VAT) fat depots. Likewise, regulation on *AQP7* expression by lipolytic and lipogenic stimuli were analysed in a human preadipocyte cell line (SGBS).
Materials and methods: 73 subjects were included in the study (19 lean, 28 overweight, and 15 obese subjects). We also included 11 patients with type 2 diabetes. Clinical and anthropometrical variables were measured in all subjects. Biochemical and metabolic parameters were quantified. Gene expression of *AQP7*, *ACC1*, *ACSS2*, *DGAT1*, *PEPCK*, *FABP4*, *PLIN*, *ATGL*, *HSL*, *APM*, *PPAR (α,δ,γ)* and *PPIA* were quantified by Real-time PCR. Human SGBS preadipocytes were differentiated and cultured overnight in serum-free medium. Cells were stimulated with several doses of insulin and isoproterenol (0 up to 100 nM) for 24h and *AQP7* gene expression was measured.
Results: Obese subjects shown higher VAT *AQP7* mRNA expression than overweight patients (p=0.006). No differences in SAT *AQP7* gene expression were found according BMI. In patients with type 2 diabetes, higher VAT *AQP7* expression was observed when comparing with their BMI-matched non-diabetic counterparts (p=0.018). *AQP7* both in SAT and VAT depots correlated positively with *APM*, genes related with lipogenesis, lipolysis, FFA transport and with the three members of the *PPAR family*. The independence of the associations was evaluated by linear regression analysis, including sex and presence of type 2 diabetes as confounding variables: In SAT, *AQP7* mRNA expression was positively associated with *APM* (B=0.380, p=0.000), *PPARγ* (B=0.768, p=0.000), *ACC1* (B=0.162, p=0.004) and negatively with *PEPCK* mRNA (B=-0.055, p=0.017). In VAT *AQP7* mRNA expression was determined by BMI (B=0.298, p=0.043), *PLIN* (B=0.283, p=0.038), *FABP4* (B=0.413, p=0.000) and *ATGL* mRNA expression (B=0.298, p=0.043). Treatment of SGBS adipocytes with 100 nM insulin and 100 nM isoporterenol for 24 h inhibited significantly AQP7 gene expression by 60% and 40%, respectively.
Conclusion: AQP7 shows a different pattern of gene expression in SAT than in VAT depots. VAT from obese and type 2 diabetic patients show higher AQP7 mRNA expression levels. A strong interdependence with lipogenic and lipolytic genes was observed. A paradoxical inhibitory response in adipocytes is observed both with lipogenic and lipolytic stimulus.
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725

Circulating and adipose tissue gene expression of zinc-alpha2-glycoprotein in obesity. Its relationship with adipokyne and lypolitic gene markers in subcutaneous and visceral fat

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Background and aims: Zinc-alpha2-glycoprotein (ZAG) is a soluble protein similar in structure to the class I major histocompatibility complex (MHC) heavy chain, which has been implicated in lipid catabolism. However the clinical significance of this lipolytic activity remains scarcely explored. The aim of this study was to analyze ZAG gene expression in human adipose tissue from lean and obese subjects in parallel with several lipolytic and adipokine genes. ZAG circulating plasma levels and its relationship with cardiometabolic risk factors were also studied.
Materials and methods: Clinical and anthropometrical variables were assessed in 73 Caucasian (43 male and 30 female) subjects. Plasma and adipose tissue [subcutaneous (SAT) and visceral (VAT)] from the same patient were studied. Real-time quantification of PPARγ, Hormone sensitive lipase (HSL), Adipose triglyceride lipase (ATGL), Adiponectin, Omentin, Visfatin and ZAG was performed. Plasma concentrations of ZAG were determined with ELISA.
Results: ZAG plasma levels showed a negative correlation with insulin (r= -0.39; p=0.008) and with the HOMA index (r= -0.36; p=0.016). *ZAG* expression in SAT was significantly reduced in overweight and obese individuals compared to lean subjects (p<0.001 and p=0.007 respectively). *ZAG* mRNA expression both in SAT and VAT depots were negatively correlated with many clinical and metabolic cardiovascular risk factors (Table 1). A positive correlation between *ZAG* mRNA expression and circulating adiponectin levels was observed (r=0.404; p<0.001 and r=0.337; p=0.007 for SAT and VAT *ZAG* mRNA expression, respectively). After multiple linear regression analysis, SAT *ZAG* was mainly predicted by *Adiponectin* mRNA expression (B=1.068; p<0.0001) and triglyceride plasma levels (B=-0.572; p=0.006). VAT *ZAG* expression was predicted by *Adiponectin* expression (B=0.539; p<0.0001), and *HSL* VAT expression (B=0.123; p=0.036).
Conclusion: The present study provides evidence of a role of *ZAG* gene in adipose tissue metabolism, with a close association with A*diponectin* gene expression in subcutaneous and visceral fat.

Table 1: Bivariate correlations between ZAG expression levels and clinical or analytical variables

	SAT ZAG	P	VAT ZAG	P
	R	p	R	p
Weight	**-0.376**	**0.001**	**-0.335**	**0.004**
BMI	**-0.366**	**0.001**	-	-
Waist	**-0.448**	**<0.001**	**-0.338**	**0.001**
Waist/Hip	**-0.370**	**0.003**	**-0.523**	**<0.0001**
SBP	**-0.401**	**0.003**	-	-
DBP	-0.259	0.063	-	-
Triglycerides	**-0.506**	**<0.001**	**-0.320**	**0.008**
HDLc	**0.289**	**0.016**	-	-
HOMA	**-0.457**	**0.001**	**-0.356**	**0.001**
Adiponectin (serum)	**0.404**	**0.001**	**0.337**	**0.007**

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726

Insulin resistance in hyperthyroidism: the role of cytokines

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Background and aims: Although insulin resistance is a common finding in hyperthyroidism, the implicated mechanisms are obscure. In muscle and adipocytes of euthyroid subjects, adiponectin has been found to stimulate glucose disposal by activating AMP-activated protein kinase, whereas leptin to reduce insulin stimulated glucose uptake. Tumor necrosis factor-α (TNF-α) and interleukin-6 (IL6) are cytokines with metabolic and weight-regulating effects. In euthyroid subjects both TNF-α and IL6 inhibit lipoprotein lipase activity and increase lipolysis. The aim of this study was to investigate whether cytokines plasma levels are altered in hyperthyroidism and if these changes could be related to the development of insulin resistance.
Materials and methods: A mixed meal was given to ten female hyperthyroid subjects (HR: age 37±4 years, BMI 24±1kg/m^2, Total fat mass (TFM)17±2.9kg T3 334±44ng/dl, TSH 0.03±0.003μU/ml) and eight female euthyroid subjects (EU: age 34±4 years, BMI 24±0.6 kg/m^2 , TFM 15.7±2kg, T3 112±8ng/dl, TSH 1.1±0.07 μU/ml). Plasma samples were taken every 30-60 min for 360 min from a vein draining the abdominal subcutaneous adipose tissue (AD) and from the radial artery for measurements of glucose, insulin, non-esterified fatty acids (NEFA), leptin, TNF-a, IL-6 and adiponectin.
Results: (1) In HR vs EU: (a) arterial glucose was similar ($AUC_{0\text{-}360}$2212±43 vs 2089±41mM*min), but insulin was increased (16333±2804 vs 10087±548mU/l*min, p=0.03) (b) HOMA was increased (2.17±0.47 vs 0.9±0.1 kg/m2, p=0.03) suggesting the development of insulin resistance in hyperthyroidism (c) plasma NEFA levels were increased ($AUC_{0\text{-}360}$138381±16488 vs 89265±7851μmol/l*min, p=0.02), suggesting increased lipolysis (d) fasting plasma levels of IL-6 (2±0.3 vs 0.9±0.1ng/ml, p=0.01), TNF-a (4±0.9 vs 1.4±0.2ng/ml, p=0.04) and leptin (12.3±2 vs EU 5.3±0.8 ng/ml, p=0.03) were increased, whereas adiponectin plasma levels were decreased (10.2±1.7 vs EU 20.8±3.5ng/ml, p=0.01). **(2)** (a) IL-6 was increased in the abdominal vein compared to the radial artery, both in HR (4.9±1.2 vs 2±0.3ng/ml, p=0.01) and in EU (5.6±1.7 vs 0.9±0.1ng/ml, p=0.01), suggesting that IL-6 is produced by the subcutaneous adipose tissue (b) IL-6 levels ($AUC_{0\text{-}360}$) were positively correlated with HOMA index (r=0.66, p=0.03) (c) TNF-α was positively correlated with arterial NEFA levels ($AUC_{0\text{-}360}$) (r=0.86, p=0.006).
Conclusion: In hyperthyroidism: **(1)** glucose and lipid metabolism are resistant to insulin **(2)** IL-6, TNF-a and leptin are increased and adiponectin is decreased supporting the development of insulin resistance **(3)** subcutaneous adipose tissue releases IL-6 which could then act as an endocrine mediator of insulin resistance **(4)** although there is no net secretion of TNF-α by the subcutaneous adipose tissue in vivo, increased systematic TNF-α levels could be part of the mechanism explaining the resistance of lipolysis to insulin.

727

The weight sparing effect of insulin detemir is associated with increased adiponectin levels and decreased adiposity in the diabetic ZDF rat

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Background and aims: It is currently unclear how changes in body weight (BW) are associated with changes in fat mass and adiponectin levels, which are reduced in conditions associated with increased risk of cardiovascular disease (CVD), such as diabetes. This study compared the effects of NPH insulin (NPH) and insulin detemir (IDet) on glycemic control (HbA_{1c}), BW, fat mass, and adiponectin levels in the ZDF rat model of type 2 diabetes.
Materials and methods: Diabetic ZDF rats were treated subcutaneously with vehicle, NPH or IDet for 4 weeks. Fat was determined non invasively using quantitative magnetic resonance.
Results: At baseline, HbA_{1c}, BW, and fat mass did not differ significantly between groups, with HbA_{1c} averaging 8.0±0.3% (mean±SD). NPH and IDet both decreased HbA_{1c} significantly (6.2±0.3%, p<0.01; 6.2±0.4%, p<0.01) compared with vehicle (9.1±0.3%). Despite comparable changes in glycemic control, IDet significantly reduced gain in BW and fat mass vs NPH. The reduction in fat mass with IDet accounted for 62% of the reduced BW gain. Four-week relative weight reduction in the IDet group was 2.1%. NPH and IDet increased adiponectin levels 48% (p<0.01) and 90% (p<0.001) respectively vs vehicle, and IDet increased adiponectin levels 28% (p<0.001) vs NPH.
Conclusion: Four weeks of basal insulin therapy (NPH or IDet) resulted in increased adiponectin levels. IDet reduced gain in BW and fat mass and resulted in a greater increase of circulating adiponectin levels in the ZDF rat than NPH, suggesting that IDet may improve the CVD risk profile compared with NPH.

728

Human adipocyte-secreted factors and vascular smooth muscle cell proliferation: an integrated proteome analysis

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Background and aims: Adipose tissue as an endocrine organ secretes a variety of proteins and peptides. In the context of obesity, especially the perivascular fat may induce inflammation and proliferation in the vascular wall and therefore play a major role in development of atherosclerosis. In this study we examined the influence of adipocyte secretion products on vascular smooth muscle cells (SMC) combining different protein profiling techniques.
Materials and methods: SMCs were incubated with conditioned media (CM) derived from isolated and *in vitro* differentiated pre-adipocytes, which comprises a complex mixture of secretion products. Performing proliferation assays revealed an increase of three-fold in response to CM. In order to examine the cellular mechanism, we performed combined protein profiling by Kinex™ Antibody Microarray and 2D-gel based Difference in Gel Electrophoresis (DIGE). Protein samples achieved under standardized conditions were either analyzed using the array approach screening by 650 different antibodies specific for several hundred distinct proteins including diverse phosphorylation states or by explorative 2D-DIGE multiplex analysis.
Results: Dependent on CM incubation, array analysis revealed 145 alterations in protein abundance or protein phosphorylation status. Investigating the proteome of these SMC by comparing more than 1800 different protein spots, indicated significant alterations for 62 different protein spots. Subsequently these protein spots were identified by MALDI-TOF-TOF analysis. Assigning identified proteins to their cellular functions and combining the results from array and gel based profiling indicates that several cellular key proteins e.g. mTOR, Rac1, STAT, FAK, Caldesmon and Zyxin are influenced through CM incubation and can be assigned to cellular signaling pathways playing a decisive role for regulation of cell cycle.
Conclusion: Factors released by human adipocytes have the potential to induce proliferation of smooth muscle cells. Therefore, integrated proteome analysis of SMCs provide new insights into intra-cellular networking and may lead to new hints for identification of atherogenic or anti-atherogenic factors and their pathways.

PS 56 GLP-1 agonists - clinical 1

729

Effects of exenatide plus rosiglitazone on measures of beta cell function and insulin sensitivity in subjects with type 2 diabetes previously treated with metformin

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Background and aims: This 20 week study was designed to study the effects of exenatide (EXE) plus rosiglitazone (ROSI) on β-cell function and insulin sensitivity in subjects with type 2 diabetes (T2D) using the hyperglycaemic and euglycaemic insulin clamp techniques.
Materials and methods: A total of 137 subjects (mean age 55.7 years, weight 92.9 kg, and HbA1c 7.8%) were randomized to either EXE (10μg twice daily [BID]), ROSI (4mg BID), or EXE (10μg BID) + ROSI (4mg BID). A subgroup (n=50) completed the clamp procedures at baseline and after 20 weeks to allow estimation of insulin secretion, insulin action, and the disposition index (*AUC*M÷I*, where AUC=insulin area under the curve [hyperglycaemic clamp], *M*=insulin sensitivity and *I*=insulin value [steady state of euglycaemic clamp]).
Results: HbA1c decreased significantly in all groups, with the greatest decrease observed for EXE+ROSI (see Table). Subjects treated with ROSI had significant weight gain, while those on EXE or EXE+ROSI had significant weight loss. At 20 weeks, total insulin secretion was significantly higher in EXE and EXE+ROSI than ROSI, and insulin sensitivity (M value) was significantly higher in EXE+ROSI and ROSI than EXE.
Conclusion: Combination therapy elicited an additive effect on glycaemia, and the combination of EXE with ROSI offset the known weight gain effects of ROSI. We confirmed the insulin sensitizing effects of ROSI with a negligible effect on the β-cell while EXE exerted a robust effect on the β-cell and also a modest insulin sensitizing effect. Combination therapy resulted in significant improvements in insulin secretion and insulin sensitivity with reductions in body weight, thus targeting the major pathophysiologic defects of T2D.
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730

Exenatide once-weekly treatment elicits sustained glycaemic control and weight loss over 2 years

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Background and aims: Treatment with the GLP-1 receptor agonist exenatide once weekly (QW) resulted in sustained improvements in HbA_{1c}, fasting plasma glucose (FPG), body weight, serum lipid profiles, and blood pressure through 52 weeks of treatment in patients with type 2 diabetes. Here we describe interim results in a cohort of these patients (on a variety of background antihyperglycaemic agents) who completed the 30-week, controlled, open-label trial in which they received either exenatide QW or exenatide BID, followed by 70 weeks of treatment with 2 mg exenatide QW during a subsequent open-ended assessment period.
Materials and methods: Of the 181 patients in this cohort who entered the trial, 75% (n=135) completed 2 years of treatment. Baseline characteristics (mean±SD) for these patients were: HbA_{1c} 8.3±1.0%, FPG 9.26±0.21 mmol/L, body weight 100±19 kg, BMI 34.7±5.0 kg/m^2, diabetes duration 8±6 y.
Results: Significant improvements (LS mean±SE) in both HbA_{1c} (-1.8±0.1%; 95% CI: -2.0 to -1.6%) and FPG (-2.04±0.22 mmol/L; 95% CI: -2.48 to -1.59 mmol/L) were maintained after 2 years of treatment, with 66% and 42% of patients achieving an HbA_{1c} ≤7.0% and ≤6.5%, respectively. Body weight was significantly reduced (-3.6±0.6kg; 95% CI: -4.8 to -2.3 kg) from baseline following 2 years of treatment. Serum lipid profiles also were significantly improved during 2 years of treatment (triglycerides: -18%, 95% CI: -24% to -12%; total cholesterol: -0.25±0.09 mmol/L, 95% CI: -0.42 to -0.07 mmol/L). Furthermore, a clinically significant reduction in systolic blood pressure (-3.2±1.2 mmHg; 95% CI: -5.5 to -0.8 mmHg) was maintained through 2 years of treatment. Nausea (predominantly mild in intensity) was the most common adverse event during the 30-week treatment period and decreased over time, occurring in 8% of patients during the open-ended treatment period. No severe hypoglycaemia was observed.

	EXE (n=45)	ROSI (n=47)	EXE+ROSI (n=45)	P EXE vs. ROSI	P EXE vs. EXE+ROSI	P ROSI vs. EXE+ROSI
ΔHbA1c (%)	-0.9±0.1	-1.0±0.1	-1.3±0.1	0.720	0.016	0.039
ΔWeight (kg)	-2.8±0.5	1.5±0.5	-1.2±0.5	<0.001	0.038	<0.001
Hyperglycaemic Clamp	(n=17)	(n=15)	(n=18)			
Insulin Incremental AUC (% increase)	372±83	3±19	286±66	<0.001	0.414	<0.001
Insulin clamp (M value) (% increase)	27±11	61±15	69±15	0.061	0.032	0.728
Disposition Index (mg/kg, Change from Baseline)	80.1±15.1	10.6±15.4	100.3±14.4	0.003	0.344	<0.001

Data presented as LS mean±standard error

Conclusion: Exenatide QW was well tolerated during the treatment period. This study demonstrated sustained glucose control and weight loss over 2 years of treatment with exenatide QW.

731

Reductions in glycaemia and weight with once-weekly dosing of LY2189265, a long-acting glucagon-like peptide 1 (GLP-1) analogue in patients with type 2 diabetes mellitus

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Background and aims: LY2189265 is a novel, long-acting glucagon-like peptide 1 (GLP-1) analog, being developed for treatment of type-2 diabetes (T2DM). LY2189265 consists of a dipeptidyl peptidase-IV (DPP-IV)-protected GLP-1 analog covalently linked to an Fc fragment of human IgG4, thereby increasing its duration of pharmacological activity. Safety, tolerability, pharmacokinetics (PK), glycemic and weight effects, and immunogenicity of weekly subcutaneous doses of LY2189265 were evaluated in a 5-week, placebo-controlled, parallel-group, patient- and investigator-blinded study of 43 patients with T2DM.
Materials and methods: Patients inadequately controlled by diet and exercise or metformin were enrolled and received 0.05 mg to 8 mg of LY2189265 or placebo. Evaluations included glucose, insulin, glucagon, C-peptide during test-meals and oral glucose tests, changes in weight, gastric emptying rate, hemoglobin A1C, and antibodies to LY2189265.
Results: The half-life of LY2189265 was ~95 hours in T2DM patients. LY2189265 at ≥1 mg significantly reduced fasting and postprandial glucose area under the concentration-time curve (AUC) and 2-hour glucose after a test meal. Glycemic reductions observed following the first dose were maintained through weekly dosing. An insulinotropic effect of LY2189265 was demonstrated by increased insulin and C-peptide AUC normalized to glucose AUC. Statistically significant reductions in A1C (-0.69% to -1.34%, p<.01) were observed across doses. Statistically significant effects on gastric emptying after the first dose and weight loss after the last dose were observed at ≥5 mg. LY2189265 was well tolerated at doses producing significant reductions in glycemia. The most commonly reported adverse events were nausea, vomiting, headache, and diarrhea. A statistically significant increase in heart rate was noted at the 5-mg dose. No patient developed antibodies to LY2189265.
Conclusion: Once-weekly administration of LY2189265 demonstrated sustained glycemic and GLP-1 effects in T2DM patients. Side effects were consistent with expected GLP-1 pharmacology. These results support further investigation of this novel long-acting GLP-1 analog.

732

Differences in baseline characteristics between patients prescribed sitagliptin versus exenatide based on a US electronic medical record database

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Background and aims: Sitagliptin (SITA) is the first once-daily oral dipeptidyl peptidase-4 inhibitor and exenatide (EX) is the first twice-daily injectable glucagon-like peptide-1 receptor agonist available for the treatment of type 2 diabetes (T2DM). This study examined differences in baseline characteristics

between patients with T2DM initiating SITA vs. EX in real-world practice in the US, where SITA and EX are both indicated for use in dual and triple combination therapy with other oral antihyperglycemic agents (AHA) and SITA is also indicated as monotherapy.
Materials and methods: The General Electric Centricity electronic medical record database, covering 9 million U.S. patients of all ages from 49 states, was used to select patients with T2DM, aged ≥30 years, who received their first SITA or EX prescription between Oct 2006 and June 2008. Included patients were new to monotherapy, dual or triple combination therapy. Although EX is not indicated as monotherapy, real world EX monotherapy use was observed and included. Patient's medical records, including demographics, diagnoses, prescriptions, and lab results were extracted for the one year period (baseline) prior to the date of the first prescription of SITA or EX. Patient baseline profiles were stratified by mono-, dual, or triple therapy and compared between regimens with SITA vs. EX.
Results: Among patients new to AHA, 1091 initiated SITA and 759 initiated EX monotherapy. Compared to patients initiating EX, at baseline, patients on SITA were older (63.8 vs. 56.9 years), and had a lower obesity rate (61.5% vs. 87.6%), higher serum creatinine (106 μmol/L vs. 88 μmol/L), higher proportion of males (46.1% vs. 35.6%), and higher prevalence rates of macrovascular conditions (18.1% vs. 10%) (all p<0.0001). Among patients started on dual combination regimens, 2261 were prescribed SITA, and 1901 were prescribed EX. Similar to the pattern seen with initial monotherapy, patients on SITA at baseline were older, and had a lower obesity rate, higher serum creatinine, higher proportion of males, and higher rate of macrovascular conditions; additionally, patients started on SITA had a higher rate of renal insufficiency (based on diagnosis of renal disease or elevated serum creatinine level >132 μmol/L for male and >106 μmol/L for female) (all p<0.0001). Among those new to triple combination therapy, patients with SITA (n=3194) compared to EX (n=2661) at baseline were also older, and had a lower obesity rate, higher serum creatinine, higher proportion of males, and higher rates of macrovascular conditions (all p<0.0001). Observed patient profile differences were consistent across all the three regimen types.
Conclusion: This study demonstrates that patients with T2DM who received SITA compared to EX in a real-world setting were generally older, more likely to be male, had higher rates of preexisting macrovascular conditions and renal insufficiency and had a lower rate of obesity than patients who received EX. This has important implications for future observational studies using electronic medical record or claims databases, in that estimated outcome measures may be biased by the channeling of sicker, higher-risk patients to SITA.
Supported by: Merck & Co., Inc.

733

Investigation of features of type 2 diabetic patients with secondary failure to oral hypoglycaemic agents associated with blood glucose response to exenatide
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Background and aims: Exenatide is a GLP-1 receptor agonist that may improve insulin secretion in T2D patients. We investigated the features of T2D patients with secondary failure to oral hypoglycemic agents (OHA), either never treated by insulin or using insulin combined with OHA for less than 12 months, which were associated with blood glucose response to exenatide.
Materials and methods: Blood glucose response to exenatide was prospectively followed-up for 6 months in 21 T2D patients with secondary failure to OHA (A1c > 7.5 %). Group A (N or mean ± SEM) included 7 women/3 men; age: 55.8 ± 3.5 years, BMI: 33.1 ± 2.6 kg/m²; waist circumference (WC): 107.5 ± 8 cm; diabetes duration (DD): 14.8 ± 1.6 years; A1c: 9.97 ± 0.42 % who never used insulin. Group B included 6 women/5 men; age: 56 ± 2.3 years; BMI: 34.1 ± 1.7 kg/m²; WC: 119.2 ± 4.7 cm; DD: 18.2 ± 3.2 years; A1c: 9.14 ± 0.36 % who had been using insulin combined with OHA for less than 12 months at baseline. Before initiation of exenatide, blood glucose levels were brought close to normal by a 48-hour IV insulin infusion after withdrawal of SC insulin (if applicable) and OHA but metformin. Insulin sensitivity (S*i*) was assessed by the amount of insulin in IU/kg/day needed during the last 24 hours of IV insulin infusion to keep blood glucose in the normal range and insulin secretion was investigated by **Δ** plasma C peptide before and 6 min after 1 mg glucagon IV. Exenatide was then initiated at 5 μg bid for at least one month and increased to 10 μg bid according to blood glucose monitoring, while OHA were restored as before IV insulin. A1c was followed-up at 2, 4, and 6 months. Responders to exenatide were defined as patients whose A1c change was greater or equal to median A1c variation after 2 months (i. e. ≥ -1.5%). Statistical analysis was performed using ANOVA for repeated measurements.
Results: Baseline characteristics of patients were not significantly different in groups A and B, in terms of S*i* : 0.56 ± 0.05 vs. 0.50 ± 0.09 IU/kg/day; *p*=0.58, and **Δ** plasma C peptide:1.04 ± 0.25 vs. 0.6 ± 0.34 ng/ml; *p*=0.15, respectively. Maximal change in A1c levels was obtained between baseline and month 2, and remained stable for 6 months in both groups. Among patient features at baseline, only **Δ** plasma C peptide was significantly higher in responders to exenatide (R; n=9) than in non-responders (NR; n=12) as shown in the table below. Moreover, previous insulin use was associated with a significantly higher non-response rate to exenatide: 10/11 vs. 2/10 (Fisher exact test, *p*=0.0019).

	Responders	Non Responders	
Age (years)	55 ± 1.74	56.6 ± 3.4	*p*=0.71
Diabetes duration (years)	15.7 ± 2.14	17.3 ±2.83	*p*=0.68
Baseline A1c (%)	9.98 ± 0.53	9.26 ± 0.31	*p*=0.22
BMI (kg/m²)	32.3 ± 2.8	34.6 ± 1.5	*p*=0.46
Waist Circumference (cm)	106 ± 9	118 ± 4	*p*= 0.17
S*i* (IU/kg/day)	0.55 ± 0.7	0.51 ± 0.6	*p*=0.68
Δ plasma C peptide (ng/ml	1.16 ± 0.26	0.55 ± 0.12	*p*=0.04

Conclusion: From our data, remaining capacity of insulin secretion appears as a significant factor associated with response to exenatide on blood glucose control in T2D patients showing secondary failure to OHA. Previous insulin use is also associated with a poor response to exenatide therapy.

734

Liraglutide, a human GLP-1 analogue, maintains greater reductions in HbA_{1c}, FPG and weight than glimepiride over 2 years in patients with type 2 diabetes: LEAD-3 extension study
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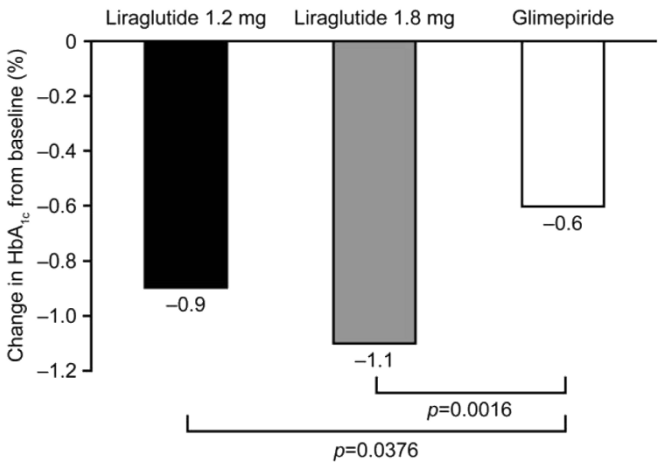
Background and aims: The human GLP-1 analogue liraglutide (1.8 and 1.2 mg QD) achieved greater reductions in HbA_{1c}, FPG, weight, hypoglycaemia and systolic blood pressure than glimepiride (8 mg QD) when used as monotherapy for one year in patients with type 2 diabetes in the randomised, double-blind, LEAD-3 study (n = 746). This open-label extension study analysed the continued efficacy and tolerability of liraglutide, as compared with glimepiride, during a further year of treatment (2 year extension).
Materials and methods: The extension period was an open-label continuation of the one-year, randomised, double-blind, parallel-group LEAD-3 study. A total of 90% (n=440) of one-year completers entered the extension study for a further one year, of whom 321 (73%) completed a further year of treatment (2-year completers: mean age = 54yrs, HbA_{1c} = 8.2%; median diabetes duration = 3.3yrs; BMI = 33kg/m²; diet/exercise only at LEAD 3 study entry = 36%; 1 OAD = 64%) Data presented are for 2-year completers.
Results: Compared with subjects taking glimepiride, after 2 years liraglutide (1.8 and 1.2mg) subjects experienced greater reductions in HbA1c (-1.1 and -0.9%, respectively, versus -0.6%; ANCOVA; p = 0.0016 and p = 0.0376, respectively) (Figure); had lower final HbA1c (6.9 ± 1.2 and 7.1 ± 1.2% versus 7.5 ± 1.2%); and a greater proportion reached HbA1c < 7.0% (58 and 53% vs. 37%; p = 0.0054 and p = 0.0269, respectively). Liraglutide also achieved greater reductions in FPG (1.5 and 1.3 versus 0.3 mmol/L , respectively). Weight loss at one year associated with liraglutide and weight gain observed with glimepiride were maintained (-2.7 and -2.1kg versus + 1.1kg; p < 0.0001), Hypoglycaemia (BG < 3.1 mmol/L) was reduced by at least 85% with the use of liraglutide 1.8 mg/day compared to glimepiride (0.28 and 0.16 vs. 1.82 events/subject/yr; p = 0.0001 and p < 0.0001). Differences in HbA1c between completers and the ITT (LOCF) population were comparable; ITT (LOCF) analyses also showed significantly greater reductions in FPG, weight and hypoglycaemia with liraglutide vs. glimepiride.
Conclusion: The clinically and statistically significant reductions in HbA_{1c}, FPG, weight and hypoglycaemia achieved after one year of liraglutide treatment are maintained after 2 years, showing benefit over glimepiride, with lower hypoglycaemic risk.



Data are for 2-year completers; n=321

Supported by: Novo Nordisk

735

Weekly, biweekly and monthly efficacy of albiglutide, a long-acting GLP-1-receptor agonist, in patients with type 2 diabetes receiving concomitant background metformin

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Background and aims: Albiglutide (ALB) consists of a DPP-4-resistant human GLP-1 dimer fused to human albumin resulting in a pharmacokinetic half-life of ~5 days that allows the potential for weekly or less-frequent dosing. This study was conducted to establish optimal ALB dose and schedule for Phase 3 trials and to assess glycemic responses and adverse events (AEs). Open-label exenatide (Ex) was used as a reference arm. The overall population is reported, and an exploratory analysis examined efficacy and tolerability of ALB in subjects previously treated with only metformin (MET), to compare treatment effects vs Ex used per label.
Materials and methods: In this randomized, multicenter, double-blind, parallel-group, Phase 2 study, 356 subjects (mean age 54 years, BMI 32.1 kg/m^2) with type 2 diabetes inadequately controlled (mean HbA_{1c} 8.0%) on diet/exercise or MET received subcutaneous placebo (PBO), ALB [weekly (4, 15 or 30 mg), biweekly (15, 30 or 50 mg) or monthly (50 or 100 mg)] or open-label Ex twice daily (on MET background, per label) for 16 weeks.
Results: Across ALB groups and PBO, 65.6-74.3% received background MET. After 16 weeks, background MET subjects experienced fasting plasma glucose (FPG) reductions of -1.26, -2.10, -1.80 and -0.07 mmol/L for the 30 mg weekly, 50 mg biweekly and 100 mg monthly doses of ALB and PBO, respectively, vs -1.44, -1.32, -1.22 and -0.10 mmol/L, respectively, for the overall population. Ex decreased FPG by -0.80 mmol/L.
In MET subjects, the highest ALB doses in each dose schedule significantly reduced HbA_{1c} similarly over 16 weeks: ALB 30 mg weekly, 0.78%; ALB 50 mg biweekly -0.83%; and ALB 100 mg monthly -0.77% vs PBO (-0.05%, $p < 0.05$); Ex reduced HbA_{1c} by -0.54%. In the overall population, HbA_{1c} was reduced -0.87, -0.79 and -0.87% by ALB 30 mg weekly, 50 mg biweekly and 100 mg monthly dosing, respectively, vs PBO (-0.17%, $p < 0.005$).
Among MET patients, HbA_{1c} <7% was achieved by 43%, 50% and 46% of subjects receiving ALB 30 mg weekly, 50 mg biweekly and 100 mg monthly, respectively, vs 15% with PBO and 35% in the Ex group. Weight loss with ALB was observed in both the MET group (-0.4 to -2.1 kg) and in the overall population (-0.9 to -1.8 kg). Documented hypoglycemia was not increased with ALB, and most commonly reported AEs were gastrointestinal events. Rates of nausea or vomiting in MET subjects were 18.2%, 47.8% and 56.5% of patients receiving 30 mg weekly, 50 mg biweekly and 100 mg monthly doses of ALB, respectively, vs 29.0%, 54.3% and 55.9% in the overall study population. Nausea or vomiting was experienced by 45.7% in the Ex group.
Conclusion: Albiglutide was effective in patients receiving background MET and provided numerically greater HbA_{1c} and FPG reductions than did exenatide. Tolerability was most favourable with the 30 mg weekly ALB group.
Supported by: GlaxoSmithKline

HbA_{1c} for Overall Population		ALB Weekly			ALB Biweekly			ALB Monthly	
	Ex n=34	4mg n=34	15mg n=34	30mg n=29	15mg n=30	30mg n=32	50mg n=34	50mg n=35	100mg n=33
Baseline HbA_{1c}	7.99	8.15	8.00	8.01	8.17	7.96	7.91	7.92	8.06
Week 16 Δ from baseline	-0.54	-0.11	-0.49	-0.87	-0.56	-0.79	-0.79	-0.55	-0.87
Week 16 Δ from PBO (treatment effect estimate; LS mean)	--	+0.20	-0.26	-0.62*	-0.22	-0.55*	-0.57*	-0.34	-0.60*
HbA_{1c} for Background MET Population		ALB Weekly			ALB Biweekly			ALB Monthly	
	Ex n=34	4mg n=22	15mg n=25	30mg n=21	15mg n=21	30mg n=21	50mg n=22	50mg n=24	100mg n=22
Baseline HbA_{1c}	7.99	8.26	8.01	8.06	8.04	8.01	7.98	7.99	8.10
Week 16 Δ from baseline	-0.54	-0.31	-0.38	-0.78	-0.43	-0.75	-0.83	-0.45	-0.77
Week 16 Δ from PBO (treatment effect estimate; LS mean)	-0.48*	-0.09	-0.23	-0.61*	-0.24	-0.61*	-0.70*	-0.32	-0.62*

*$p < 0.05$

736

Improvement in glycaemic control when adding liraglutide to existing therapy: results from a meta-analysis of six large randomised clinical trials

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Background and aims: In modern clinical practice, worsening glycaemic control associated with the progression of type 2 diabetes is usually addressed by adding additional drugs to existing therapy. Liraglutide is a once-daily human glucagon-like peptide-1 (GLP-1) analogue for the treatment of type 2 diabetes. Liraglutide was tested as monotherapy or in combination with various therapies, including lifestyle, in six randomised, controlled phase 3 trials in 4442 patients.
Materials and methods: Meta-analysis using logistic regression of the subset of patients who maintained their pre-trial regimen of oral antidiabetic drugs during these trials (n=1683) examined the efficacy of combining liraglutide with existing therapy. Only those patients who maintained their pre-trial regimen, and added liraglutide, were included. Existing therapy was diet and exercise in one trial, metformin in one trial, SU in one trial, metformin+SU in two trials and metformin+TZD in one trial. Impact on glycaemic control, body weight (BW) and systolic blood pressure (SBP) was assessed for liraglutide 1.8 mg, liraglutide 1.2 mg, and placebo.
Results: ANCOVA analysis of the improvements in HbA_{1c} from baseline for liraglutide 1.8 and 1.2 mg as add-on to prior treatment showed reductions of 1.5% and 1.3%, respectively ($p<0.0001$ for both). In the placebo group, HbA_{1c} was reduced from baseline by 0.3% ($p=0.0008$). As add-on therapy compared to placebo, liraglutide 1.8 mg and 1.2 mg reduced HbA_{1c} by 1.2% and 1.0%, respectively ($p<0.0001$ for both). The proportion of subjects (with 95% CIs) reaching target HbA_{1c}<7% with add-on therapy was 71% [65%; 75%] for liraglutide 1.8 mg; 59% [51%; 67%] for liraglutide 1.2 mg; and 18% [12%; 26%] for placebo. The odds-ratios of reaching target HbA_{1c} <7% with add-on therapy for liraglutide 1.2 mg vs placebo was 6.6 ($p<0.0001$), and for liraglutide 1.8 mg vs placebo was 11.1 ($p<0.0001$). The odds ratio of reaching target HbA_{1c} <7% for liraglutide 1.8 mg vs liraglutide 1.2 mg was 1.7 ($p=0.0202$). FPG was improved by 1.8 mmol/L with liraglutide 1.8 mg, and 1.7 mmol/L with liraglutide 1.2 mg, while an increase of 0.5 mmol/L was observed for placebo. Body weight was reduced by 2.2 kg for liraglutide 1.8 mg, 1.4 kg for liraglutide 1.2 mg, and 0.4 kg for placebo. SBP improved by 2.9 mmHg with liraglutide 1.8 mg, by 1.4 mmHg with liraglutide 1.2 mg, and by 0.4 mmHg with placebo.
Conclusion: When added to existing treatment as per clinical practice, greater improvement in glycaemic control was achieved for liraglutide than previously reported from individual trials where background medication was changed for many subjects.

Comparison of liraglutide with placebo, as add-on therapy (summary statistics)

		HbA_{1c} (%)	FPG (mmol/L)	BW (kg)	SBP (mmHg)
Liraglutide 1.8 mg	Baseline	8.3	9.6	90.0	132.0
	End of trial	7.0	7.7	88.0	129.0
	Change	-1.4	-1.8	-2.2	-2.9
Liraglutide 1.2 mg	Baseline	8.3	9.5	89.3	128.6
	End of trial	7.1	7.7	88.1	127.0
	Change	-1.3	-1.7	-1.4	-1.4
Placebo	Baseline	8.3	9.3	89.1	132.4
	End of trial	8.0	9.8	88.7	131.8
	Change	-0.3	0.5	-0.4	-0.4

Add-on group includes subjects on prior SU in LEAD 1, prior met in LEAD 2, prior diet/exercise in LEAD 3, prior met and TZD in LEAD 4, prior met and SU in LEAD 5, and all subjects in LEAD 6. Data are ITT LOCF.

Supported by: Novo Nordisk

PS 57 GLP-1 agonists - clinical 2

737

Liraglutide, a human GLP-1 analogue, lowers HbA_{1c} independent of weight loss

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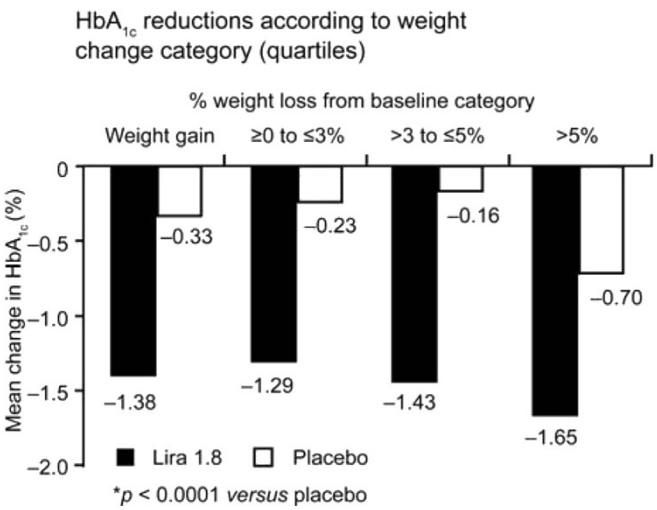
Background and aims: GLP-1 receptor agonists offer the potential to both improve glycaemic control and promote weight loss. Weight loss is known to improve glycaemic control in people with type 2 diabetes. The objective of this meta-analysis comparing reductions in HbA_{1c} with liraglutide 1.8 mg daily and placebo was to investigate whether improvements in HbA_{1c} induced by liraglutide were independent or dependent on concomitant weight loss.
Materials and methods: A meta-analysis of six phase 3 randomised controlled trials (RCT) including 2739 patients with type 2 diabetes compared HbA_{1c} reductions among patients in different categories of weight change from baseline (weight gain, weight loss ≥0 to ≤3%, weight loss >3 to ≤5%, and weight loss >5%), following treatment with liraglutide 1.8 mg once daily or placebo. HbA_{1c} reductions and weight changes were assessed at 26 weeks in all trials.
Results: Seventy-six per cent of patients treated with liraglutide lost weight from baseline compared with 60% in the placebo group. Greater than 5% weight loss occurred in 24% of patients in the liraglutide group and 10% of patients in the placebo group. Weight loss of >3% to ≤5% occurred in 17% and 13% of patients in the liraglutide and placebo groups, respectively. A similar percentage of patients in both groups had weight change from baseline of 0 to 3% (35% with liraglutide and 37% with placebo). Liraglutide induced statistically and clinically significant reductions in least-squared (LS)-mean HbA_{1c} from baseline of between 1.29% and 1.65% in all weight change groups compared with reductions of 0.16% to 0.70% in the placebo group ($p < 0.0001$) (Figure 1). The effect of liraglutide on change in HbA_{1c} was independent of weight loss category ($p = 0.71$).
Conclusion: A greater percentage of patients treated with liraglutide lost weight from baseline compared with placebo and almost a quarter of patients treated with liraglutide experienced >5% weight loss. Liraglutide treatment also induced significantly greater reductions in HbA_{1c} compared with placebo across all weight loss categories. HbA_{1c} change with liraglutide was independent of weight loss category.



Supported by: Novo Nordisk

738

Exenatide compared with long acting insulin to achieve glycaemic control with minimal weight gain in patients with type 2 diabetes mellitus: results of the Helping Evaluate Exenatide in patients with diabetes compared to Long-Acting insulin (HEELA) study

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Many add-on therapies for glycaemic control in patients with T2DM are associated with weight gain. The HEELA study was designed to examine whether the GLP-1 receptor agonist, exenatide, could provide better control of HbA1c and weight than basal insulin glargine for 26 weeks. Overweight patients with at least one other cardiovascular risk factor (previous cardiovascular event, peripheral vascular disease, abnormal lipids or blood pressure) and T2DM inadequately controlled with 2 or 3 OADs were randomised to add-on exenatide 10 µg b.i.d. (n=118) or insulin glargine o.d. (n=117; median final dose 34.0 IU/day). Patients had overall mean (SD) age 56.5 (9.1) years, HbA1c 8.57 (0.67)% and BMI 34.1 (5.3) kg/m^2 at baseline, and 58.5% were taking 2 OADs. The proportion of patients achieving HbA1c ≤7.4% with weight gain ≤1 kg was greater in the exenatide group (53.4%) than the insulin glargine group (19.8%, P<0.001). Improvements in LS mean [SEM] HbA1c following treatment with exenatide (-1.25 [0.09]%) and insulin glargine (-1.26 [0.09]%, P=0.924) were similar. In contrast, mean body weight decreased with exenatide but increased during insulin glargine treatment. The between-group difference in weight changes was significant after 4 weeks (P<0.001) and was maintained to 26 weeks (-5.71 [0.44] kg, 95%CI: -6.58 to -4.84 kg, P<0.001). Treatments did not differ in overall or severe hypoglycaemia, but nocturnal hypoglycaemia incidence was lower with exenatide. In overweight patients with T2DM and cardiovascular risk factors, additional exenatide treatment resulted in significantly more patients achieving better glycaemic control with minimal weight gain compared with insulin glargine.

Supported by: Eli Lilly & Co. Ltd.

739

DURATION-2: exenatide once weekly demonstrated superior glycaemic control and weight reduction compared to sitagliptin or pioglitazone after 26 weeks of treatment

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Background and aims: The once weekly GLP-1 receptor agonist exenatide (EQW) has demonstrated sustained improvements in glycaemic control and body weight through 2 years of treatment.
Materials and methods: DURATION-2, a randomised, double-blind, double-dummy, 26-week study, compared the efficacy, safety, and tolerability of weekly treatment with EQW (2 mg; n=160) to maximum daily doses of a DPP-4 inhibitor (sitagliptin 100 mg; n=166) or a TZD (pioglitazone 45 mg; n=165) in patients with type 2 diabetes on a stable metformin background (ITT N=491; A1C 8.5±1.1%, FPG 9.1±2.6 mmol/L, weight 88.0±20.1 kg).
Results: EQW produced a clinically and statistically superior reduction in HbA_{1c} compared to both sitagliptin and pioglitazone that was first evident at Week 6. A significantly greater proportion of EQW patients achieved HbA_{1c} targets of ≤7.0% and ≤6.5%. EQW resulted in significantly greater weight loss versus sitagliptin from Week 4 through Week 26; patients on pioglitazone gained weight (+5.1 kg weight difference vs. EQW at Week 26). Significant improvements from baseline in systolic BP, albumin:creatinine, hsCRP, BNP, and adiponectin were observed with EQW; improvements in hsCRP and adiponectin also occurred with sitagliptin and pioglitazone. All treatments were generally well tolerated, with over 80% of patients completing the trial. Nausea was transient and predominantly mild (24% EQW; 10% sitagliptin; 5% pioglitazone; 1 withdrawal due to nausea in each treatment arm). No major hypoglycaemia was observed. Pancreatitis, including one case of necrotising pancreatitis, occurred in 2 patients treated with pioglitazone; no EQW patients experienced pancreatitis.
Conclusion: Once weekly treatment with exenatide was well tolerated and elicited superior improvements in glucose control and body weight compared to maximum daily doses of sitagliptin and pioglitazone on a background of metformin.

Week 26	Exenatide QW	Sitagliptin	Pioglitazone
Δ HbA_{1c} (%)	-1.55±0.10	-0.92±0.10*	-1.23±0.10*
% to HbA_{1c} ≤7.0 / ≤6.5%	66 / 43	42*/ 18*	56*/ 33*
Δ FPG (mmol/L)	-1.8±0.2	-0.9±0.2*	-1.5±0.2
Δ Body Weight (%)	-2.7±0.4	-0.9±0.4*	+3.2±0.4*

ITT LS mean±SE for A1C, FPG, weight; Evaluable (n=387) for % to HbA_{1c} target. *P<0.05 vs. EQW.

740

Can the glycaemic effect of incretin mimetics be predicted prior to therapeutic application?

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Background and aims: Incretin mimetics are a new class of antihyperglycaemic agents that have the possibility to improve glycaemic control similar to natural incretin hormones. Exenatide, the first incretin mimetic on the market, mediates the re-establishment of glucose-dependent insulin secretion, suppression of elevated postprandial glucagon secretion, and slowing of gastric emptying. Initial application of Exenatide has also demonstrated that some patients meet the expected effects on glycaemic control but others failed. At present, no pre-clinical method is available to predict low or high Exenatide responders. It was therefore the aim of this project to develop and to verify a method allowing identification of low or high responders prior to the therapeutic application of incretin mimetics.
Materials and methods: The Karlsburg Diabetes-Management System KADIS® was used to develop an *in silico* method for outcome prediction of therapeutic application of the incretin mimetic Exenatide. For this purpose, KADIS® was adapted to the special requirements needed to meet the aim of this study. The modified KADIS®-supported program comprises: (1) continuous glucose monitoring (CGM) which was performed first in each study patient; (2) KADIS®-based identification of the current individual metabolic situation of each patient; (3) *in silico* testing of the metabolic effect of the application of 20µg Exenatide on the 24-h glycaemic pattern; (4) estimation of an Exenatide equivalence factor by replacing *in silico* Exenatide by a long acting insulin and titration of the insulin dose required to achieve the same blood glucose lowering effect as Exenatide; (5) relating the patients to high or low responders according to the estimated Exenatide equivalence factor of insulin action. To verify the proposed procedure, 58 non-insulin treated type 2 diabetic patients were involved into the study.
Results: The overall glycaemic lowering effect of 20µg Exenatide was estimated to be 0.81± 0.50 mmol/l and equals an insulin dose of 12.6 ±4.8 IU. 41% of the study patients could be identified to be high responders (equivalence factor of insulin action >12.6 IU) to an Exenatide therapy and 59% were low responders (equivalence factor of insulin action <12.6 IU). In the group of high responders the mean daily glucose pattern (MBG) dropped down by 1.15 ±0.57 mmol/l which equals an insulin dose of 17.2 ±3.2 IU, whereas in the group of low responders the MBG dropped down by 0.57 ±0.23 mmol/l which equals an insulin effect of 9.3 ±2.6 IU. In the group of high responders to an Exenatide therapy a higher BMI (31.4 vs. 28.5 kg/m^2), an enhanced endogenous insulin supply (62.2 vs. 49.7 IU/day), and a higher HbA1c (6.7 vs. 6.2%) was observed at baseline.
Conclusion: The metabolic effect of an Exenatide therapy can be predicted prior to therapeutic application by using CGM in combination with KADIS®-based *in silico* simulation strategy.

741

Association of British Clinical Diabetologists (ABCD) nationwide exenatide audit

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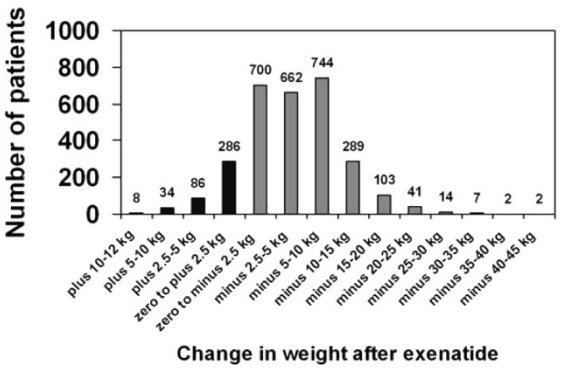
Background and aims: In December 2008, 18 months after the launch of exenatide in the UK, ABCD launched a 9 month project to accelerate understanding of the new agent, through a nationwide audit of its use in real clinical practise.
Materials and methods: A password protected on-line questionnaire for collection of anonymised patient data. Diabetes specialists in the UK were given persistent email encouragement to submit their data.
Results: There has been a dramatic response such that already we have data promised on 7559, data submitted on 5313 and data available for analysis on 3913 patients (mean (+/- SD) age 54.6 (+/-10.4) years, 2167/3913 (55.4%) male), with all these numbers rising relentlessly. First analysis of the data thus far showed that in response to exenatide mean (+/- SD) HbA1c, weight and body mass index fell as follows: HbA1c by 0.75 from 9.42 (+/- 1.19) to 8.65 (+/- 1.22)% ($p<10^{-126}$), weight by 4.9 from 114 (+/- 23.3) to 109.1 (+/- 22.6) kg ($p<10^{-15}$), BMI by 1.74 from 39.89 (+/- 7.5) to 38.15 (+/-7.24) kg/m^2 ($p<10^{-16}$). The weight reduction was variable with some patients showing dramatic response (see figure). A similar wide variability in HbA1c response was observed. Factors accounting for variability in weight response included: patients with osmotic symptoms who increased weight as exenatide controlled glycaemia; patients in whom discontinuation or reduction of insulin, glitazones and/or sulphonylureas contributed to weight loss. Factors accounting for variability in HbA1c response included: patients in whom discontinuation or reduction of other antidiabetic drugs led to worsening glycaemic control; patients with dramatic weight loss and concurrent dramatic reduction in HbA1c. Other reported benefits included return of ovulation in polycystic ovary syndrome and improvement in obstructive sleep apnoea. Gastrointestinal side effects were reported in 1122/3913 (28.7%) (transient in 773/3913 (19.7%), stopped exenatide temporarily in 67/3913 (1.7%), stopped exenatide permanently in 282/3913 (7.2%)). 1763/3913 (45%) patients were on insulin prior to exenatide. Hypoglycaemia rate increased from 104/3913 (2.7%) prior to 177/3913 (4.5%) after exenatide. 7/3913 (0.18%) cases of pancreatitis were reported.
Conclusion: The ABCD nationwide exenatide audit has already generated a very substantial clinical database which is expanding rapidly. First analysis has revealed responders and non responders and that the responses are very varied but sometimes dramatic. The 9 month project is scheduled to finish by September 2009. Further detailed analysis of the expanding database will be undertaken to define the factors associated with different degrees of response, and the extent to which factors such as change in medication, initial weight, BMI and HbA1c, and duration of diabetes contribute to response. Detailed analsis of the response against time and cases of pancreatitis will also be performed.

Difference between last weight after exenatide and weight before exenatide in 2977 patients



Supported by: Eli Lilly to facilitate staff and IT

742

A phase I/II study of the safety, pharmacokinetics and pharmacodynamics of albiglutide in Japanese subjects with type 2 diabetes

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Background and aims: Albiglutide is a long-acting GLP-1-receptor agonist shown to improve indices of glycemia in white and Hispanic populations when dosed weekly, once every other week (biweekly) and monthly. The aim of this study was to assess the pharmacokinetics, pharmacodynamics, and safety and tolerability of albiglutide in Japanese subjects with type 2 diabetes.
Materials and methods: A 28-day, single-blind, randomized, placebo-controlled study was conducted. A total of 40 subjects (mean age 54.5 years, BMI 24.5 kg/m^2, HbA_{1c} range 6.3-10.3%) were given (subcutaneous, to the abdomen) placebo or albiglutide in the following doses: 15 mg or 30 mg once weekly; 50 mg biweekly; or 100 mg monthly.
Results: Albiglutide had a plasma half life of 5.3 d, systemic clearance (CL/F) of 68.7 mL/hr and volume of distribution (V/F) of 12.6 L. Albiglutide was generally well-tolerated; GI events were comparable with placebo in all doses except 100 mg monthly and were numerically lowest in the 30 mg weekly group. In the 100 mg monthly group, the most common adverse events (AEs) were flatulence (n=3, 38%), vomiting (n=3, 38%) and nausea (n=2, 25%). No serious AEs were reported. Fasting plasma glucose (FPG) and weighted mean glucose AUC_{0-4} were improved as early as day 3. At day 29, all doses of albiglutide except 100 mg monthly significantly reduced FPG and weighted mean glucose AUC_{0-4} from baseline, compared with placebo; all doses significantly reduced HbA_{1c}. At the day 43 follow-up visit, albiglutide also significantly reduced FPG (all doses except 100 mg monthly) and HbA_{1c} (all doses) compared with placebo.

	Albiglutide Dose			
Placebo-adjusted change from baseline (LS Means Difference)	15mg Weekly (n=8)	30mg Weekly (n=8)	50mg Biweekly (n=8)	100mg Monthly (n=8)
FPG (day 3), mmol/L	-0.85	-0.98*	-1.33*	-0.97
FPG (day 29), mmol/L	-1.91*	-1.98*	-1.74*	-0.73
FPG (day 43), mmol/L	-1.43*	-1.16*	-1.18*	-0.29
AUC_{0-4} glucose, mmol/L (day 29)	-2.86*	-3.58*	-2.51*	-1.44
HbA_{1c}, % (day 29/43)	-0.58*/ -0.87*	-0.57*/ -0.78*	-0.63*/ -0.79*	-0.51*/ -0.59*

*p < 0.05 vs placebo

Conclusion: Weekly or biweekly doses of albiglutide significantly improved glycemic control, with a favorable safety and tolerability profile, in Japanese subjects with type 2 diabetes.
Supported by: GlaxoSmithKline

743

Attaining a clinically relevant endpoint of HbA_{1c}<7.0%, no weight gain and no hypoglycaemia with liraglutide as compared to other therapies in type 2 diabetes mellitus: meta-analysis of the LEAD studies

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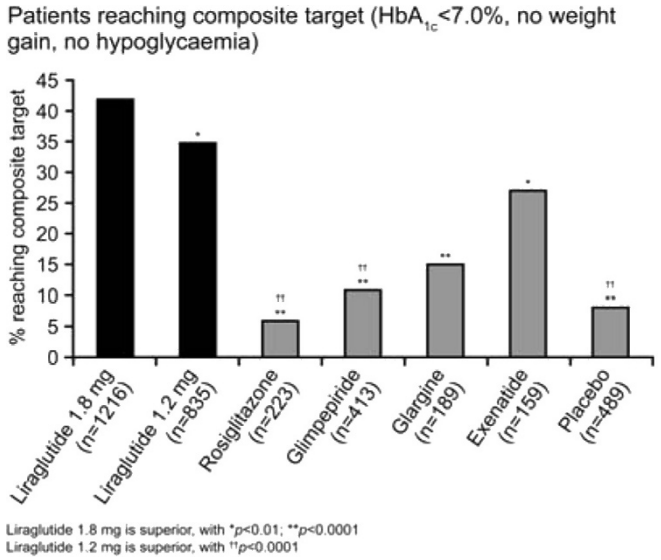
Background and aims: Therapies for type 2 diabetes mellitus (T2DM) commonly are associated with undesirable hypoglycaemia and weight gain, limiting success in achieving HbA_{1c} clinical practice recommendations. In this context we conducted a post-hoc meta-analysis of 6 large randomised controlled trials of liraglutide, a human GLP-1 receptor agonist, versus other diabetes therapies to determine the proportions of subjects achieving a composite endpoint: HbA_{1c} <7.0% with no major or minor hypoglycaemia and no weight gain.
Materials and methods: The meta-analysis was performed on 26-week data from the six LEAD (Liraglutide Effect and Action in Diabetes) trials

(n=3967), which compared liraglutide as follows: liraglutide vs glimepiride (GLM) as monotherapy; liraglutide +metformin (MET) vs GLM+MET; liraglutide + GLM vs rosiglitazone (ROS)+GLM; liraglutide +ROS + MET vs placebo + ROS + MET; liraglutide + MET + GLM vs insulin glargine + MET + GLM; liraglutide + MET and/or GLM vs exenatide + MET and/or GLM. Analyses were ITT and LOCF, and results were adjusted for baseline HbA1c and weight.

Results: At 26 weeks, the composite target was achieved by 42% of the liraglutide 1.8 mg group, 35% of the liraglutide 1.2 mg and 6-27% of those treated with comparators (Figure). Odds ratios (p values) for reaching the composite endpoint with liraglutide 1.8 mg vs comparators were as follows: vs liraglutide 1.2 mg, 1.38 (0.0047); ROS, 11.15 (<0.0001); GLM, 6.05 (<0.0001); glargine, 4.22 (<0.0001); exenatide, 1.96 (0.0067); and placebo, 7.87 (<0.0001). For liraglutide 1.2 mg, odds ratios (p values) for reaching the composite endpoint were as follows vs competitors: vs ROS, 8.06 (<0.0001); GLM, 4.37 (<0.0001); and placebo, 5.68 (p<0.0001).

Conclusion: The composite endpoint reflects three clinically relevant outcomes in T2DM (HbA1c, weight control and hypoglycaemia). The odds of reaching this composite endpoint were significantly increased with the GLP-1 receptor agonist liraglutide. These observations provide clinically relevant information for selecting pharmacotherapies for T2DM.



Supported by: Novo Nordisk

744

The human GLP-1 analogue liraglutide given once daily provides excellent metabolic control in Japanese patients either as monotherapy or in combination with SU during 52 weeks of treatment

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Background and aims: Liraglutide is a once daily human GLP-1 analogue. To investigate its safety and efficacy in Japanese subjects with type 2 diabetes, two large phase 3 trials were performed.

Materials and methods: The first trial (trial A) compared liraglutide 0.9 mg (n=268) vs. glibenclamide 1.25-2.5 mg (n=139), both as monotherapy. The second trial (trial B) compared liraglutide 0.6 mg or 0.9 mg in combination with sulphonylurea (SU) vs SU monotherapy (n=88 all groups). The trials consisted of a 24-week double blind part followed by a 28-week unblinded part.

Results: In trial A HbA_{1c} decreased from baseline by 1.48±1.12% in the liraglutide group vs. 0.95±1.06% in the glibenclamide group, the difference being -0.49% [-0.71, -0.27] after 52 weeks of treatment. The percentage of patients reaching HbA_{1c} <7.0% was significantly greater for liraglutide (36.9%) compared to glibenclamide (18.2%). In trial B the corresponding decrease from baseline was 1.09±0.84 (liraglutide 0.6 mg); 1.30±0.91 (liraglutide 0.9 mg) and 0.06±1.29 (SU); the difference (liraglutide vs SU) being -0.96% [-1.25, -0.67] and -1.33% [-1.62, -1.04], respectively. More patients reached HbA_{1C} <7.0% with liraglutide (0.6 mg: 46.5%, 0.9 mg: 70.5%) vs SU (14.8%) Fasting plasma glucose (PG) and 7-point profiles showed that mean PG and mean PG increments were lower with liraglutide in both trials. Beta-cell function measured as pro-insulin/insulin and pro-insulin/C-peptide was improved in both trials for patients treated with liraglutide. The liraglutide safety profiles in the two trials did not raise any concerns. In both trials more subjects reported AEs from the GI tract during the first 4 weeks of the trials; however after 4 weeks the GI-related AEs were at comparator level. No major hypoglycaemic events were reported in the two trials. In trial A patients on liraglutide experienced 0.7 hypoglycaemic episodes per patient year, vs 3.8 in the glibenclamide group. In combination with SU (trial B) the rates were similar in the groups (3.1 vs 3.7 vs 3.0 episodes/patient year); however in the period from 24 to 52 weeks the rates in the liraglutide groups were lower than those in the SU group. Despite markedly improved glycaemic control, liraglutide in both trials was weight-neutral. Approximately 15% of patients treated with liraglutide developed liraglutide antibodies in the two trials. Antibodies did not have an influence on HbA_{1c} and the mean decrease in HbA_{1c} in both trials was similar in patients with antibodies as in the entire group.

Conclusion: Once-daily administration of liraglutide as monotherapy provided safe and sustained metabolic control superior to glibenclamide without causing weight gain and low frequency of hypoglycaemia. Addition of liraglutide in people poorly controlled with SU further improves and sustains glycaemic control in a dose-dependent manner. Both trials demonstrate improved beta-cell function with liraglutide and significant long-term clinical benefit in typical Japanese patients with type 2 diabetes.

Supported by: Novo Nordisk

745

DURATION 2: weight-related quality of life, psychological well-being, and satisfaction with exenatide once weekly compared to sitagliptin or piaglitazone after 26 weeks of treatment

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Background and aims: In this 26-week randomised, double-blind, double-dummy study, patients (ITT N=491) with type 2 diabetes on a stable metformin background received once weekly exenatide (exenatide QW 2 mg; n=160), or maximum daily doses of sitagliptin (100 mg; n=166) or pioglitazone (45 mg; n=165). Weight-related quality of life (QOL), psychological general well being, diabetes treatment satisfaction, and general health status were assessed using the Impact of Weight on Quality of Life Questionnaire-Lite (IWQOL), Psychological General Well-being (PGWB) index, Diabetes Treatment Satisfaction (DTSQ), and EQ-5D at Baseline (BL) and Week 26.

Materials and methods: The ANCOVA model was used to estimate least squares (LS) mean group changes in IWQOL, PGWB, DTSQ, or EQ-5D from BL to Week 26 and compare the treatment effects between groups. The model included factors for treatment group, country, HbA_{1c} stratum (<9% vs. ≥9%), and baseline score for IWQOL, PGWB, DTSQ, or EQ-5D as a covariate.

Results: Exenatide QW produced a statistically superior reduction (%±SE) in HbA_{1c} (-1.55±0.10) versus sitagliptin (-0.92±0.10) and pioglitazone (-1.23±0.10). Exenatide QW resulted in significantly greater weight loss (-2.7%±0.4) versus sitagliptin (-0.9%±0.4) and pioglitazone (+3.2%±0.4). Exenatide QW and sitagliptin groups experienced significant improvement in all five domains of weight-related QOL (physical function, self-esteem, sexual life, public distress, work) and IWQOL total score, with no significant differences between exenatide QW and sitagliptin. The pioglitazone group significantly improved only on self-esteem. The exenatide QW group experienced significantly greater improvement in physical function, public distress, work, and total IWQOL score versus pioglitazone. All groups experienced significant improvements in all six domains of the PGWB (anxiety, depressed mood, positive well-being, self-control, general health, and vitality) and on the PGWB global scale, as well as in overall treatment satisfaction. The exenatide QW group experienced significantly greater improvement in overall treatment satisfaction versus sitagliptin. Patients in the exenatide QW and sitagliptin groups experienced significant improvements in overall health status.

Conclusion: Exenatide QW resulted in superior improvements in glucose control and body weight compared to sitagliptin and pioglitazone on a background of metformin. Patients on exenatide QW and sitagliptin experienced significant improvement on all domains of weight-related QOL. Significant improvements in weight-related QOL were observed for exenatide QW versus pioglitazone, consistent with differences in body weight change. All

groups experienced significant improvement in all domains of psychological general well-being and overall treatment satisfaction; greater improvement in overall treatment satisfaction was observed with exenatide QW versus sitagliptin.

Parameter	Exenatide QW	Sitagliptin	Pioglitazone
Baseline IWQOL total score	80.67 ± 1.51	80.74 ± 1.49	79.32 ± 1.50
Δ IWQOL total score	5.15 ± 1.04*	4.56 ± 1.02*	1.20 ± 1.06†
Δ Physical function	6.78 ± 1.35*	5.81 ± 1.33*	2.00 ± 1.38†
Δ Self-esteem	5.88 ± 1.39*	5.79 ± 1.36*	3.11 ± 1.41*
Δ Sexual life	5.80 ± 1.61*	5.02 ± 1.61*	2.41 ± 1.63
Δ Public distress	3.86 ± 1.17*	2.40 ± 1.14*	-0.63 ± 1.18†
Δ Work	2.79 ± 1.28*	3.02 ± 1.25*	-1.28 ± 1.29†
Baseline PGWB global score	67.54 ± 1.38	69.96 ± 1.30	71.60 ± 1.16
Δ PGWB global score	6.82 ± 1.00*	6.97 ± 0.98*	4.78 ± 1.02*

ITT LS mean change ± SE; positive scores indicate improvement on all domains and items of the PGWB and IWQOL.
*P<0.05 for Baseline to Week 26.
†P<0.05 vs. Exenatide QW.

746

Glycaemic outcomes following initiation of insulin glargine or exenatide in type 2 diabetes patients treated with oral agents in US

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Background and aims: Randomized controlled trials can demonstrate the safety and efficacy of glucose-lowering agents in specific populations under well-defined circumstances for regulatory purposes, but assessing these agents in general clinical practice remains challenging.
Materials and methods: We analyzed US managed care databases of 47 health plans from May 2005 to September 2007 to evaluate therapeutic outcomes after initiating insulin glargine (GLAR, n=15,695) or exenatide (EX, n=21,516) in patients previously treated with only oral antidiabetic drugs (OADs).
Results: Baseline characteristics (6 months prior to study initiation) for GLAR vs EX, respectively, were as follows: mean age, 54 vs 53 years; female, 43% vs 54%; glycated hemoglobin A1C (A1C), 9.5% vs 7.9%; and rate of hypoglycemia on OAD only, 4.8% vs 2.7%. Of note, 16% on GLAR vs 31% on EX visited an endocrinologist at baseline. The unadjusted change in A1C 1 year after initiation was -1.22% for GLAR vs -0.41% for EX (P<0.001). The medication discontinuation rate at any time following initiation was 37% for GLAR and 50% for EX (P<0.001). One year after initiation, 11% of EX patients started insulin and 5% of GLAR patients started EX. In patients without (n=35,877) and with (n=1,334) hypoglycemia at baseline, respectively, the 1-year incidence of hypoglycemia was 6% vs 3% (odds ratio [OR] = 1.97, P<0.001) and 35% vs 43% (OR=0.71, P=0.003) for GLAR vs EX. The 1-year adjusted A1C was 7.2% vs 7.8% (Δ=0.6%, P<0.001) for GLAR (n=2,117) vs EX (n=3,450), after multivariable adjustment for age, sex, baseline A1C, hypoglycemia, hospitalization, Charlson comorbidity score, statin use, concomitant medications and timing of A1C measurement. The Figure shows increased glycemic benefits with higher baseline A1C in patients initiated GLAR instead of EX. Specifically, the higher the baseline A1C the greater the difference in adjusted A1C between the groups during the period of 1 year follow-up.
Conclusion: Although a seemingly apparent choice of therapy as a function of baseline A1C level, this study suggests that initiation of GLAR relative to EX was associated with a lower and better adjusted 1-year A1C after controlling for the baseline A1C and additional confounding factors. However, these results should be interpreted with caution, as GLAR or EX appeared to be used in different populations.

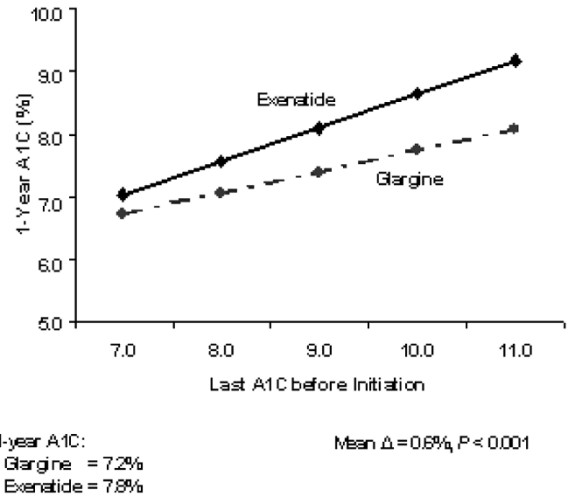

Figure 1: Adjusted 1-year A1C after Initiation of Glargine or Exenatide

Editorial support provided through the sanofi-aventis US Group

PS 58 DPP-IV inhibition - clinical

747

Initial combination therapy with sitagliptin and pioglitazone improves glycaemic control and measures of beta cell function compared with pioglitazone alone in patients with type 2 diabetes

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Background and aims: Initial combination therapy with sitagliptin (SITA) + pioglitazone (PIO) was compared with PIO alone in drug-naïve patients (pts) (HbA1c 8-12%) with type 2 diabetes.
Materials and methods: After a 2-week single-blind placebo run-in, 520 pts (mean baseline HbA1c=9.5%) were randomized (1:1) to SITA 100 mg q.d.+ PIO 30 mg q.d. or PIO 30 mg q.d. monotherapy for 24 weeks.
Results: Initial treatment with SITA+PIO led to a reduction from baseline in HbA1c of -2.4% compared with -1.5% with PIO monotherapy (between-group difference for SITA+PIO vs. PIO -0.9%;p<0.001). A greater HbA1c reduction was observed in pts with a baseline HbA1c ≥10% (change from baseline with SITA+PIO -3.0% vs. -2.1% with PIO). Significantly more pts in the SITA+PIO group vs. PIO group had an HbA1c of <7% at Wk 24 (60% vs. 28%, respectively;p<0.001). Fasting plasma glucose was reduced by -63.0 mg/dL with SITA+PIO compared to -40.2 mg/dL with PIO monotherapy (between-group difference SITA+PIO vs. PIO -22.8 mg/dL;p<0.001), and 2-hr post meal glucose was reduced by -113.6 mg/dL with SITA+PIO compared to -68.9 mg/dL with PIO (between-group difference SITA+PIO vs. PIO -44.7 mg/dL;p<0.001). Regarding measures related to β-cell function, the insulinogenic index and the postprandial proinsulin/insulin ratio were significantly improved with SITA+PIO vs. PIO (p≤0.001). Additionally, in response to a meal challenge, the areas under the curves for glucose, insulin, and C-peptide were significantly improved with SITA+PIO vs. PIO monotherapy (p<0.05 for all parameters). SITA+PIO was generally well tolerated compared with PIO monotherapy, with similar incidences of hypoglycemia (1.1% vs. 0.8%, respectively), gastrointestinal adverse events (5.7% vs. 6.9%, respectively), and edema (2.7% vs. 3.5%, respectively).
Conclusion: In summary, initial combination therapy with sitagliptin and pioglitazone substantially improved glycemic control and was generally well tolerated compared with pioglitazone monotherapy.

748

Twelve weeks treatment with the DPP-4 inhibitor sitagliptin improves glycaemic control, but does not improve GLP-1 secretion, in patients with type 2 diabetes - a randomised trial

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Background and aims: Subjects with type 2 diabetes (T2DM) have been shown to display reduced postprandial secretion of glucagon-like peptide 1 (GLP-1), possibly as a consequence of the diabetic state. We examined whether 12 weeks treatment with the dipeptidyl peptidase-4 (DPP-4) inhibitor sitagliptin would improve glycemic control and increase the postprandial response of total GLP-1. Furthermore, intact GLP-1 and total and intact glucose-dependent insulinotropic polypeptide (GIP) were examined.
Materials and methods: A double-blinded, placebo-controlled study was performed over 12 weeks, in which 24 patients with T2DM were randomized to receive either sitagliptin (Januvia®) 100 mg qd or placebo as add-on therapy to ongoing treatment with metformin (≥1000mg). In week 0, 1, and 12, subjects were examined with a 240 min standardized meal test (2,370 KJ). HbA_{1c} was measured before and after the treatment period. Intact and total (the intact hormone and the metabolite) GLP-1 and GIP responses were calculated as the area under the meal test response curves (AUC).
Results: 22 subjects (sitagliptin n=12, mean age 60 years, mean HbA_{1c} 8.3%; placebo n=10, mean age 61 years, mean HbA_{1c} 7.6%) were included in the post-study analysis. Two subjects originally randomised to placebo were excluded due to a deliberate 12% body weight loss. From week 0 to 12, sitagliptin induced a placebo-corrected reduction in HbA_{1c} of 0.9% (p=0.005). From week 0 to week 1, sitagliptin increased the levels of intact GIP 2-fold (p=0.002) and intact GLP-1 3-fold (p=0.003). In week 12, levels of intact GIP remained elevated, whereas intact GLP-1 did not (p=0.6, week 0 *vs* week 12). Sitagliptin had no effect on the levels of total GLP-1 (p=0.2) or total GIP (p=0.3) from week 0 to 12. The increases in intact GLP-1 and GIP in week 1 were significantly different from the changes in the placebo group (both p<0.001). There were no significant changes in the placebo group.
Conclusion: 12 weeks treatment with sitagliptin in subjects with T2DM significantly improved glycemic control. However, this improvement did not increase the postprandial response of total GLP-1. Sitagliptin efficacy was retained despite the fact that the plasma level of intact GLP-1 was not increased in week 12. This would suggest that factors in addition to an increase in the peripheral concentration of intact GLP-1 may contribute to the therapeutic efficacy of DPP-4 inhibitor treatment.
Supported by: Merck and Co, Inc.

749

Initial therapy with combination alogliptin plus pioglitazone in type 2 diabetes patients inadequately controlled with diet and exercise

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Background and aims: Initial combination therapy is an emerging strategy to address the multifactorial nature of T2DM from the outset. This study investigated the efficacy and tolerability of initial combination therapy with the DPP-4 inhibitor alogliptin (ALO), which improves islet cell function, and the TZD pioglitazone (PIO), which reduces insulin resistance.
Materials and methods: This randomized, double-blind study compared the effects of two doses of ALO in combination with PIO vs ALO alone and PIO alone in T2DM inadequately controlled with diet and exercise (A1C 7.5%-11%). Subjects were randomized (1:1:1:1) to ALO 25 mg/d (n=164), PIO 30 mg/d (n=163), ALO 12.5 + PIO 30 mg/d (n=164) and ALO 25 + PIO 30 mg/d (n=164). Sequential efficacy analysis on the intent-to-treat population using ANCOVA compared first ALO 25 + PIO 30 vs ALO and PIO alone and, if significant, then ALO 12.5 + PIO vs PIO alone.
Results: Mean baseline characteristics (N=655; age 53 yrs; diabetes duration 3.2 yrs; A1C 8.8%; fasting plasma glucose (FPG) 191 mg/dL; BMI 31 kg/m^2) were similar between groups. Greater improvements in the least-squares (LS) mean change from a baseline A1C 8.8% were observed in the ALO 25 + PIO (-1.71%) vs either ALO (-0.96%) or PIO (-1.15%) (P<0.001) and for ALO 12.5 + PIO (-1.56%) vs PIO (P<0.001). The decreases in A1C for both combination treatment groups vs PIO monotherapy reached statistical significance at week 4 and remained through week 26. Percentages of subjects with A1C ≤7% at study end were: 24, 34, 53 and 63% for ALO, PIO, ALO 12.5 + PIO and ALO 25 + PIO; respectively. The LS mean FPG change from baseline at Week 26 was -25.8, -37.3, -48.5, and -50.2mg/dL, for ALO, PIO, ALO 12.5 + PIO, and ALO 25 + PIO, respectively. Weight changes were -0.3kg, +2.2kg, +2.5kg and +3.1kg, respectively; edema was not increased in the ALO + PIO groups (1.2- 2.4%) compared to PIO alone (5.5%). Hypoglycemia was rare, occurring in 0.6%, 1.8%, 1.8%, and 3.0% of subjects in the four groups, respectively.
Conclusion: In conclusion, this study demonstrates the potential for initial combination therapy with ALO + PIO to significantly improve glycemic parameters beyond either monotherapy component.
Supported by: Takeda Global Research & Development Center, Inc.

750

Vildagliptin added to basal insulin glargine improves glycaemic control and reduces glycaemic excursions in type 2 diabetes

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Background and aims: The majority of patients with Type 2 diabetes who fail to achieve adequate glycaemic control on once daily basal insulin tend to be given the option of trying twice daily mix insulins or other more inten-

sive insulin regimes. The introduction of the new class of oral hypoglycaemic agents, dipeptidyl peptidase-IV inhibitors (DPP-IV inhibitors), provides clinicians with more choices to treat patients with Type 2 diabetes, particularly in those who are obese. The aim of our audit was to evaluate the efficacy and safety of adding vildagliptin 50mg bd to patients inadequately controlled (HbA1c >8.0%) on once daily insulin glargine.
Materials and Methods: We observed 14 patients with type 2 diabetes, who were on at least 25 units of insulin glargine per day and who took at least 1g/day of metformin. Hypoglycaemic events (capillary blood glucose < 4 mmol l^{-1} and/or symptoms) were recorded 4 weeks prior to the initiation of vildagliptin. Before the addition of vildagliptin, all patients underwent 48 hours of continuous subcutaneous glucose monitoring (CSGM). Two days after the initiation of vildagliptin, patients underwent a further 48 hours of CGSM. Weight and HbA1c were recorded prior to treatment and repeated again 8 weeks after initiation of vildagliptin.
Results: Mean HbA1c prior to and 8 weeks after initiation of vildagliptin was 8.4% and 7.3% respectively. Fasting glucose reduced by 11% and post-prandial glucose reduced by 29%. The total of hypoglycaemic events was 6 in the previous 8 weeks prior to the addition of vildagliptin and a total of 7 events for 8 weeks on vildagliptin. Mean weight before and 8 weeks after initiation of vildagliptin was 83.7kg and 83.3kg respectively.
Discussion: We have demonstrated that the addition of vildagliptin to insulin glargine improves HbA1c and does not induce any significant increase in hypoglycaemia. More importantly it did not induce weight gain associated with increasing insulin dosage. The addition of vildagliptin to basal insulin therapy is an attractive option to current treatment options in people with type 2 diabetes.

751

Initial therapy with the Fixed-Dose Combination (FDC) of sitagliptin and metformin (JANUMET™) in patients with type 2 diabetes mellitus provides superior glycaemic control and HbA_{1c} goal attainment with lower rates of abdominal pain and diarrhea vs metformin alone

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Background and aims: Initiating oral therapy with the combination of 2 agents is a potential approach to achieve earlier and more effective glycemic goal attainment.
Materials and methods: This large, multicenter, double-blind study evaluated the efficacy and safety of initial therapy with the combination of sitagliptin and metformin [SITA/MET] (administered as a FDC tablet) compared with metformin (MET) alone in drug-naïve pts with type 2 diabetes mellitus (HbA1c≥7.5%). After a 1-wk screening period, 1250 pts (mean baseline HbA1c=9.9%) were randomized 1:1 to twice-daily (BID) SITA/MET or MET for 18 wks. SITA/MET 50/500 mg BID and MET 500 mg BID was uptitrated over 4 wks to 50/1000 mg BID and 1000 mg BID, respectively.
Results: At Wk 18, mean HbA1c reductions from baseline were -2.4% (95% CI:-2.5,-2.2) for SITA/MET and -1.8% (95% CI:-1.9,-1.6) for MET alone, resulting in a significant between-group difference of -0.6% favoring SITA/MET (p<0.001). More pts in the SITA/MET group achieved an HbA1c goal of <7% vs. MET alone (49% vs. 34%; p<0.001). Reductions in fasting plasma glucose were significantly greater with SITA/MET (-69.5 mg/dL) compared with MET alone (-53.5 mg/dL) (p<0.001). A reduction from baseline in body weight of -1.6 kg (95% CI:-2.1,-1.1) was seen in each group. The incidences of overall clinical adverse experiences were similar in both groups. The incidences of hypoglycemia were low and not significantly different between the 2 groups (2.1% for SITA/MET; 1.8% for MET; p=0.686). The incidences of abdominal pain (1.1% and 3.8%, respectively; p=0.002) and diarrhea (12.0% and 16.8%, respectively; p=0.015) were significantly lower with SITA/MET vs. MET alone, while the incidences of nausea and vomiting were similar in both groups.
Conclusion: In summary, initial treatment with the combination of SITA/MET provided superior glycemic improvements, resulted in more pts achieving HbA1c goals, similar weight loss and lower incidences of abdominal pain and diarrhea vs. MET alone over 18 wks.

752

Effect of alogliptin combined with pioglitazone on glycaemic control in metformin-treated patients with type 2 diabetes

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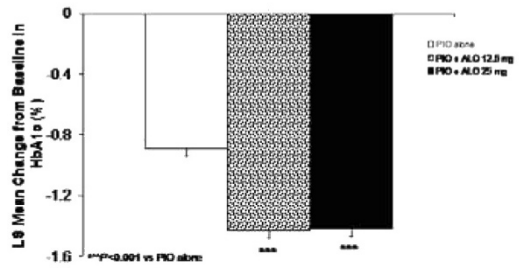
Background and aims: The efficacy and safety of alogliptin (ALO) combined with pioglitazone (PIO) in patients with type 2 diabetes inadequately controlled on metformin monotherapy was assessed in a randomized, double-blind, placebo-controlled, 26-week study.
Materials and methods: Individual treatment arms were placebo, ALO alone (12.5 or 25 mg qd), PIO alone (15, 30, or 45 mg qd), and the combinations of each ALO dose with each PIO dose. The primary analyses compared PIO monotherapy (all doses pooled; n = 387) with ALO 12.5mg plus any dose of PIO (n = 390) or ALO 25mg plus any dose of PIO (n = 390).
Results: There were statistically significant (P<0.001) decreases from baseline in the mean HbA1c levels at Week 26 in subjects treated with ALO12.5 + PIO or ALO25 + PIO vs PIO alone. These decreases were seen as early as Week 4 and were sustained through Week 26. The least squares (LS) mean change from baseline was -0.89%, -1.43%, and -1.42%, for PIO alone, ALO12.5 + PIO, and ALO25 + PIO groups, respectively (Figure A). Each of the individual combination therapy arms had significantly greater efficacy at lowering HbA_{1c} than either component monotherapy (Figure B). For fasting plasma glucose (FPG), the LS mean change from baseline at Week 26 was -28, -45, and -44mg/dL for the PIO alone, ALO12.5 + PIO, and ALO25 + PIO groups, respectively. Weight gain in the ALO + PIO groups was within the expected range previously reported for PIO. Also, weight gain was not associated with peripheral edema, and a parallel increase in edema-related adverse events was not observed.
Conclusion: In conclusion, ALO combined with PIO was well tolerated and produced statistically significant reductions in HbA_{1c} and FPG. The combinations of ALO (12.5 or 25mg qd) plus PIO (15, 30, or 45mg qd) were significantly more effective than either component monotherapy.

A.



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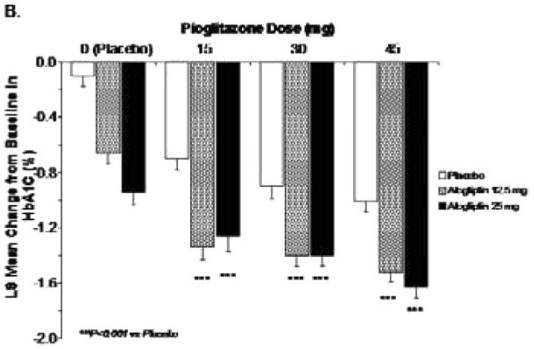

Supported by: Takeda Global Research & Development Center, Inc.

753

Reaching HbA_{1c} goals with saxagliptin in combination with metformin or sulfonylurea

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Background and aims: Saxagliptin (SAXA) is a potent, selective dipeptidyl peptidase-4 (DPP-4) inhibitor, specifically designed for extended inhibition of the DPP-4 enzyme. Metformin (MET) and sulfonylurea (SU) are two of the most commonly used agents to treat type 2 diabetes (T2D). Three multicenter, randomized, double-blind, 24-wk phase 3 trials (CV181-) assessed the efficacy and safety of SAXA as add-on to background MET (-014), as initial combination therapy with MET (-039), or as add-on to the SU glyburide (GLY) (-040) in patients (pts) with T2D and inadequate glycemic control. Previously-treated pts with inadequate glycemic control (-014, -040) and treatment-naïve pts with high mean baseline (BL) HbA_{1c} (-039) were studied; data for SAXA 5 mg (the proposed usual clinical dose) vs control are presented with a focus on the proportion of pts reaching predefined HbA_{1c} goals of <7.0% and ≤6.5%.
Materials and methods: In the add-on to MET study, 743 pts inadequately controlled on MET alone (HbA_{1c} 7.0%-10.0%; mean BL HbA_{1c} 8.0%; mean T2D duration 6.5 yrs) were randomized to SAXA or placebo (PBO) in addition to each pt's ongoing dose of MET. In the initial combination study, 1306 drug-naïve pts (HbA_{1c} 8.0%-12.0%; mean BL HbA_{1c} 9.5%; mean T2D duration 1.7 yrs) were randomized to SAXA + MET, SAXA + PBO, or MET + PBO. In the add-on to SU study, 768 pts inadequately controlled on SU alone (HbA_{1c} 7.5%-10.0%; mean BL HbA_{1c} 8.4%; mean T2D duration 6.9 yrs) were randomized to SAXA or uptitrated GLY + PBO in addition to open-label GLY. Blinded uptitration was allowed in the uptitrated GLY + PBO arm to a maximum total daily dose of GLY 15 mg. Efficacy analyses for continuous variables were performed using an ANCOVA model with last-observation-carried-forward methodology; the proportion of pts reaching HbA_{1c} goals were examined using the Fisher exact test. HbA_{1c} goals were predefined for each study.
Results: At 24 wks, pts treated with SAXA + MET as add-on or initial combination therapy or with SAXA + GLY demonstrated statistically significantly greater decreases from BL in HbA_{1c} vs control. In all three studies, statistically significantly greater proportions of SAXA-treated pts achieved HbA_{1c} goals of <7.0% and ≤6.5% vs control at 24 wks (Table). More than twice as many pts treated with SAXA added to MET (-014) or GLY (-040) achieved the HbA_{1c} goal of <7% and ≤6.5% relative to control at 24 wks. For all three studies, the frequency of adverse events (AEs) was generally similar for SAXA vs control (Table).
Conclusion: Saxagliptin 5 mg + MET, as either add-on or initial combination therapy, and saxagliptin 5 mg + SU, significantly improved glycemic control and more patients achieved predefined HbA_{1c} goals vs control. Saxagliptin in combination with MET or SU was generally well tolerated and may be a suitable combination partner in a broad range of patients with T2D.

Efficacy & Safety Variables at Wk 24	Add-on MET		Initial Combination With MET		Add-on SU	
	SAXA 5 mg + MET	PBO + MET	SAXA 5 mg + MET	PBO + MET	SAXA 5 mg + GLY	PBO + UP-GLY
	N=191	N=179	N=320	N=328	N=253	N=267
Adjusted mean Δ from BL in HbA_{1c} (SE), %	−0.7 (0.1)[a]	0.1 (0.1)	−2.5 (0.1)[a]	−2.0 (0.1)	−0.6 (0.1)[a]	0.1 (0.1)
HbA_{1c} <7%, %	43.5[a]	16.6	60.3[a]	41.1	22.8[a]	9.1
HbA_{1c} ≤ 6.5%, %	22.0[b]	8.0	45.3[a]	29.0	10.4[a]	4.5
Overall AEs, (%)	70.2	64.8	55.3	58.5	72.3	76.8

[a]P<.0001 vs PBO; [b]P=.0002 vs PBO; [c]P=.0117 vs PBO + UP-GLY.
GLY=glyburide; UP-GLY=uptitrated glyburide.

Supported by: BMS and AZ

754

Long-term efficacy with sitagliptin as monotherapy or add-on therapy to metformin: improvement in glycaemic control over 2 years in patients with type 2 diabetes

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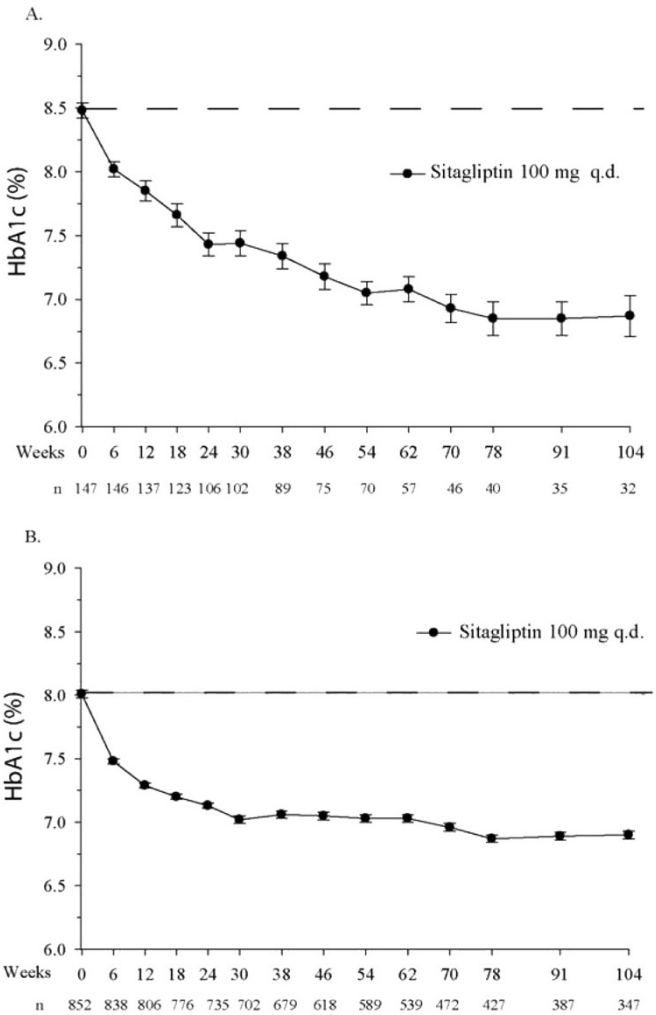
Merck Research Laboratories, Rahway, United States.

Background and aims: The long-term efficacy of sitagliptin as monotherapy or as add-on therapy to metformin was evaluated over 2 years in 2 pooled populations of patients with type 2 diabetes (T2DM).
Materials and methods: For the monotherapy analysis, data from 2 clinical trials were pooled, and the HbA_{1c} response over time was examined in the cohort of 147 patients not on an antihyperglycemic agent at screening and with a common baseline HbA_{1c} of 7.5%-10%. For the add-on therapy to metformin analysis, data from 2 other clinical trials were pooled for this cohort of 852 patients on ≥1500 mg/day and with a common baseline HbA_{1c} of 7%-10%. Data following initiation of an additional antihyperglycemic agent (used as glycemic rescue therapy in some studies) were treated as missing. Missing data were not imputed.
Results: Mean baseline values for the pooled monotherapy cohort included: age 53 years, HbA_{1c} 8.5%, fasting plasma glucose (FPG) 187 mg/dL, and duration of T2DM 4.9 years. Sitagliptin monotherapy decreased mean HbA_{1c} from 8.5% at baseline to 6.9% at 2 years (Figure A). Mean baseline values for the pooled add-on to metformin cohort included: age 56 years, HbA_{1c} 8.0%, FPG 173 mg/dL, and duration of T2DM 6.5 years. The addition of sitagliptin to ongoing metformin therapy decreased mean HbA_{1c} from 8.0% at baseline to 6.9% at 2 years (Figure B). Treatment with sitagliptin was generally well tolerated over 2 years in each of the 4 clinical trials from which the pooled populations were drawn.
Conclusion: Sitagliptin as monotherapy or as add-on therapy to metformin in patients with T2DM provided substantial improvement in glycemic control over 2 years.



Supported by: MRL

PS 59 Incretin-based therapies - cardiovascular risk factors

755

Weight loss and associated changes in glycaemic control and cardiovascular biomarkers in patients with type 2 diabetes mellitus receiving incretin therapies in a large cohort database

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Background and aims: In clinical trials, incretin therapies improve glycemic control and cardiovascular (CV) risk factors such as blood pressure (BP) and lipids. GLP-1 agonists also produce weight loss (WL), and DPP-IV inhibitors are weight neutral. However, the effect of GLP-1 agonist-induced WL on glycemic control and CV biomarkers has not been examined in a large cohort database.
Materials and methods: We analyzed retrospectively the GE Centricity electronic medical records database to explore the association between WL and glycemic control, changes in BP, and changes in lipids in patients with type 2 diabetes (T2DM) who initiated exenatide (EXN), sitagliptin (SIT), or insulin (INS) in 2005-2007 in the United States. Patients had baseline and 1 yr follow-up data for body weight, A1C, fasting glucose (FPG), BP, total cholesterol (TC), LDL-C, HDL-C, and triglycerides (TG). Multivariate regression and correlation analyses were conducted, adjusting for baseline factors and outcome measures.
Results: A total of 6280, 5861, and 32,398 patients on EXN, SIT, and INS, respectively, were studied (baseline factors shown in the Table). Patients on EXN and SIT had WL (mean± SE) of 3.0±0.09 and 1.1±0.07 kg, respectively, whereas INS was associated with a slight weight gain (0.6±0.05 kg). WL was associated with reductions in BP in all groups ($p<0.0001$) and reductions of TG in the EXN and SIT groups ($p<0.05$). Moreover, EXN showed improvements in A1C, FPG ($p<0.0001$ both), TC ($p<0.0001$), and LDL-C ($p=0.004$), which were correlated with WL after multivariate adjustment. There was no correlation with WL and A1C and FPG for SIT, and correlations were negative with INS ($p<0.0001$). EXN patients who lost ≥4.5 kg were more likely to achieve A1C<7% with an adjusted odds ratio [OR] of 2.01[95% CI 1.52, 2.67] compared with those who lost no weight; where this OR was 1.39 [1.00,1.93] and 1.50 [1.25,1.80] for SIT and INS; respectively.
Conclusion: Thus, in a large cohort of patients with T2DM, even a small degree of WL was associated with significant improvement of BP. Furthermore, GLP-1 agonists provided additional benefits on lipids when WL was ≥4.5 kg, and significant incremental benefits in glycemic control were associated with WL. Therefore, the greater WL associated with GLP-1 agonists and its associated improvement in glycemic control, BP, and lipids may convey long-term CV benefits in T2D.

Mean±SD	EXN	SIT	INS
HbA1c (%)	7.7±1.54	7.7±1.53	8.8±2.28
BW (kg)	110±24.7	97±23.4	96±25.0
SBP (mmHg)	130±16.1	130±17.0	132±19.7
DBP (mmHg)	77±10.0	76±10.5	75±11.8
TG (mg/dL)	209±166.9	188±166.3	223±248.3
LDL-C (mg/dL)	95±35.8	96±35.7	100±40.4
HDL-C (mg/dL)	43±11.6	44±12.4	45±14.3
TC (mg/dL)	177±45.7	175±44.7	183±53.0

756

Exenatide once weekly reduced HbA_{1c}, weight, and cardiovascular risk factors across a wide BMI range

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Background and aims: Type 2 diabetes (T2DM) is often accompanied by cardiometabolic complications including obesity, hypertension, and dyslipidemia that elevate patients' risk of cardiovascular (CV) disease. In a 30-week open-label phase 3 study, exenatide once weekly (QW 2 mg) improved glycaemic control with associated weight reduction and improvement in CV risk factors in patients with T2DM (N = 148, HbA_{1c} 8.3 ± 1.0%, weight 102 ± 19 kg, BMI 35 ± 5 kg/m^2, diabetes duration 7 ± 5 y, mean ± SD). HbA_{1c} and weight reductions from baseline to Week 30 were -1.9 ± 0.1% and -3.7 ± 0.5 kg, respectively (LS mean ± SE). Because higher BMI has been associated with an increased risk of diabetes complications, we examined the relationship between baseline BMI and changes in CV risk factors with exenatide QW treatment.
Materials and methods: The association between BMI at baseline and HbA_{1c} reduction at Week 30 in the overall ITT population was examined using the Pearson correlation coefficient. We also examined the cardiometabolic profile in subgroups of the population stratified by baseline BMI (<30 [n = 24], 30 to <35 [n = 58], 35 to <40 [n = 44], ≥40 kg/m^2 [n = 22]). Comparisons across the BMI subgroups for each parameter were made using an ANCOVA model with baseline BMI stratum as the factor and baseline value of the parameter as a covariate.
Results: The analysis indicated that HbA_{1c} reduction was not significantly correlated with baseline BMI (Pearson r = 0.02) and that improvements in HbA_{1c} and CV risk factors were apparent in all BMI subgroups (Table). Further, comparison across the BMI subgroups demonstrated no statistically significant differences between subgroups in change from baseline for HbA_{1c}, weight, blood pressure, total cholesterol, LDL, or triglycerides. Exenatide QW was generally well tolerated. Nausea was reported in 26% of patients and was predominantly mild in intensity and transient. No major hypoglycaemia was observed.
Conclusion: In this study, exenatide QW improved glycaemic control, weight, blood pressure, and lipids for patients with T2DM across a wide range of BMI, from mildly overweight to morbidly obese.

Endpoint Δ from Baseline	Baseline BMI Subgroup (kg/m^2)				ANCOVA P-value[†]
	<30	30 to <35	35 to <40	≥40	
HbA_{1c} (%)	-1.6 ± 0.2*	-1.8 ± 0.1*	-1.4 ± 0.1*	-1.5 ± 0.2*	0.09
Weight (%)	-2.9 ± 1.3*	-3.5 ± 0.7*	-3.6 ± 0.8*	-5.4 ± 1.4*	0.66
Systolic BP (mmHg)	-3.0 ± 2.5	-4.7 ± 1.5*	-3.8 ± 1.7*	-5.7 ± 2.4*	0.86
Diastolic BP (mmHg)	-2.7 ± 1.6	-0.6 ± 1.0	-0.6 ± 1.1	-1.8 ± 1.6	0.63
Cholesterol (mmol/L)	-0.21 ± 0.14	-0.35 ± 0.09*	-0.19 ± 0.1	-0.29 ± 0.15	0.64
LDL-C (mmol/L)	-0.07 ± 0.11	-0.19 ± 0.07*	-0.01 ± 0.09	-0.06 ± 0.12	0.42
Triglycerides (%)	-16 ± 6*	-15 ± 4*	-15 ± 5*	-15 ± 7	1.00

Triglycerides and weight are presented as LS mean ± SE % change. All other data are LS mean ± SE. *P<0.05, significant vs baseline; [†]Comparison across BMI subgroups using one-way ANCOVA with baseline BMI stratum as the factor and baseline value of the parameter as a covariate.

757

Effects of exenatide vs insulin glargine on central haemodynamics in subjects with type 2 diabetes

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Background and aims: Exenatide (EXN), a GLP-1 agonist, is an effective glucose lowering therapy, primarily resulting in the reduction of postprandial glycemia. Data are emerging that GLP-1 may have beneficial effects on the cardiovascular system, including reduction in peripheral blood pressure and improvement in endothelial function. It is not known whether EXN has any effects on central hemodynamic parameters, which may be better markers for cardiovascular risk than peripheral blood pressure. We assessed the effects of EXN compared to insulin glargine (IG) on central hemodynamics and endothelial function in subjects with DM2.
Materials and methods: 56 subjects with DM2 (HbA_{1c} 8.15 ± 1.14%) were randomized to 3 months of treatment with either EXN or IG to obtain similar reductions in HbA_{1c}. A liquid mixed meal test (MMT) using Boost [360 kcal, 61.5g Carbohydrate, 15g Protein, and 6g Fat] was performed before and after 3 months of therapy. Flow mediated dilation (FMD) of the brachial artery

and pulse wave analysis (PWA) were performed fasting, 2 hours, and 4 hours after the MMT before and after 3 months of therapy. Measurements of central hemodynamics by PWA included central pulse pressure (CPP), augmentation pressure (AP), and augmentation index (AI).
Results: 28 subjects were randomized to treatment with EXN and 28 to treatment with IG. The EXN and IG groups had similar reductions in HbA_{1c} [-1.37 ± 1.16 vs. -0.85 ± 0.71%, respectively (p=.NS)]. The EXN group had a significant reduction in the systolic blood pressure (SBP) (-8.1 ± 12 (p=.002)] and diastolic blood pressure (DBP) [-4.9 ± 7.3 (p=.002)], while the IG group did not have a significant decrease in SBP or DBP. The EXN group had a significant reduction in CPP and AP at all time points, and in AI 4 hours after the MMT (Table 1). The IG group had no improvement in any parameters of central hemodynamics. There was no effect of either treatment on FMD (Table 1) or response to nitroglycerin (data not shown).
Conclusion: In subjects with DM2, treatment with EXN, compared to IG, resulted in a reduction in SBP and DBP, as has been previously reported. Treatment with EXN also resulted in a reduction in CPP and AP, which has not been previously described. Reduction in CPP may more strongly predict a reduction in cardiovascular risk than the reduction in peripheral blood pressure, and EXN may thus impart cardiovascular risk reduction by its effect on lowering CPP. These effects appear to be independent of overall glycemic control.

Table 1

		CPP (mmHg)	AP (mmHg)	AI (%)	FMD (%)
EXN	**Pre-Tx 0h**	51.0 (15.3)	11.78 (6.4)	22.5 (9.2)	4.6 (2.8)
	Post-Tx 0h	44.3 (12.3)*	9.7 (5.2)*	21.8 (8.9)	4.7 (2.4)
	Pre-Tx 2h	50.4 (15.8)	10.8 (6.1)	20.2 (9.7)	4.1 (1.9)
	Post-Tx 2h	42.7 (14.9)*	8.6 (6.6)*	18.8 (10.3)	3.7 (2.1)
	Pre-Tx 4h	48.4 (14.3)	10.6 (5.8)	22.0 (9.0)	5.6 (3.3)
	Post-Tx 4h	43.3 (13.1)†	8.8 (5.3)*	19.4 (8.7)*	5.5 (2.3)
IG	**Pre-Tx 0h**	50.1 (12.1)	13.3 (6.2)	26.3 (7.9)	4.7 (2.6)
	Post-Tx 0h	49.4 (14.1)	13.3 (5.1)	27.1 (6.6)	5.0 (2.3)
	Pre-Tx 2h	47.0 (14.6)	11.3 (5.9)	23.2 (8.6)	4.4 (2.3)
	Post-Tx 2h	46.5 (15.4)	10.7 (6.1)	22.3 (8.5)	4.9 (2.3)
	Pre-Tx 4h	51.3 (18.8)	13.1 (8.2)	24.9 (9.4)	4.8 (2.5)
	Post-Tx 4h	49.4 (15.2)	12.9 (6.7)	25.5 (8.1)	5.2 (2.7)

Numbers in parenthesis are standard deviations
* p <.05 Pre-Tx vs Post-Tx
† p = .05 Pre-Tx vs Post-Tx

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758

Exenatide once weekly improved cardiometabolic risk factors in subjects with type 2 diabetes during one year of treatment
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Background and aims: Patients with type 2 diabetes (T2DM) often exhibit a number of cardiometabolic abnormalities including hypertension, obesity, and dyslipidemia, which increase the risk for cardiovascular (CV) disease and nonalcoholic fatty liver disease (NAFLD). In a controlled, open-label trial, 30 weeks of exenatide once weekly (QW) improved glycaemic control, reduced weight, and improved markers of CV risk in subjects with T2DM.
Materials and methods: We examined the cardiometabolic profile in subjects who continued exenatide QW for a total of one year (n=120, baseline HbA_{1c} 8.3±1.0%, BMI 35.2±5.0 kg/m^2, age 56±9 y, mean±SD). Changes in concomitant antihypertensive and lipid lowering medications were allowed only if deemed necessary by the investigator.
Results: One year of exenatide QW improved HbA_{1c} (-2.0±0.1%; LS mean±SE), FPG (-2.60±0.16 mmol/L), and weight (-4.1±0.6 kg), with nearly all subjects (98%) exhibiting improvement in HbA_{1c}. Blood pressure, fasting lipids, and the hepatic injury biomarker ALT were also improved, with greater improvements in subjects with abnormal baseline values. Weight change was mild to moderately correlated (r≤0.3125; p<0.05) with changes in DBP, LDL, cholesterol, and ALT, but not significantly correlated with SBP or triglycerides (TG). Nausea, predominantly mild in intensity, occurred in 29% of subjects.
Conclusion: Improvements in cardiometabolic risk factors were sustained during one year of exenatide QW treatment; patients with abnormal baseline cardiometabolic risk factors exhibited the greatest improvements in those parameters. These data indicate that, in addition to improving glycaemic control and body weight, exenatide QW has the potential to improve the overall cardiometabolic risk profile in patients with T2DM.

	SBP (mmHg)	DBP (mmHg)	LDL (mmol/L)	TG (mmol/L)	Cholesterol (mmol/L)	ALT (IU/L)
All Patients						
Baseline	127.8±1.1	77.7±0.7	2.31±0.08	2.22±0.16	4.39±0.10	34.6±1.5
Δ at 52 weeks	-6.2±1.2*	-2.8±0.8*	-0.06±0.06	-0.45±0.13*	0.20±0.08*	-7.1±1.4*
Abnormal Baseline	≥130	≥80	≥2.59	≥1.69	-	>19/30 (F/M)
Baseline	139.4±1.2	84.7±0.6	3.29±0.63	3.20±0.25	-	42.4±1.9
Δ at 52 weeks	-11.4±1.9*	-6.5±1.2*	-0.41±0.11*	-0.95±0.23*	-	-11.4±2.0*
% Acheiving target for each parameter	50%	51%	31%	38%	-	54%

Δ = LS mean±SE for blood pressure, mean±SE for lipids and ALT; *p<0.05 vs. baseline.

759

Cardiovascular safety of exenatide BID: an integrated-analysis from long-term controlled clinical trials in subjects with type 2 diabetes
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Background and aims: Exenatide BID (Ex) is a first-in-class GLP-1 receptor agonist, with more than 750,000 patient-years of marketed exposure accumulated worldwide, that is an antidiabetic medication associated with weight loss, improvements in lipid profile, reduction in blood pressure, and a low risk of hypoglycaemia in the absence of a sulphonylurea. To evaluate cardiovascular (CV) safety, a meta-analysis of CV events was performed.
Materials and methods: An integrated database of 12 completed, randomised controlled clinical trials ranging from 12 to 52 weeks was used to compare the relative risk (RR) of CV events with Ex (5 and 10 μg) versus a pooled comparator group (PC) treated with either placebo or insulin. CV events included: stroke, myocardial ischaemia, myocardial infarction, cardiac mortality, arrhythmia, revascularisation procedures, and congestive heart failure. Events were identified by preferred terms according to the Medical Dictionary for Regulatory Activities (MedDRA 11.0). In this integrated analysis, 2,279 Ex subjects and 1,629 PC subjects were treated for a total of 1,063 and 780 patient-years of exposure, respectively.
Results: Demographics were comparable (mean age 56 y, 53-55% M, BMI 31-32 kg/m^2, HbA_{1c} 8.3-8.4%). The unadjusted incidence of experiencing at least one CV events was 2.0% for Ex and 2.6% for PC; the relative risk between Ex and PC was 0.69 (95% CI: 0.46-1.04) (calculated using Cochran-Mantel-Haenszel estimate using study as a stratification factor). The Exposure Adjusted Incidence Rate (expressed as per 1000 patient years) was 43.7 for Ex and 54.4 for PC with a relative risk 0.80 (95% CI: 0.53-1.22) (calculated using a log-normal approximation method).
Conclusion: This analysis is consistent with long-term clinical trial data demonstrating favourable effects of Ex on CV risk factors and suggests a potential benefit of Ex treatment, warranting further evaluation in an appropriately powered prospective outcomes study.

760

Effects of exenatide vs insulin glargine on cardiovascular risk factors in subjects with type 2 diabetes
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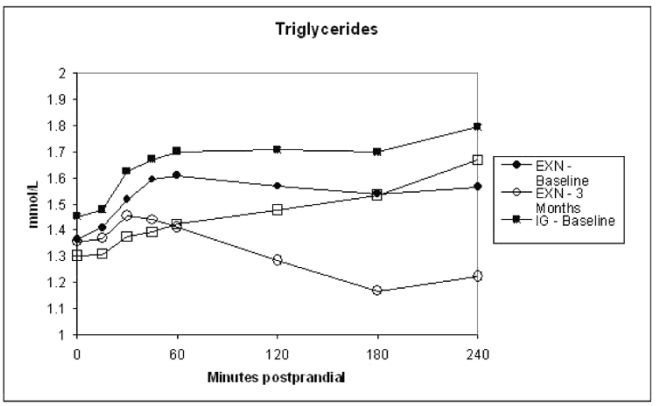
Background and aims: Multiple factors contribute to the increased risk for cardiovascular (CV) disease in people with type 2 diabetes (DM2), including postprandial hyperglycemia and hypertriglyceridemia. Exenatide (EXN), a GLP-1 agonist, is an effective glucose lowering therapy, primarily resulting in the reduction of postprandial glycemia. Data in healthy adults suggest GLP-1 may also improve postprandial lipemia, and studies in DM2 show lower postprandial triglycerides (TG) after 1 year of treatment with EXN. We assessed the effects of EXN compared to insulin glargine (IG) on postprandial lipemia and other CV risk factors in subjects with DM2 after 3 months of treatment.

Materials and methods: 56 subjects with DM2 (HbA_{1c} 8.15 ± 1.14%) were randomized to 3 months of treatment with either EXN or IG to obtain similar reductions in HbA_{1c}. Markers of CV risk were assessed, including changes in blood pressure (BP), weight, BMI, and waist circumference. A liquid mixed meal test (MMT) using Boost was done at baseline and after 3 months of therapy. Glucose, insulin, C-peptide, lipids, and free fatty acids were measured prior to and during the 4 hours following the mixed meal.
Results: 28 subjects were randomized to treatment with EXN and 28 to treatment with IG. The EXN and IG groups had similar reductions in HbA_{1c} [-1.37 ± 1.16 vs. -0.85 ± 0.71%, respectively (p=.NS)]. The EXN group had significantly greater reductions in weight [-3.4 ± 2.7 vs. 0.4 ± 1.6 kg (p<.0001)], BMI [-1.16 ± 0.92 vs. 0.14 ± 0.55 kg/m^2 (p<.0001)], and waist circumference [-2.95 ± 3.5 vs. -0.56 ± 3.4 cm (p=.02)] compared to the IG group. There was also a significantly greater reduction in diastolic BP [-4.85 ± 7.3 vs. 0.15 ±9.7 mmHg (p=.038)], and a trend towards a greater reduction in systolic BP [-8.1 ± 12 vs. -2.5 ± 13 mmHg (p=NS)] in the EXN group compared to the IG group. Similar decreases in fasting glucose occurred. (-2.63 ± 2.86 vs -2.2 ± 2.04 mmol/L, p=NS) in the EXN and IG groups, respectively. There were no significant changes in fasting LDL, HDL, total cholesterol or TG in either group. As expected, during the MMT, the decrease in the incremental area under the curve (iAUC) for glucose was significantly greater in the EXN group than in the IG group (iAUC -554 ± 436 vs. -44 ± 355 mmol/L/4h, p=0.000). Importantly, there was also a significantly greater decrease in TG (iAUC -61.2 ± 80.6 vs -6.4 ± 49.8 mmol/L/4h, p=.005) (Fig 1).
Conclusion: In subjects with DM2, treatment with EXN for 3 months, compared to IG, resulted in greater reductions in multiple cardiovascular risk factors, including postprandial changes in plasma glucose and serum triglycerides, and reductions in body weight, BMI, waist circumference, and diastolic BP. These effects appear to be independent of overall glycemic control.



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761

A meta-analysis of six clinical trials demonstrates that the once-daily human GLP-1 analogue liraglutide reduces systolic blood pressure

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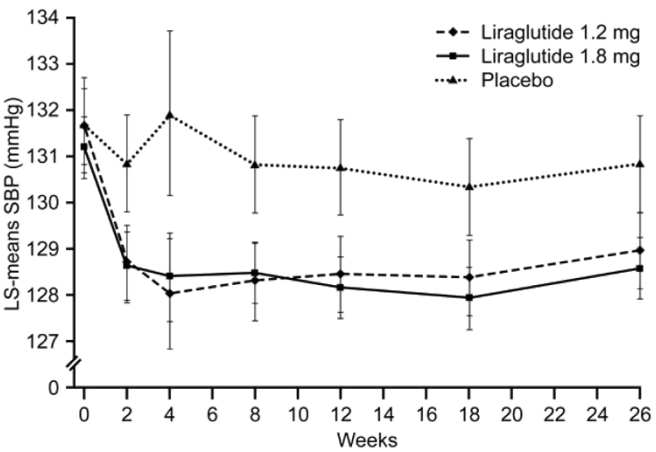
Background and aims: Type 2 diabetes (T2D) patients frequently have hypertension, which contributes to the elevated cardiovascular risk observed in this population. The six large phase 3 Liraglutide Effect and Action in Diabetes (LEAD) studies have shown that, in T2D patients, the once-daily human GLP-1 analogue liraglutide reduces HbA_{1c} by 1.0-1.5% with concomitant mean weight loss of approximately 3 kg and, importantly, that it reduces systolic blood pressure (SBP) by 2.7-6.6 mmHg (min, max baseline SBP, mmHg: 128/76-134/81). We now have investigated the effect of liraglutide on SBP by quartile of baseline SBP.
Materials and methods: A meta-analysis across the LEAD studies was performed. Patients treated with liraglutide 1.8 mg (n=1362), liraglutide 1.2 mg (n=896) or placebo (PBO n=520) were stratified by quartile of baseline SBP. An ANCOVA model adjusted for trial, baseline SBP and baseline SBP quartile was employed to analyse mean SBP changes from baseline. Baseline SBP quartile limits were 80 mmHg≤SBP≤120 mmHg in Q1; 120 mmHg <SBP≤130 mmHg in Q2; 130 mmHg <SBP≤140 mmHg in Q3 and 140 mmHg <SBP≤190 mmHg in Q4. The investigators did not receive specific instructions on lowering blood pressure with medications during the trials, in which HbA_{1c} reduction was the primary endpoint.
Results: Overall, mean SBP decreased significantly from baseline with liraglutide 1.8 mg and liraglutide 1.2 mg by 2.6 mmHg (p=0.0008) and 2.5 mmHg (p=0.0030), respectively, and was sustained for 26 weeks. No significant change was seen with PBO (-0.2 mmHg, p=0.7828). Repeated measure analysis with a model adjusted for baseline SBP quartile was used to investigate the longitudinal effect on SBP from baseline to week 26. Full impact on SBP was achieved at first visit after 2 weeks (Figure) before any major weight loss had occurred. The effect associated with baseline SBP quartile was significant (p<0.0001). The greatest reductions were observed in the quartile with highest baseline SBP. Thus, changes from baseline of -11.4 mmHg in Q4, -4.7 mmHg in Q3, -0.4 mmHg in Q2 and +4.8 mmHg in Q1 were observed with liraglutide 1.8 mg. Similarly, changes of -11.4 mmHg in Q4, -6.2 mmHg in Q3, +0.4 mmHg in Q2 and +5.4 mmHg in Q1 were observed with liraglutide 1.2 mg.
Conclusion: Overall, liraglutide treatment reduces and sustains SBP over 26 weeks, especially in patients with elevated SBP at baseline. The impact on SBP is achieved within 2 weeks and cannot be explained by weight loss alone. Thus, liraglutide treatment may improve the cardiovascular risk profile of T2D patients, with patients with elevated SBP possibly experiencing a greater benefit.

LS-Means ± 95% CIs for SBP over 26 weeks with liraglutide 1.8 mg, liraglutide 1.2 mg and placebo.



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762

Meta-analysis demonstrates that liraglutide, a once-daily human GLP-1 analogue, significantly reduces lipids and other markers of cardiovascular risk in type 2 diabetes

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Background and aims: Type 2 diabetes (T2D) is characterised by abnormal lipid profiles and raised levels of biomarkers that predict cardiovascular risk, including brain natriuretic peptide (BNP), high sensitivity C reactive protein (hsCRP), and apolipoprotein B (Apo B). In clinical trials, the once-daily human GLP-1 analogue liraglutide was shown to improve HbA_{1C} by 1.0-1.5%, produce sustained weight reductions of 2-3 kg, and reduce SBP by 2-6 mmHg when given for up to 52 weeks. We aimed to assess the impact of liraglutide on lipids and other cardiovascular (CV) risk markers using meta-analysis of a clinical trial database.
Materials and methods: We conducted a meta-analysis (ANCOVA, ITT, LOCF) of six randomised controlled phase 3 trials that compared liraglutide 1.8 mg OD with other T2D therapies (glimepiride, rosiglitazone, insulin glargine, exenatide) and placebo (n=3967).

Results: After 26 weeks of liraglutide, total cholesterol, low-density lipoprotein-cholesterol (LDL-C), free fatty acids (FFA) and triglycerides (TG) all decreased significantly compared with baseline (p<0.01 for all) (Table). With other therapies, these lipids did not all decrease significantly vs baseline (Table). BNP decreased vs baseline by 11.9% with liraglutide (p<0.01), but increased by 30.9% with rosiglitazone (p<0.0001); changes with other therapies were not significant (NS). hsCRP decreased by 23.1% (liraglutide, p<0.0001), 42.6% (rosiglitazone, p<0.0001), 12.3% (glimepiride, p<0.05), 15.6% (exenatide, p<0.05), but was NS with glargine or placebo. ApoB levels did not change significantly with any treatments. High-density lipoprotein decreased significantly from baseline with all interventions except rosiglitazone.
Conclusion: In addition to significantly improving levels of total cholesterol, LDL-C, FFA, and TG in patients with T2D, treatment with liraglutide for 26 weeks also significantly reduced BNP and hsCRP, two markers of CV risk. Liraglutide may reduce CV risk by additional mechanisms in addition to its known effects on glycaemia, weight and systolic blood pressure.

Change in lipid levels from baseline to 26 weeks.

	Liraglutide; n=1363[a]	Rosiglitazone; n=896[a]	Glimepiride; n=231[a]	Glargine; n=490[a]	Exenatide; n=231[a]	Placebo; n=524[a]
Lipid change (mmol/L)						
Total cholesterol (TC)	-0.13**	0.29**	-0.05	0.02	-0.05	0.01
Low density lipoprotein (LDL)	-0.20***	0.06	-0.12*	-0.07	-0.15*	-0.13*
Very low density lipoprotein (VLDL)	0.10**	0.22***	0.12**	0.12**	0.16**	0.16***
High density lipoprotein (HDL)	-0.04***	0.02	-0.04**	-0.04**	-0.05**	-0.03*
Free fatty acids (FFA)[b]	-0.09***	-	-0.05**	-	-0.03	-0.06*
Triglycerides (TG)	-0.20**	-0.05	-0.16	-0.15	-0.05	0.02
CV risk marker relative change (%)						
Brain natriuretic peptide (BNP)[c]	-11.9**	30.9***	0.1	10.2	-3.9	1.4
High sensitivity C reactive protein (hsCRP)[c]	-23.1***	-42.6***	-12.3*	2.8	-15.6*	-3.0

[a]n values show all patients in each group. n values for each analysis were smaller, depending on availability of data. Meta-analysis of LEAD 1-6, except as follows: [b]LEAD 2,3,6; [c]LEAD 1-2, 4-6. *p<0.05; **p<0.01; ***p<0.0001 vs baseline.
Supported by: Novo Nordisk

763

Assessing the cardiovascular safety of vildagliptin: a meta-analysis of adjudicated cardiovascular and cerebrovascular events from a large phase 3 population

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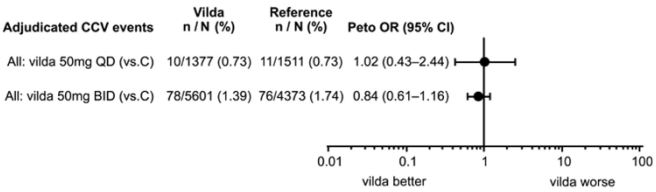
Background and aims: While the direct cardiovascular benefits of improving glycemic control are still not clearly established in type 2 diabetes (T2DM), there has recently been an intense discussion of the cardiovascular safety of some anti-diabetic agents. It is therefore critical to provide a thorough evaluation of the cardiovascular and cerebrovascular (CCV) safety of new anti-diabetic agents, even before the results of long-term outcome studies become available.This work aimed to assess the CCV safety of vildagliptin, a potent and selective DPP-4 inhibitor, in a large population representative of T2DM patients, applying methodology put forward by a new FDA guidance.
Materials and methods: Twenty phase 3 double-blind clinical trials (monotherapy and combination therapy; ranging in duration from 24 to more than 104 weeks) were included. A meta-analysis using Peto fixed effects odds ratio was performed to compare vildagliptin treatment groups (doses of 50 mg qd and 50 mg bid; n=6978) to the all comparators group (placebo and active-controlled treatment groups; n=4773) using a composite endpoint of adjudicated CCV events. These events were adjudicated as "confirmed" by the blinded assessment of an independent CCV adjudication committee; categories included were acute coronary syndrome, sudden cardiac death, stroke and transient ischemic attack with imaging evidence of infarction. This meta-analysis combined within-study treatment comparisons and therefore indirectly adjusted for study level factors that could affect the occurrence of adverse events (e.g. different durations of exposure or different study populations).
Results: The phase 3 population was well representative of a T2DM population, with mean age ~56 years, slight male predominance (~55/45%), mean BMI ~31.4 kg/m^2 (> 50% were obese), disease duration of ~4.2 years and mean baseline HbA1c of ~8.1%. About 18% of patients had medical history in the 'Cardiac Disorders' System Organ Class category, ~43% had dyslipidemia and ~ 58% hypertension. The incidences and odds ratios for confirmed adjudicated CCV events are shown below. Vildagliptin was not associated with any increased risk relative to all comparators, the odds ratios being 1.02 for vildagliptin 50 mg qd and 0.84 for vildagliptin 50 mg bid; the 95% CIs included 1 (0.43 - 2.44 and 0.61 - 1.16, respectively).



Reference = Comparators; (vs.C) = vs. Comparators.
Comparators = all non-vilda treatment groups.
Peto fixed odds ratios estimates only includes studies with at least one event.

Conclusion: This large meta-analysis demonstrated that vildagliptin is not associated with an increased risk for adjudicated CCV events relative to all comparators, confirming the favorable cardiovascular safety profile of the drug.
Supported by: Novartis Pharmaceuticals

PS 60 Incretins - safety

764

Hepatic safety profile of vildagliptin, a new DPP-4 inhibitor for the treatment of type 2 diabetes

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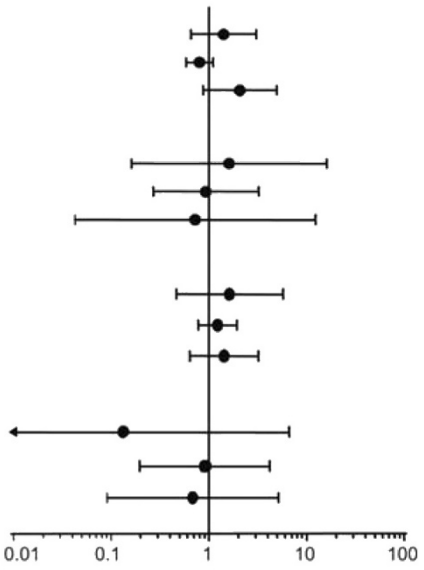
Background: Vildagliptin is a potent and selective DPP-4 inhibitor that improves islet function by increasing α- and β-cell responsiveness to glucose. Vildagliptin does not have the preclinical characteristics of a hepatotoxic agent. The product information describes rare cases of potential drug-induced hepatitis and recommends hepatic enzyme monitoring. The results of the most recent hepatic analysis are presented based on an a data integration done in November 2008.
Objective: To characterize the hepatic safety profile of vildagliptin in diabetic patients.
Methods: Safety data from 36 phase 2 and 3 clinical trials (monotherapy and combination therapy, ranging in duration from 12 to more than 104 weeks) were pooled including 3 open-label studies. A meta-analysis using Peto fixed effects odds ratio was performed to compare vildagliptin treatment groups to the all comparators group (placebo and active-controlled treatment groups pooled) with respect to the risk of hepatic adverse events and persistent hepatic enzyme elevations defined as 2 consecutive elevations or an elevation at the last visit.
Results: More than 11000 patients have been treated with vildagliptin. The overall incidences of hepatic events for vildagliptin were generally similar to the comparator incidences with corresponding Peto ORs slightly above or below 1. A similar pattern was observed for serious hepatic events, with overall very small event numbers (Figure).The risk of having an ALT/AST ≥3 x ULN was slightly increased for vildagliptin relative to all comparators with no dose response relationship (Figure). In contrast, for ALT/AST ≥3 x ULN accompanied by bilirubin >ULN the odds ratios were less than 1.0 for each vildagliptin dose regimen (Figure). Hepatic enzyme elevations ALT/AST ≥10 x ULN; or 3 x ULN accompanied by bilirubin ≥2ULN were rare with a incidence similar to comparators. 2 cases of ALT/AST ≥3 x ULN with bilirubin ≥2ULN were attributed to vildagliptin by an independent and blinded expert panel. Both cases were asymptomatic, did not require hospitalization, and resolved on cessation of treatment without clinical sequelae.
Conclusion: Although a slightly higher risk of milder hepatic enzyme elevations was observed with vildagliptin, this does not translate into an increased risk of more severe hepatic enzyme elevations or an increased risk of actual hepatic adverse events. In a clinical setting, however, hepatic enzymes should be monitored consistent with the label and clinical practice.
Figure: Incidence and odds ratio for selected hepatic adverse events (excluding open label safety population) and treatment-emergent persistent on-treatment hepatic enzyme elevations by treatment (including open label safety population).

Selected hepatic events	Vilda n / N (%)	Reference n / N (%)	Peto OR (95% CI)
All: vilda 50mg QD (vs.C)	15/1456 (1.01)	12/1618 (0.74)	1.42 (0.66–3.07)
All: vilda 50mg BID (vs.C)	75/5601 (1.34)	81/4373 (1.85)	0.81 (0.58–1.11)
All: vilda 100mg QD (vs.C)	15/1501 (1.00)	6/1253 (0.48)	2.09 (0.88–5.00)
Selected hepatic serious events			
All: vilda 50mg QD (vs.C)	2/1468 (0.13)	1/1618 (0.06)	1.62 (0.16–16.20)
All: vilda 50mg BID (vs.C)	5/5601 (0.09)	5/4373 (0.11)	0.93 (0.27–3.26)
All: vilda 100mg QD (vs.C)	1/1501 (0.07)	1/1253 (0.08)	0.73 (0.04–12.46)
ALT/AST>= 3xULN			
All: vilda 50mg QD (vs.C)	6/1390 (0.43)	4/1531 (0.26)	1.62 (0.46–5.68)
All: vilda 50mg BID (vs.C)	48/5376 (0.89)	30/4109 (0.73	1.23 (0.78–1.94)
All: vilda 100mg QD (vs.C)	17/3100 (0.55)	8/2012 (0.40)	1.43 (0.64–3.22)
ALT/AST>= 3xULN and Bilirubin > ULN			
All: vilda 50mg QD (vs.C)	0/1384 (0.00)	1/1528 (0.07)	0.13 (<.01–6.64)
All: vilda 50mg BID (vs.C)	4/5366 (0.07)	3/4100 (0.07)	0.91 (0.19–4.20)
All: vilda 100mg QD (vs.C)	2/2936 (0.07)	2/1923 (0.10)	0.68 (0.09–5.13)



Reference=comparators (vs.C) = vs. Comparators.
Comparators= all non-vilda treatment groups. Arrows represent estimates outside of axis range. n= number of patients meeting the criterion (i.e who are notably abnormal). Total= number of patients with evaluable criterion. Notable abnormalities summarized are those which are treatment emergent (i.e. not present at any pre-treatment visit). Persistent evaluations are those which meet the criterion at consecutive on-treatment measurements or at last on-treatment visit.
Peto fixed odds ratios estimates only includes studies with at least one event.

Supported by: Novartis Pharmaceuticals

765

Alogliptin use in the elderly: a pooled analysis from Phase 2-3 studies

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Background and aims: Here we report the results of a pooled analysis of elderly type 2 diabetes patients treated with the DPP-4 inhibitor, alogliptin (ALO).
Material and methods: Six phase 2-3 double-blind, placebo (PBO)-controlled, randomized studies evaluated the efficacy and safety of ALO 12.5 or 25 mg qd for up to 26 weeks. Among the subset of elderly subjects (≥65 yrs), 350 received ALO; among the subset of younger subjects (<65 yrs), 1482 received ALO. Comparisons were made on the PBO-corrected data between ALO dose groups and cohorts using an ANCOVA analysis with missing data imputed using the LOCF method (P<.05=significant).
Results: Among ALO-treated younger subjects, 74.3% completed the study vs 83.4% of elderly subjects. Mean age in the younger and elderly cohorts was 52 and 70 yrs, respectively. Mean baseline and LS mean change from baseline (CFB) values for A1c, fasting plasma glucose, and weight showed minor variations between age cohorts. However, there were no statistically significant age-group differences in the PBO-corrected LS mean CFB for any measured parameter between age cohorts. ALO was generally well tolerated in elderly subjects. Overall, the rate of any serious AE and AEs leading to discontinuation were somewhat higher in the elderly subjects.
Conclusion: Elderly patients realized the same beneficial effects of ALO on glycemic endpoints as younger patients. Alogliptin is an effective and well tolerated treatment option for elderly patients with type 2 diabetes.

Patients ≥65 yrs

	PBO N=105		ALO 12.5 mg N=175		ALO 25 mg N=175	
	BL	Δ±SE	BL	Δ±SE	BL	Δ±SE
A1c (%)	8.15	-0.18 (0.083)	8.00	-0.69 (0.063)†	8.02	-0.77 (0.063)†
FPG (mg/dL)	178.4	-6.8 (4.73)	168.6	-20.6 (3.60)†	168.7	-23.1 (3.58)†
Weight (kg)	83.9	-0.22 (0.229)	83.4	-0.03 (0.227)	83.9	-0.05 (0.227)
Any AE	68 (64.8%)	117 (66.9%)	111 (63.4%)			
AEs leading to dc	2 (1.9%)	6 (3.4%)	7 (4.0%)			
Any Serious AE	6 (5.7%)	8 (4.6%)	13 (7.4%)			
Hypoglycemic event	11 (10.5%)	14 (8.0%)	13 (7.4%)			

Patients <65 yrs

	PBO N=429		ALO 12.5 mg N=747		ALO 25 mg N=735	
	BL	Δ±SE	BL	Δ±SE	BL	Δ±SE
A1c (%)	8.42	-0.07 (0.041)	8.23	-0.53 (0.030)†	8.22	-0.61 (0.030)†
FPG (mg/dL)	181.6	3.3 (2.31)	175.6	-8.8 (1.72)†	175.2	-12.6 (1.74)†
Weight (kg)	90.7	0.37 (0.147)	88.7	0.58 (0.109)	88.4	0.41 (0.110)
Any AE	279 (65.0%)	492 (65.9%)	475 (64.6%)			
AEs leading to dc	9 (2.1%)	15 (2.0%)	15 (2.0%)			
Any Serious AE	14 (3.3%)	28 (3.7%)	29 (3.9%)			
Hypoglycemic event	31 (7.2%)	62 (8.3%)	48 (6.5%)			

†$P<.05$ for comparison vs placebo within age group.

Supported by: Takeda Global Research & Development Center, Inc.

766

Efficacy and safety of saxagliptin 5 mg once-daily therapy in elderly patients with type 2 diabetes mellitus

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Background: The prevalence of type 2 diabetes mellitus (T2DM) increases with age and most patients require a combination of antidiabetic drugs to achieve glycaemic control (HbA_{1c} <7%). The antihyperglycaemic agent saxagliptin (SAXA) is a potent, selective dipeptidyl peptidase-4 (DPP-4) inhibitor, specifically designed for extended inhibition of the DPP-4 enzyme.
Aims: This subgroup analysis examined the efficacy and safety of SAXA 5 mg treatment in younger (<65 years) and older (≥65 years) patients with T2DM.
Methods: SAXA 5 mg treatment was investigated in 882 patients (18-77 years of age) with inadequately controlled T2DM (HbA_{1c} ≥7.0%) in five double-blind, placebo-controlled, Phase III studies. Following a placebo run-in period, patients were randomised to SAXA 5 mg once daily (o.d.; n=882) or placebo (n=799). The data presented focus on patients who received SAXA 5 mg monotherapy or placebo o.d. (CV181-011; CV181-038), or SAXA 5 mg or placebo o.d. plus each patient's stable dose of metformin (MET; CV181-014), glibenclamide (GLIB; CV181-040), or thiazolidinedione (CV181-013) for 24 weeks. In study CV181-040, blinded up-titration of GLIB was allowed in the GLIB-only arm. The primary endpoint of all studies was HbA_{1c} change from baseline at Week 24.
Results: Treatment groups were well balanced for baseline characteristics within each study. At Week 24, for each age subgroup, larger reductions in pooled adjusted-mean HbA_{1c} change from baseline were observed with SAXA 5 mg, compared with placebo (Table). Adverse events (AEs) rates were similar with SAXA 5 mg compared with placebo in both older and younger patients.
Conclusion: The combined analysis of saxagliptin monotherapy and add-on therapy trials demonstrated similar efficacy (HbA_{1c} reductions) and tolerability in younger (<65 years) and older (≥65 years) patients with inadequately controlled T2DM.

	<65 years of age		≥65 years of age	
	Pooled SAXA 5 mg (*n*=723)*	Pooled placebo (*n*=643)*	Pooled SAXA 5 mg (*n*=138)*	Pooled placebo (*n*=136)*
HbA_{1c}**	-0.68 [-0.75, -0.61]	-0.01 [-0.09, 0.07]	-0.73 [-1.04, -0.42]	-0.17 [-0.45, 0.10]
Difference from placebo	-0.67 [-0.77, -0.56]		-0.55 [-0.97, -0.14]	

*Total of evaluable patients for the HbA_{1c} efficacy endpoint.
**Adjusted-mean % change from baseline [95% two-sided confidence intervals].

Supported by: AZ/BMS Clinical Research Programme

767

Albiglutide, a long-acting GLP-1-receptor agonist, for the treatment of type 2 diabetes: an analysis of gastrointestinal adverse events over time

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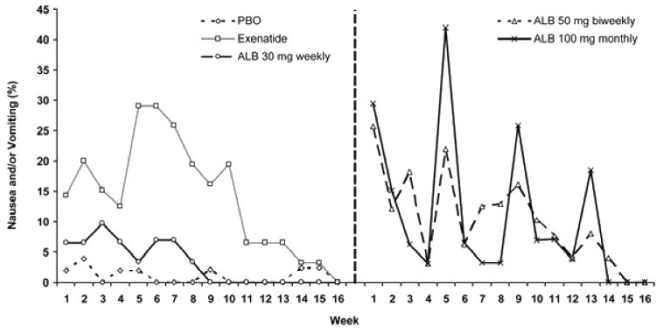
Background and aims: Gastrointestinal (GI) adverse events can limit adherence to GLP-1-receptor agonist therapies. The goal of this analysis was to systematically assess the time course of nausea or vomiting following treatment with albiglutide (ALB), a GLP-1-receptor agonist suitable for once-weekly or less-frequent dosing.
Materials and methods: A 16-week randomized, multicenter, double-blind, parallel-group study was conducted. A total of 356 subjects (mean age 54 years, BMI 32.1 kg/m^2) with type 2 diabetes (mean duration 5 years, HbA_{1c} 8.0%) received placebo, ALB [weekly (4, 15 or 30 mg), every other week (biweekly; 15, 30 or 50 mg) or monthly (50 or 100 mg)], or exenatide twice daily (open-label reference arm administered per label) over 16 weeks. Rates of nausea or vomiting were captured on a weekly basis.
Results: The incidence of nausea and/or vomiting was 11.8% with placebo and 45.7% with exenatide (used as a clinical reference to put ALB-related nausea/vomiting in perspective). The incidence of nausea and/or vomiting reported for the maximum dose in each ALB schedule was: 29% for 30 mg weekly; 54.3% for 50 mg biweekly; and 55.9% for 100 mg monthly. Of note, the percentage of subjects receiving ALB having nausea or vomiting for more than 7 days ranged from 0-15.6%, compared with 31.4% with exenatide. All nausea or vomiting events for 30 mg weekly ALB were mild, and > 90% of events with 50 mg biweekly or 100 mg monthly dosing were mild or moderate. In the exenatide group, 63.6% of events were mild and 36.4% were moderate. The incidence of nausea or vomiting was lower with more frequent, smaller ALB doses than with less frequent, larger ALB doses. Nausea and/or vomiting correlated with ALB exposure and clearly decreased over time. There was a low incidence of nausea or vomiting in the 30 mg weekly dose of ALB, with no nausea or vomiting reported after week 8. The 50 mg biweekly dose was generally associated with a low incidence of nausea and/or vomiting that decreased gradually over time. For the 100 mg monthly dose, rates of nausea and/or vomiting increased following each injection but markedly dissipated during the interim period between injections. Rates of nausea and/or vomiting for exenatide increased after 4 weeks, when uptitration from 5 μg to 10 μg occurred. Of the 2 symptoms, the incidence of nausea predominated; vomiting followed a similar time course, but with lower incidence. However, more vomiting (with or without nausea) than nausea alone was observed for the 100 mg monthly dose and was associated with the occurrence of peak plasma ALB concentrations.
Conclusion: Weekly 30 mg ALB administration shows a favorable GI tolerability profile as compared with other ALB regimens and exenatide, and in a manner associated with the level of exposure.



Supported by: GlaxoSmithKline

768

Safety and tolerability of exenatide BID in patients with type 2 diabetes: integrated analysis of 3854 patients from 11 comparator controlled clinical trials

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Background and aims: Exenatide BID (Ex) is a first-in-class GLP-1 receptor agonist approved for the treatment of type 2 diabetes (T2DM). The objective of this integrated analysis was to further examine the safety profile of Ex (5 and 10 μg) compared to a population of pooled comparators (PC) treated with placebo or insulin in T2DM patients.
Materials and methods: Data from 11 completed, randomised, controlled clinical trials including 3854 patients followed for 12 to 52 weeks were pooled and analyzed. Incidence rates (IR), exposure-adjusted incidence rates (EAIR) per 100 patient-years (PY), and 95% confidence intervals (CI) around differences (Diff) between cohorts were calculated for adverse event (AE), system organ class by preferred term, and serious AEs.
Results: Baseline demographics were comparable between cohorts (Ex: N=2251; PC: N=1603; 53-55% M; age 56 y; BMI 31-32 kg/m^2; HbA_{1c} 8.3-8.4%; mean days exposure time Ex: 172; PC: 176). The overall IRs of serious AEs were 3.6% vs 3.5% and non-serious AEs were 79% vs 64% for Ex-treated and PC groups, respectively. Transient, mild-to-moderate nausea (Ex: 39%; PC: 9%) was the most frequent AE. Other AEs (≥5%) included vomiting (Ex: 13%; PC: 3%), diarrhea (Ex: 10%; PC: 4%), nasopharygitis (Ex: 8%; PC: 8%), headache (Ex: 8%; PC: 7%), upper respiratory infection (Ex: 7%; PC: 6%), and dizziness (Ex: 6%; PC: 3%). Discontinuations due to AEs were 8% and 2% for the Ex-treated and PC groups, respectively. The IR of mild-moderate hypoglycaemia without concomitant sulphonylurea (SU, Ex: 9%; PC: 8%) or with SU (Ex: 37%; PC: 31%) was comparable between groups. Severe hypoglycaemia was rare [No SU: Ex: 0.2%; PC: 0.5% vs SU: Ex: 0.5%; PC: 0.5%]. Finally, composite EAIRs (rates per 100 PY) were not statistically different between treatment groups for pancreatitis (Ex: 0.28; PC: 0.26; Diff: 0.025 [-0.46, 0.51]), biliary disease (Ex: 0.66; PC: 0.65; Diff: 0.017 [-0.74, 0.77]), and renal impairment (Ex: 1.7; PC: 1.8; Diff: -0.11 [-1.3, 1.13]).
Conclusion: Overall, based on this integrated analysis of 3854 patients representing 1058 PY of Ex exposure, exenatide BID was well tolerated in patients with T2DM.

769

Vildagliptin therapy is not associated with an increased risk of pancreatitis

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Background and aims: Vildagliptin is a potent and selective DPP-4 inhibitor that has been shown to enhance the physiological effects of incretin hormones thereby increasing islet alpha-and beta-cell responsiveness to glucose. Patients with type 2 diabetes may be at increased risk of pancreatitis compared to patients without diabetes and whether the use of pharmacological agents may exacerbate this risk remains unclear. This work aimed at assessing whether treatment with the DPP-4 inhibitor vildagliptin is associated with an increased risk of pancreatitis in patients with type 2 diabetes in a large clinical database.
Materials and methods: Safety data from 24 phase 2 and 3 double-blind controlled clinical trials (monotherapy and combination therapy; ranging in duration from 12 to more than 104 weeks) were pooled. A meta-analysis using the Peto fixed effects odds ratio was performed to compare vildagliptin treatment groups (doses of 50 mg qd and 50 mg bid; n= 7087) to the all comparators group (placebo and active-controlled treatment groups pooled, n= 4880) with respect to the risk of pancreatitis related AEs. Selected preferred terms for pancreatitis-related AEs were used for this analysis.
Results: The incidences in controlled clinical trials and odds ratios for pancreatitis-related events are shown in Figure 1.The odds ratio for pancreatitis-related AEs was < 1 for vildagliptin 50 mg qd and for vildagliptin 50 mg bid (OR = 0.90 and 0.78, respectively), indicating no increased risk relative to all comparators.
Conclusion: There was no evidence of an increased risk of pancreatitis-related AEs following treatment with the DPP-4 inhibitor vildagliptin at the marketed doses of 50 mg qd and 50 mg bid relative to the all comparators group.

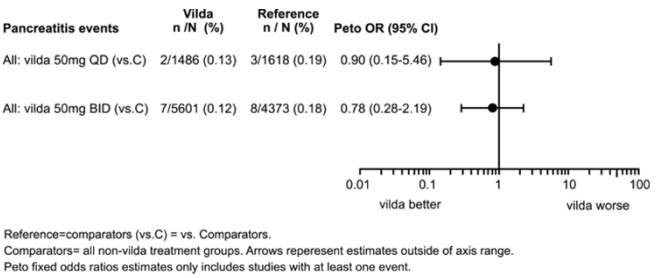

Fig 1. Incidence and odds ratio of pancreatitis-related AEs (all studies safety population)

Supported by: Novartis Pharmaceuticals

770

Surveillance of lipase and amylase levels in type 2 diabetes patients assessed during a randomized clinical study: the EGO study experience

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Background and aims: Serum lipase and amylase elevations may be diagnostic for pancreatitis yet their incidence and prevalence in patients with type 2 diabetes mellitus (T2DM) is not well characterized. Aims of this analysis included 1) cross-sectional analysis of lipase and amylase levels in a cohort of 440 patients with T2DM but without signs or symptoms of pancreatitis or other gastrointestinal (GI) disease and 2) longitudinal surveillance in those patients who participated in the EGO study (clinicaltrials.gov ID: NCT00630825) of the long-acting, glucagon-like peptide-1 (GLP-1) analog LY2189265 (LY).
Materials and methods: EGO was a double-blind, randomized phase 2 clinical trial comparing LY versus placebo in overweight/obese patients with suboptimal control of T2DM despite therapy with 2 oral anti-hyperglycemic agents. Study participants underwent detailed history and physical examination including specific scoring for GI symptoms and were randomized to once-weekly subcutaneous injections for 16 weeks of placebo; LY 1.0 mg; LY 0.5 mg, 4 weeks then 1.0 mg; or LY 1.0 mg, 4 weeks then 2.0 mg. Simple linear regression was used to test associations between rank order of increase in lipase at endpoint and 21 factors including LY treatment, demographic characteristics, laboratory parameters and concomitant medications. Factors with p<.10 were analyzed by multiple linear regression using backward selection with type I error of .10; two-way interactions were examined for significance with type I error of .10.
Results: Of 440 patients screened [48% female, 57±11 years; baseline HbA1C 8.3±1.6% (mean ±SD)], 13% had elevated lipase (≥60 IU/dL), 5% had elevated amylase (≥112 IU/dL), 16% had either enzyme elevated and 3% had both enzymes elevated; comparable frequencies of elevations were observed for randomized study participants [n=262; 49% female; mean age 57±12 years;

mean HbA1C 8.2±0.9%). At study endpoint increases in amylase and lipase, maximum values and changes in categorical lipase or amylase levels, shown in the table below, were not significantly different between treatment groups (all p>.05). Tobacco non-use at baseline; statin use at baseline; and lower endpoint HbA1C were associated in univariate analysis with increase in lipase during study and HbA1C (p=.090) and tobacco non-use (p=.017) were retained in the final multivariate model. Replacing HbA1C with LY in the final model did not result in statistical significance for LY (p=.41).

Amylase and Lipase Measurements

Assessment	Treatment	Baseline Mean (SD), IU/dL	Endpoint Change Mean (SD), IU/dL	Endpoint Maximum Value, IU/dL	Became abnormal during study N (%)	Normalized during study N (%)
Lipase	Placebo	40 (18)	13 (64)	513	8/59 (13.6)	1/3 (33.3)
	0.5/1.0mg LY	40 (14)	20 (87)	709	13/58 (22.4)	1/3 (33.3)
	1.0/1.0mg LY	45 (33)	20 (53)	439	9/49 (18.4)	5/11 (45.5)
	1.0/2.0mg LY	47 (32)	14 (63)	455	11/49 (22.4)	5/12 (41.7)
Amylase	Placebo	57 (21)	6 (18)	177	4/58 (6.9)	0/4
	0.5/1.0mg LY	58 (27)	10 (34)	261	4 /54 (7.4)	1/3 (33.3)
	1.0/1.0mg LY	57 (33)	10 (30)	215	5/53 (9.4)	0/2
	1.0/2.0mg LY	61 (26)	9 (21)	153	6/51 (11.8)	1/5 (20.0)

Conclusion: Elevations in lipase and amylase occur at greater frequency in this population than may be expected and were observed irrespective of treatment. Prospective evaluations are warranted to explore the potential clinical significance of these findings.

771

Exenatide significantly ameliorates increases in pancreatic enzyme secretion in a rat model of acute pancreatitis

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Background and aims: Exenatide (Ex) is a GLP-1 receptor agonist that produces similar glucoregulatory actions to the endogenous incretin GLP-1, via enhancement of glucose-dependent insulin secretion, regulation of gastric emptying, decreased postprandial glucagon secretion and decreased food intake. CCK-8 is a peptide hormone secreted from the duodenum that physiologically stimulates release of digestive enzymes. Caerulein (CRN) is a decapeptide agonist of CCK receptors, which potently stimulates gastric, biliary and pancreatic secretion and is known as an agent that induces acute pancreatitis in experimental animal models. The present study examined the effect of exenatide on basal, CCK-stimulated and CRN-hyperstimulated pancreatic enzyme secretion and pancreatic morphology in rats.
Materials and methods: Fasted, male, anesthetised Sprague Dawley (SD) rats were injected subcutaneously with saline or Ex (0.3, 1 or 3μg/kg) 15 minutes before saline (100μl), CCK-8 (10μg/kg) or CRN (10μg/kg) IP administration. Plasma amylase and lipase concentrations were measured for up to 6 hours after IP injections (t= -15, 0, 5, 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min). At completion of the experiment, pancreata were weighed and prepared for histological evaluation.
Results: Exenatide had no significant effect on fasting basal (unstimulated) plasma amylase and lipase activity over the 6-hour time period. Following CCK-8 injection, 3μg/kg Ex showed a tendency to reduce plasma amylase and lipase secretion compared to saline controls, but the reduction was not statistically significant (amylase AUC_{180} 107929 ± 8401U/L (Ex) vs 136369 ± 14029U/L (sal), P=0.12; lipase AUC_{180} 6982 ± 1851U/L (Ex) vs 14957 ± 4546U/L (sal), p=0.15). Following CRN injection, 3μg/kg Ex significantly reduced CRN-induced increases in plasma amylase (AUC_{180} 212730 ± 16675U/L (Ex) vs 331654 ± 36241U/L (sal), P<0.05 ANOVA: Dunnett's test) and lipase (AUC_{180} 39428 ± 5149U/L (Ex) vs 79710 ± 13566U/L (sal), P<0.05 ANOVA: Dunnett's test). CRN induced very mild changes in pancreatic acinar cell vacuolisation and necrosis and exenatide did not significantly change these responses.
Conclusion: Acute treatment with exenatide at doses up to 30-fold those given therapeutically to lower glucose did not change basal pancreatic amylase or lipase secretion in non-diabetic SD rats. Furthermore, exenatide significantly suppressed the increases in pancreatic enzyme activity observed in this rat model of acute, caerulein-induced pancreatitis.

772

Efficacy and safety of early combination of vildagliptin and metformin in comparison to placebo in patients with type 2 diabetes

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Background and aims: To reach treatment goals in type 2 diabetes early combination of antidiabetic drugs without the risk of hypoglycemia is requested. As promising new approach, inhibiting of dipeptidyl peptidase-4 (DPP-4) enhances endogenous glucagon-like peptide-1 activity, improves β-cell insulin secretion, and suppresses hyperglucagonemia and excessive hepatic glucose output. The current study investigated the use of early combination of the highly selective DPP-4 inhibitor vildagliptin in patients inadequately controlled with metformin.
Materials and methods: In this double-blind, randomized, placebo-controlled 24 weeks study with 12 weeks open-label extension 405 patients (HbA1c 6.8-8.0%) have been randomized (ratio 2:1 vildagliptin:placebo). Patients with 18-85 years of age were pre-treated with the maximal tolerated dose of metformin for at least 3 months. During the double-blind and open-label period the dose of vildagliptin was 100 mg o.a.d.
Results: The primary endpoint, change in HbA1c-levels after 24 weeks versus placebo, could be documented in 395 patients. Mean HbA1c-levels were reduced from 7.3% to 6.9% under vildagliptin. In contrast, HbA1c-levels slightly increased from 7.2% to 7.4% in the placebo group. The difference of 0.5% between groups was statistically significant (p<0.0001). FPG-values declined under vildagliptin from 123 mg/dl to 114 mg/dl and from 124 mg/dl to 122 mg/dl under placebo. During the following 12 weeks open-label extension under vildagliptin HbA1c-levels and FPG-levels kept stable. 31 % of patients treated with vildagliptin did reach HbA1c-levels below 6.5% versus 18% under placebo. Rate of SAEs was 5.2% in the vildagliptin group versus 9.4% under placebo. There were no significant changes in liver enzymes in either group. Mild hypoglycemia was reported in 0.4% of patients under vildagliptin and 0.8% of patients under placebo. No severe hypoglycemia occurred during the study.
Conclusion: Early combination of vildagliptin with metformin is a well tolerated and efficacious treatment option of patients with type 2 diabetes.
Supported by: Novartis Pharma

773

Lack of vildagliptin effects on the immune system

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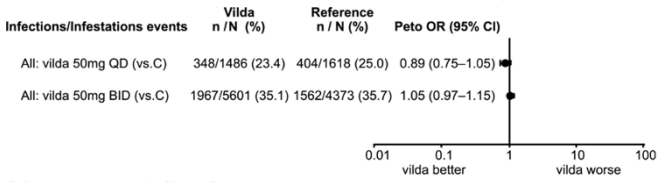
Background and aims: Vildagliptin is a potent and selective DPP-4 inhibitor that improves overall glycemic control in patients with type 2 diabetes by reducing both fasting and postprandial glucose levels via GLP-1's effect to increase the sensitivity to glucose of the α- and β-cells of the pancreas. Since DPP-4 is also known as CD26, an effect on the immune system was recognized as a potential side effect of DPP-4 inhibition from the beginning of the development of vildagliptin, particular efforts were made in both the animal toxicology studies and human studies to identify any potential effects of vildagliptin on the immune system.
Materials and methods: The assessment of the immune system's capability to respond to foreign proteins with the generation of specific antibodies was carried out according to the European guideline for repeated dose toxicity testing of medicinal products in rats at doses of 0,50,225, and 900 mg/kg/day. The exposure resulting from a dose of 900 mg/kg/day is 135 times the exposure resulting from the maximum 100 mg daily dose in man. In human studies, the most sensitive and relevant immune system parameter is the infection rate. A meta-analysis using Peto fixed effects odds ratio was performed to compare vildagliptin (at doses of 50 mg qd and 50 mg bid) to the all comparators group (placebo and all active comparators pooled) using adverse events in the System Organ Class of Infections and Infestations from double-blind clinical trials.
Results: In rats, vildagliptin at daily oral doses up to 900 mg/kg was well tolerated and did not affect the capability of the immune system to respond

to foreign proteins with the generation of specific antibodies. Furthermore, in all other toxicity studies in mice, dogs and monkeys no effects related to the immune system were identified. In human studies where patients on vildagliptin doses of 50 mg qd or 50 mg bid were studied there were also no immune system signals. As can be seen from the figure below vildagliptin was not associated with an increased risk of infections and infestations relative to all comparators, the odds ratios being 0.89 (95% CI 0.75-1.05) and 1.05 (95% CI 0.97 - 1.15) for vildagliptin 50 mg qd and 50 mg bid, respectively.
Conclusion: Based on animal and human data there is no evidence to suggest that vildagliptin treatment is associated with any adverse effects related to the immune system.



Reference=comparators (vs.C) = vs. Comparators.
Comparators= all non-vilda treatment groups. Arrows reperesent estimates outside of axis range.
Peto fixed odds ratios estimates only includes studies with at least one event.

Supported by: Novartis Pharmaceuticals

PS 61 Incretins pharmacology and pharmacokinetics

774

Comparative pharmacology of the Dipeptidyl Peptidase-4 (DPP-4) metabolites of GLP-1 and the GLP-1 analogue, liraglutide
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Background and aims: The human incretin, glucagon-like peptide-1 (GLP-1), is a glucoregulatory peptide secreted by intestinal L-cells. Mimetics of GLP-1 fall into two structural classes, classified by their peptide backbones. The "exendin class" comprises analogs of exendin-4, a peptide derived from the saliva of *Heloderma suspectum*, and includes exenatide, which is approved for the treatment of type 2 diabetes mellitus (T2DM). The "GLP-1 class" comprises analogs of human GLP-1 peptide, and includes liraglutide, a drug under clinical and regulatory evaluation as a potential T2DM therapy. While exenatide and liraglutide are both GLP-1 receptor (GLP-1R) agonists, they are pharmacologically distinct. For example, exenatide and liraglutide differ in their susceptibility to proteolytic cleavage and inactivation by DPP-4, which removes the N-terminal two amino acids from GLP-1 and liraglutide, but not from exenatide.
Materials and methods: The *in vitro* pharmacological activity of the DPP-4 metabolite of liraglutide, (γ-L-glutamyl)(N-α-hexadecanoyl)-Lys^{26}Arg^{34}-GLP-1 (9-37), was compared to the DPP-4 metabolite of GLP-1, GLP-1 (9-36 amide), in the GLP-1R-expressing rat thyroid C-cell line, 6-23 (ATCC), and in CHO cells stably transfected with the human GLP-1R and β-arrestin (DiscoverX, Freemont, CA), by measuring cAMP production and β-arrestin recruitment, respectively.
Results: In both cell assays, (γ-L-glutamyl)(N-α-hexadecanoyl)-Lys^{26}Arg^{34}-GLP-1 (9-37) and GLP-1 (9-36 amide) were qualitatively and quantitatively similar. Neither metabolite demonstrated appreciable GLP-1R agonist activity. However, both GLP-1 (9-36 amide) and (γ-L-glutamyl)(N-α-hexadecanoyl)-Lys^{26}Arg^{34}-GLP-1 (9-37) demonstrated functional GLP-1R antagonism with apparent K_b values of 1.49-2.10 and 0.42-0.95 μM, respectively.
Conclusion: Proteolysis of liraglutide by DPP-4 yields a biologically active metabolite, (γ-L-glutamyl)(N-α-hexadecanoyl)-Lys^{26}Arg^{34}-GLP-1 (9-37), which, like GLP-1 (9-36 amide), is a functional antagonist of GLP-1 *in vitro*. Further studies on the effects of the liraglutide metabolite, (γ-L-glutamyl)(N-α-hexadecanoyl)-Lys^{26}Arg^{34}-GLP-1 (9-37), *in vivo* are warranted.

775

Liraglutide, the once-daily human GLP-1 analogue, has a protracted profile based on both delayed absoprtion and a long plasma half-life
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Background and aims: Liraglutide is a once-daily human GLP-1 analogue that has been developed for the treatment of type 2 diabetes and obesity. Liraglutide has a unique structure with a simple fatty acid and a spacer attached covalently to the peptide backbone (C16-γGlu-Lys26,Arg34GLP-1(7-37)) that provides both delayed absorption and long plasma half-life. The mechanism for the protraction is based on app. 99% binding to albumin, providing the long plasma half-life, and heptamer formation in the formulation, providing a delayed absorption. Also, DPP(Di-Peptidyl Peptidase)-IV stability is obtained. We designed a series of analogues of liraglutide that provides insight into the mechanism of protraction by investigating the importance of the length of the fatty acid.
Materials and methods: The pharmacokinetic profiles were measured in pigs. DPP-IV stability was measured by mass spectroscopy. Binding to albumin was measured indirectly, by adding increasing amounts of albumin to a GLP-1 receptor binding assay. Multimer formation was assessed by circular dicroism spectroscopy.
Results: Pharmacokinetic experiments in pigs illustrate how the length of the fatty used is an important parameter for obtaining protraction, both when looking at delayed absorption and plasma half-life. Decreasing the length of the fatty acid decreased half-life after s.c administration (16, 5.1 and 0.8h) and Tmax (7, 1.3 and 0.9h), as well as half-life after i.v. administration (10, 1.7

and 0.3 h) for liraglutide (C16), C11- and C10-analogs, respectively. Stability towards degradation by the DPP-IV enzyme correlated with the length of the fatty acid. The half-life for DPP-IV degradation in vitro was 2.4 min. for GLP-1, and 937, 383, 156 and 108 min. for liraglutide, C14-, C12-, C10-analogs, respectively. Binding to albumin decreased with the length of the fatty acid, measured as the shift in receptor activation by addition of 4% albumin (45, 45, 41, 14 for liraglutide, C14, C12, C10). The un-acylated analogue did not bind to albumin. The apparent K_d for multimer formation in the formulations increased by decreasing fatty acid length. $K_{d,app}$ was <0.1 µmol/l for liraglutide, and 2, 10 and 50 µmol/l for C14, C12 and C10, respectively.
Conclusion: The protraction mechanism for liraglutide includes albumin binding, heptamer formation and DPP-IV stability, resulting in delayed absorption and long plasma half-life, allowing once-daily dosing in humans.

776

Effect of the GLP-1 receptor agonist AVE0010 on the absorption of concomitant oral drugs: acetaminophen and ethinylestradiol/levonorgestrel
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Background and aims: Glucagon-like peptide-1 (GLP-1) receptor agonists slow gastric emptying, which may alter the rate or extent of absorption of oral drugs. The present study looked at the potential influence of AVE0010 - a new GLP-1 receptor agonist under development for the treatment of type 2 diabetes mellitus - on the absorption of acetaminophen or an oral contraceptive in healthy volunteers.
Materials and methods: The effect of 10 µg AVE0010 on the absorption of 1 g acetaminophen (AC; used as a probe drug for gastric emptying) or an oral contraceptive (OC; 0.03 mg ethinylestradiol [EE]/0.15 mg levonorgestrel [LN]) was assessed in two single-centre, single-blind, placebo (PBO)-controlled, randomised, 5-treatment, 5-period, crossover studies in healthy subjects. In Study 1 (n=15; male and female), AVE0010 or PBO were administered subcutaneously 30 min before breakfast and a single oral AC dose was given -1, +1, or +4 h relative to AVE0010 and -1 or +1 h relative to PBO, with 2-day washout periods between each of the 5 schedules. In Study 2 (n=25 post-menopausal women), AVE0010 or PBO were administered 30 min before dinner/breakfast and a single oral OC dose given in accordance with the following 5 schedules: 1) PBO 8 pm Day 1, OC 7 am Day 2, PBO 8 am Day 2; 2) AVE0010 8 pm Day 1, OC 7 am Day 2, PBO 8 am Day 2; 3) AVE0010 8 pm Day 1, OC 7am Day 2, AVE0010 8 am Day 2; 4) PBO 8 pm Day 1, AVE0010 8 am Day 2, OC 9 am Day 2; 5) PBO 8 pm Day 1, AVE0010 8 am Day 2, OC 12 noon Day 2; with a 7-day washout period between each schedule. All subjects underwent 24-h (Study 1) or 72-h (Study 2) blood sampling to determine drug concentrations. Pharmacokinetic (PK) parameters estimated included area under the plasma concentration-time curve (AUC), maximum plasma concentration (C_{max}) and time to C_{max} (t_{max}), and terminal half-life ($t_{½}$). Between-schedule ratios for AUC and C_{max} were expressed as means with 90% confidence limits (CI). Lack of PK interaction was demonstrated if the lower CI for AUC and C_{max} was greater than the 0.80 non-inferiority specification value.
Results: In Study 1, the absorption of AC was unchanged when given 1 h before AVE0010. When given 1 or 4 h after AVE0010, the C_{max} of AC decreased to 71% [57%, 87%] and 69% [56%, 85%], respectively, of the PBO values, whereas the AUC parameters were unchanged. Median t_{max} increased from <1 to 4.5 h when AC was given 1 h after AVE0010. Food slowed AC absorption, with a reduction in PBO post-meal C_{max} to 52% [42%, 64%] of pre-meal values and an increase in median t_{max} (0.25 to 2 h). In Study 2, EE and LN absorption rates were unchanged when OC was given 11 h after or 1 h before AVE0010. When given 1 or 4 h after AVE0010, t_{lag} (time to concentration exceeding lower limit of quantitation) and t_{max} were increased and C_{max} was reduced to 48% [43%, 53%] (1 h) or 61% [56%, 68%] (4 h) of control values for EE, and 54% [48%, 62%] or 80% [70%, 92%] of control values for LN. AUC parameters and $t_{½}$ were unchanged. AVE0010 was well tolerated in both studies.
Conclusion: These results are consistent with a slowing of gastric emptying by AVE0010. The absorption of AC was completely unaltered if it was administered 1 h before AVE0010, and the absorption of OC was unaltered if it was administered 11 h after or 1h before AVE0010. However, the rate of absorption of AC or OC was altered when given 1 or 4 h after AVE0010. Total drug exposure was unchanged.
Supported by: sanofi-aventis

777

Liraglutide pharmacokinetic profile following s.c. dosing is unaltered by co-administration with sitagliptin in Göttingen minipigs
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Background and aims: Liraglutide is a once-daily human Glucagon-Like Peptide-1 (GLP-1) analog that has completed phase 3 clinical testing. Liraglutide is a fatty-acid derived analog of human GLP-1 that binds to albumin as its main mechanism of protraction. Liraglutide is also stabilized against the DPP-IV enzyme. However, liraglutide is subject to a minor degradation *in vivo*, and thus it is interesting if co-administration with a DPP-IV inhibitor lead to increases in liraglutide concentrations. Sitagliptin is a DPP-IV inhibitor that increases endogenous levels of GLP-1, which is the main mechanism for its blood glucose lowering ability. Since both compounds lead to increases in GLP-1-like concentrations (either liraglutide or native GLP-1), we investigated if they could be combined by investigating the pharmacokinetics of liraglutide following co-administration of liraglutide and sitagliptin.
Materials and methods: The study was carried out in minipigs because only pigs have a similar absorption of injectable peptide analogs as compared to humans. Sitagliptin is dosed once daily PO (100 mg) in patients, resulting in a steady state trough plasma concentration of 41 ng/mL. Liraglutide is dosed once-daily SC (1.2 or 1.8 mg). The study was conducted in two parallel groups (+/- sitagliptin) of mini-pigs (n=4). Both groups were dosed SC with liraglutide in a dose of 2 nmol/kg, equivalent to app. 0.6 mg pr 75 kg. The sitagliptin group was dosed three times daily in order to obtain a target steady state trough plasma concentration of sitagliptin above 40 ng/mL.
Results: The key pharmacokinetic parameters (mean ±SD) of liraglutide are given in Table 1. Sitagliptin had no significant effect on the pharmacokinetics of liraglutide as evaluated by T_{max}, C_{max}, AUC/Dose and half-life. The mean plasma concentration of sitagliptin was verified to be above 40 ng/mL.
Conclusion: In conclusion, the DPPIV inhibitor sitagliptin did not alter the pharmacokinetics of liraglutide following SC administration in mini-pigs.

Table 1

Treatment	T_{max} [hr]	C_{max} [pM]	AUC/Dose [hr·pM/pmol/kg]	T½ [hr]
- Sitagliptin	8.5 ±2.5	18000 ±3700	278 ±32	22 ±2.6
+ Sitagliptin	8.0 ±2.8	19600 ±2300	322 ±53	20 ±1.4
p-value (t-test)	0.80	0.52	0.21	0.20

778

Inhaled GLP-1 and exenatide: different effects on pancreatic and gastric activity following a single dose in type 2 diabetes mellitus
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[1]MannKind Corporation, Valencia, United States, [2]PRA International, Zuidlaren, Netherlands.

Background and aims: MKC253 is GLP-1 adsorbed onto Technosphere microparticles for oral inhalation. A single MKC253 dose in healthy, normal volunteers produced high circulating concentrations of GLP-1 (10 min) with a dose-dependent insulin release, a reduction in fasting plasma glucose, and no nausea or vomiting. This double-blind, double-dummy, placebo- and active-control crossover trial compared the effects of MKC253 (1.5 mg) and s.c. injection of exenatide (EXE; 10 µg) on postprandial glucose (PPG) excursions, postprandial insulin, gastric emptying, and GLP-1 pharmacokinetics.
Materials and methods: Included were 20 nonsmoking subjects with type 2 diabetes mellitus (HbA_{1c} 6.2%-8.5%) on a stable oral antidiabetic regimen. Subjects received five treatments 2 days apart. All received MKC253 or inhaled placebo (InhP) while fasting. EXE or s.c. placebo (SCP) was given 15 min premeal, and MKC253 or InhP was given premeal (Pre) or 30 min postmeal (Post). Subjects received in randomized order: SCP + MKC253 Pre + InhP Post; SCP + MKC253 Pre + MKC253 Post; EXE + InhP Pre + InhP Post; or SCP + InhP Pre + InhP Post. Gastric emptying was measured by absorption of ^{13}C-octanoate from a 575 kcal standardized meal.
Results: MKC253 produced a rapid spike in insulin. Mean maximum insulin concentration occurred <10 min after dosing and fell sharply thereafter. Mean peak insulin was 60 µU/ml. Mean pharmacokinetics of insulin release closely tracked mean GLP-1 kinetics. The insulin response following EXE was slower

and less pronounced. In fasting subjects with baseline glucose <9 mmol/l, MKC253 produced a mean maximal decrease in glucose of 0.75 mmol/l about 30 min after inhalation. Subjects with baseline glucose >9 mmol/l had a 1.2 mmol/l decrease in glucose ≈45 min after inhalation. MKC253 reduced PPG excursions. Compared with InhP, MKC253 Pre decreased PPG by ≥1 mM/l for >1 h and MKC253 Pre + Post decreased PPG for >3 h. Reductions were longer than that predicted by a GLP-1 half-life of <2 min. MKC253 had little or no effect on gastric emptying. In contrast, EXE also reduced PPG but acted by delaying gastric emptying. More than 90% of ^{13}C-octanoate ingested was unabsorbed 4 h after the meal compared with <60% with MKC253. After EXE injection, the insulin response to meal challenge was much smaller than with MKC253. Only 1 of 20 subjects reported nausea with MKC253 vs. rates >66% from literature with injected GLP-1.
Conclusion: Both MKC253 and EXE reduce PPG excursions, but appear to do so by different mechanisms.

779

Response to inhaled GLP-1 is dependent on baseline glucose
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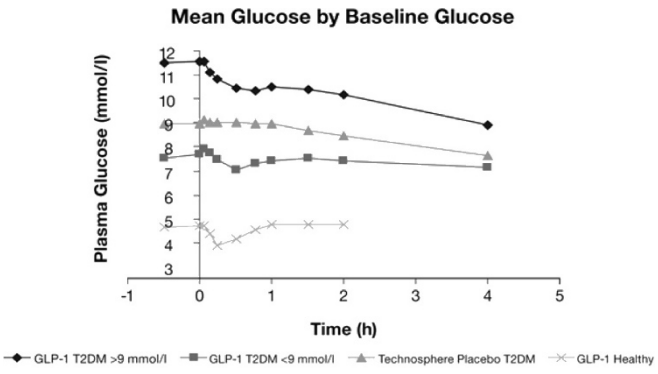
[1]MannKind Corporation, Valencia, United States, [2]PRA International, Zuidlaren, Netherlands.

Background and aims: MKC253 is GLP-1 adsorbed to Technosphere microparticles for oral inhalation. This report presents data from two studies: MKC253001 evaluated the effect of MKC253 under fasting conditions in healthy subjects, and MKC253002 evaluated MKC253 in subjects with type 2 diabetes.
Materials and methods: All subjects received 1.5 mg GLP-1 via inhalation. In MKC253001, six healthy subjects received GLP-1. In MKC253002, 15 subjects with type 2 diabetes received GLP-1, and five subjects with type 2 diabetes received Technosphere placebo.
Results: Of the 15 subjects in MKC253002 who received GLP-1, 11 had baseline glucose >9 mmol/l and four had baseline glucose <9 mmol/l. All subjects were nonsmokers with normal lung function. In healthy subjects, MKC253 produced a transient decrease in glucose of 0.8 mmol/l. Minimum levels occurred approximately 15 min after inhalation of MKC253. Following the decrease, glucose returned to baseline levels by 1 h. The duration of response was much longer than the half-life of GLP-1 (≤2 min). Response to GLP-1 in subjects with type 2 diabetes depended on baseline glucose. Subjects with baseline glucose <9 mmol/l had a mean maximum decrease of 0.75 mmol/l. The time to reach the minimum was about 0.5 h. Although glucose values recovered, they did not return to baseline levels after 4 h. Subjects with baseline glucose >9 mmol/l had a 1.2 mmol/l decrease in glucose. The duration of response was longer--the minimum occurred 45 min after inhalation, with no return from the minimum levels. Placebo subjects had no change in glucose over the first 2 h after inhalation.
Conclusion: It has been shown previously that inhalation of MKC253 produces a sharp spike in plasma insulin. This rapid pulse of insulin can produce a long-lasting decline in plasma glucose in fasting subjects with type 2 diabetes.



780

A phase 1b study of ITCA 650: continuous subcutaneous delivery of exenatide via DUROS® device lowers fasting and postprandial plasma glucose
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[1]Intarcia Therapeutics, Hayward, [2]Medpace, Cincinnati, [3]Cetero, Miami Gardens, FL, [4]Cetero, San Antonio, United States.

Background and aims: Exenatide is a proven effective treatment for type 2 diabetes that improves glucose control and induces weight loss. However, use of exenatide has been limited due to the twice-daily self-injection schedule and frequent nausea which may be associated with peak concentrations of drug. The DUROS® technology is a subcutaneous continuous delivery system which has been utilized in the Viadur® device, an FDA-approved therapy for prostate cancer. It is an osmotic delivery device consisting of a small sterile titanium cylinder (4mm x 45 mm) that is placed subcutaneously for extended periods of time. ITCA 650 is a DUROS device engineered to deliver exenatide at a continuous and consistent rate for treatment durations of 3 to 12 months and over a broad range of dose levels. Preclinical studies have shown that ITCA 650 delivers exenatide at a consistent rate for >6 months with preserved stability of the drug product.
Materials and methods: A phase 1b study to evaluate the safety and tolerability of ITCA 650 treatment is being conducted in subjects with inadequately controlled type 2 diabetes for 28 days. In this study, subjects were randomized to receive either 10 mcg/day or 20 mcg/day of exenatide.
Results: Changes in fasting plasma glucose (FPG) from 24 hours to 2 weeks are indicated in the table below. In addition to changes in FPG, a decrease in the 2-hour postprandial glucose was also observed at two weeks. ITCA 650 was well tolerated primarily with observations of anticipated mild bruising, itching and local pain at the insertion site, as well as transient mild nausea and vomiting in a small number of the subjects. Based on the favorable tolerability observed at the studied doses, higher doses of ITCA 650 are currently under clinical evaluation.
Conclusion: ITCA 650 represents a novel method to deliver exenatide for extended periods of time with 100% compliance for the long-term treatment of type 2 diabetes.

Changes in Fasting Plasma Glucose (FPG)

Dose Arm	Baseline FPG	FPG at 24 hours	FPG at 1 week	FPG at 2 weeks
10 mcg/day (n=12)	161±34 mg/dL	146±30 mg/dL	143±33 mg/dL	139± 36 mg/dL
20 mcg/day (n=11)	170 ±23 mg/dL	145±33 mg/dL	139±28 mg/dL	127±15 mg/dL

781

Safety, tolerability, pharmacokinetics, and insulinotropic activity of single subcutaneous doses of LY2189265, a long-acting glucagon-like peptide 1 analogue in healthy subjects
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Background and aims: LY2189265 is a novel, long-acting glucagon-like peptide 1 (GLP-1) analog being developed for treatment of type 2 diabetes (T2DM). LY2189265 consists of a dipeptidyl peptidase-IV (DPP-IV)-protected GLP-1 analog covalently linked to an Fc fragment of human IgG4, thereby increasing its duration of pharmacological activity. Safety, tolerability, pharmacokinetics (PK), glucose-dependent insulin secretion, and immunogenicity of LY2189265 were evaluated in healthy subjects in this first-time-in-human Phase 1 trial.
Materials and methods: The study was a 3-period, crossover, double-blind, placebo-controlled investigation of single subcutaneous doses of LY2189265 (0.1 mg to 12 mg). Twenty subjects randomly received 2 escalating doses of LY2189265 and placebo given ≥3 weeks apart; 16 received all 3 doses and 4 received 1 dose. The PK profile was assessed over 14 days. Insulinotropic activity was measured following a graded glucose infusion (GGI) and an oral glucose tolerance test (OGTT).
Results: LY2189265 was well tolerated up to 6 mg. The most frequent adverse event was dyspepsia. Headache, injection site irritation, nausea, and vomiting increased with increased dose. Compared with placebo, statistically significant ($p<.01$) dose-dependent increases were observed in supine heart rate at ≥0.3 mg and in supine diastolic blood pressure at ≥1 mg. No case of hypoglycemia occurred. No subject developed antibodies to LY2189265.

The half-life of LY2189265 was ~90 hours in healthy subjects. The maximum concentration (C_{max}) occurred between 24 and 48 hours. With each doubling of dose, the increase in area under the concentration-time curve from 0 to infinity and C_{max} were less than double (1.84-fold and 1.88-fold respectively). There were glucose-dependent increases in insulin secretion following GGI and suppression of serum glucose following OGTT at all doses of LY2189265 compared with placebo.
Conclusion: LY2189265 exhibited expected GLP-1 pharmacology and safety profiles. PK and sustained pharmacodynamic effects support further evaluation of LY2189265 for once-weekly dosing in patients with T2DM.
Page 1 of 2

782

Pharmacokinetics, pharmacodynamics, safety and tolerability of single dose exenatide in very elderly patients (≥75 years) with type 2 diabetes
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Background and aims: This study assessed the effect of exenatide on pharmacokinetic (PK) and pharmacodynamic (PD) parameters, safety and tolerability in very elderly patients with Type 2 Diabetes (T2D).
Materials and methods: This randomised, placebo-controlled, 3-treatment, crossover study compared control (45-65 years [y], n=15) and very elderly (≥75y, n=15) groups. Patients received single subcutaneous doses of placebo, exenatide 5µg or exenatide 10µg, 15min before a standardised breakfast for 3 consecutive days. Serial blood samples were collected for plasma exenatide and serum glucose concentrations. These PK data were further integrated with 6 other clinical pharmacology studies: 139 control (≤65y) and 28 elderly (>65y) subjects to evaluate exenatide clearance in a larger dataset.
Results: Mean ages (±SD) for control and very elderly groups were 57±5.5y and 78±3.3y, respectively; and renal insufficiency was present in 5 control and 15 very elderly patients. Dose-normalised exenatide maximum concentration and $AUC_{0-\infty}$ was not significantly different between groups. Glucose lowering was dose-dependent and comparable between groups. No hypoglycaemia or serious adverse events were reported, and exenatide was generally well tolerated in both groups. The integrated analysis showed that exenatide clearance was significantly related to renal clearance (test of slope=0, p<0.001), with no additional effect from age (Figure 1).
Conclusion: No clinically relevant differences in PK, PD, safety or tolerability were observed in very elderly patients in this study. These data suggest that exenatide clearance is highly correlated to renal clearance, with no additional effect of age on exenatide clearance besides declining renal function. These results warrant no further dosage changes of exenatide in very elderly patients with T2D.

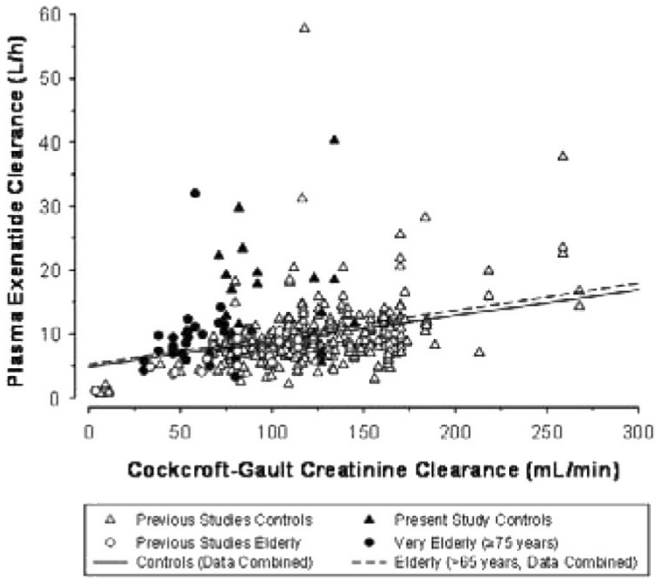

Figure 1: Exenatide Clearance vs. Creatinine Clearance

All authors are employees and stockholders of Eli Lilly and Company

PS 62 Incretins - experimental

783

Postprandial glucose levels and long-term glucose control are improved with taspoglutide, a human once-weekly GLP-1 analogue, in an animal model of type 2 diabetes
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Background and aims: Several epidemiological studies confirmed that elevated PPG is a significant predictor of cardiovascular mortality and morbidity in type 2 diabetes (T2D). In this study post-challenge response following an oral glucose tolerance test (oGTT) was used to evaluate the effect of the human once-weekly GLP-1 analogue, taspoglutide (R1583), after acute and chronic treatment on PPG and long-term glucose control in ZDF rats.
Materials and methods: Acute effects of taspoglutide treatment were measured during an oGTT after single sc administration of taspoglutide (0.1, 1 and 10 µg/kg) in 9-wk-old ZDF rats. Long-term glucose homeostasis was assessed 3 wks after single administration of taspoglutide (1 mg/animal, sc, formulated to mimic human exposure) or vehicle to 5-wk-old ZDF rats (n=6/group) during an oGTT. In addition, PPG levels (4 hrs after food removal) were measured on days 7, 14, and 21 and 1,5-anhydroglucitol (1,5-AG) levels (using GlycoMark®) and HbA1c were assessed at the end of the study.
Results: Acute efficacy measured during an oGTT after single sc administration of taspoglutide (0.1, 1 and 10 µg/kg) in 9-wk-old ZDF rats showed significant improvement in glucose tolerance over 2 hrs (AUC_{Glu}: -15; -49 and -71%) and an increase in insulin secretion (AUC_{Ins}: +128; +236 and +184%) in a dose-dependent manner compared to vehicle. Chronic treatment with taspoglutide significantly reduced glucose excursion during the week 3 oGTT at 30, 60, and 120 min and $AUC_{Glu,0\text{-}120\ min}$ (-50%) without affecting insulin levels as compared with vehicle. PPG levels were significantly decreased in rats treated with taspoglutide in comparison to vehicle at Days 14 and 21. An improvement in long-term glucose control with a significant increase in 1,5-AG levels at Day 21 was also shown after single administration of taspoglutide (7.0±1.0 µg/mL) vs. vehicle: (2.3±0.85: µg/mL; p<0.005). Taspoglutide treatment also improved insulin sensitivity assessed by HOMA (taspoglutide: 20.0±2.6 vs. vehicle: 29.4±5.2) and ISI Matsuda (taspoglutide: 360±38 vs. vehicle: 230±27; p< 0.02).
Conclusion: Taspoglutide showed a significant reduction in PPG levels and long-term glucose control in a model of T2D progression, thus improving a significant risk factor for microvascular and macrovascular complications.

784

Chronic administration of the glucagon-like peptide-1 analogue, liraglutide, delays diabetes onset and improves lipids in a novel model of type 2 diabetes, the UCD-T2DM rat
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Background and aims: Due to the increasing prevalence of type 2 diabetes (T2DM), there is a need to identify strategies for diabetes prevention. We have investigated the efficacy of liraglutide, a human glucagon-like peptide-1 (GLP-1) analog, to prevent or delay T2DM in UCD-T2DM rats, a model of polygenic obese T2DM that is more similar in etiology to T2DM in humans than other existing rodent models.
Materials and methods: At 2 months of age, rats were divided into three groups: control (CTRL), liraglutide (LIRA) and energy restricted (RSTR) (*n*=32). Rats received LIRA (0.2 mg/kg) or vehicle injections BID for up to 10 months. RSTR rats were food restricted to equalize body weights to LIRA rats. Non-fasting blood glucose was measured weekly to determine diabetes onset (<200 mg/dl) and fasting blood samples were taken monthly from all animals. At 6 months of age 16 animals from each group were euthanized for tissue analysis.
Results: Energy intake and body weight at 4 months of age were lower in LIRA and RSTR rats compared with CTRL rats: (CTRL: 103±3 kcal/d, 618±6 g; RSTR: 80±1 kcal/d, 544±5 g; LIRA: 89±2 kcal/d, 549±6, *P*<0.0001, *n*=32).

Greater energy intake in LIRA compared with RSTR rats, despite identical weight gain suggests that LIRA increases energy expenditure. Compared with CTRL, RSTR and LIRA delayed diabetes onset by ~2 and ~3 months, respectively (P<0.01). Mean ages of diabetes onset were: CTRL 4.9±0.5, RSTR 6.9±0.5, and LIRA 7.8±0.5 months. By 6.5 months of age, only 3/32 LIRA treated animals had become diabetic, whereas 12/32 RSTR and 26/32 CTRL animals were diabetic. Thus, LIRA significantly delayed diabetes onset compared with both the CTRL and RSTR groups (P<0.05). At 6 months of age, fasting plasma glucose and HbA1c concentrations were higher in CTRL (180±27 mg/dl; 9.1 ± 0.6%) compared with both LIRA (119±8 mg/dl; 5.5 ± 0.4%) and RSTR (121±5; 4.9 ± 0.2%) animals (all P< 0.001). Plasma triglycerides (TG) were reduced in LIRA treated (93 ± 5 mg/dl) compared with either CTRL (137 ± 10 mg/dl) or RSTR (175 ± 16 mg/dl) animals (P<0.05). Fasting plasma insulin at 4 months of age was lower in LIRA (1.4 ± 0.1 ng/ml) treated compared with either CTRL (2.9 ± 0.2 ng/ml) or RSTR (2.6 ± 0.2 ng/ml) animals (P<0.001). After excluding animals that had become diabetic and started losing weight before euthanasia, mesenteric and total white adipose tissue weights were lower in LIRA (8.0 ± 0.6g, 74 ± 4g, n=14) and RSTR (8.2 ± 0.4g, 81 ± 5g, n=14) compared with CTRL (11.1 ± 0.5g, 98 ± 4g, n=6) animals (P<0.05), but when expressed as percent body fat only LIRA was significantly lower compared to CTRL (CTRL: 15.1 ± 0.6%, RSTR: 14.1 ± 0.7%, LIRA: 12.8 ± 0.5%, P<0.05). Liver TG content was significantly lower in LIRA (13.2 ± 1.1 mg/g liver) and RSTR (13.0 ± 1.7 mg/g liver) compared to CTRL (23.0 ± 1.4 mg/g liver) animals (P<0.01).

Conclusion: Liraglutide delays the development of diabetes in UCD-T2DM rats and the delay in onset likely involves effects on food intake, weight gain, insulin sensitivity and energy and lipid metabolism. We are currently investigating other potential mechanisms including effects on islet morphology and function.

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785

Effects of long-term dipeptidyl peptidase-IV inhibiton on body composition and glucose tolerance under high-fat diet in mice

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Background and aims: Incretins are a group of peptide hormones released from the gastrointestinal tract in response to meal ingestion and potentiate glucose-stimulated insulin secretion. Glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP) are major incretins. In addition to their action on the enteroinsular axis, GLP-1 decreases body weight through suppression of appetite, while GIP directly facilitates energy accumulation in adipose tissue. DPP-IV inhibitor increases plasma active GLP-1 and GIP. However, the magnitude of the effects of enhanced GLP-1 and GIP signaling by long-term DPP-IV inhibition on body weight and insulin secretion has not been determined. In this study, we compared the effects of long-term DPP-IV inhibition on body composition and insulin secretion of high fat diet (HFD)-fed wild-type (WT) and GLP-1R knockout (GLP-1R$^{-/-}$) mice.

Materials and methods: WT and GLP-1R$^{-/-}$ mice (7-week-old) were fed high fat chow with or without DPP-IV inhibitor by drinking water. Body weight and food intake were measured during 8 weeks of the study. After 8 weeks, Oral glucose tolerance test (OGTT) and CT-based analysis of body composition were performed. And batch incubation study for insulin secretion and mRNA expression of incretin (GLP-1 and GIP) receptors were measured using isolated islets from WT and GLP-1R$^{-/-}$ mice treated with or without DPP-IV inhibitor.

Results: DPP-IV inhibitor had no effect on food and water intake and body weight, but increased body fat mass in DPP-IV inhibitor-treated GLP-1R$^{-/-}$ mice compared to that of untreated GLP-1R$^{-/-}$ mice. DPP-IV inhibitor-treated WT and GLP-1R$^{-/-}$ mice both showed increased insulin secretion and improved glucose tolerance in OGTT. In isolated islets of DPP-IV inhibitor-treated WT and GLP-1R$^{-/-}$ mice, insulin secretion in response to 11.1mM glucose was increased and insulin secretion in response to GLP-1 or GIP was preserved compared to those in DPP-IV inhibitor-untreated WT and GLP-1R$^{-/-}$ mice. The mRNA expression levels of GLP-1 and GIP receptors in the islets of DPP-IV inhibitor-treated and untreated WT mice were similar, as were the mRNA expression levels of GIP receptor in the islets of DPP-IV inhibitor-treated and untreated GLP-1R$^{-/-}$ mice.

Conclusion: Long-term DPP-IV inhibition does not alter body composition, possibly due to the counteracting effects of enhanced GLP-1 and GIP, but does improve glucose tolerance by the synergistic insulinotropic effects of enhanced GLP-1 and GIP, as well as by improved glucose responsiveness in pancreatic islets.

786

Novel fatty acid derivatised forms of glucose-dependent insulinotropic polypeptide with improved glucose-lowering and insulin-releasing properties

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Background and aims: Glucose-dependent insulinotropic polypeptide (GIP) is a physiological insulin-releasing hormone. However, metabolic instability, rapid renal clearance and relative ineffectiveness of native GIP limit its use as a potential therapeutic for type 2 diabetes. The present study reports effects of novel fatty acid (myristic, palmitic or stearic acid) derivatised forms of GIP on metabolic stability, *in vitro* biological characterisation and *in vivo* glucose-lowering and insulin-release.

Materials and methods: Stability of GIP peptides (5 μg) was assessed by incubation (0, 2, 4, 8 and 24 h; 37°C) with DPP-IV (5 mU; n=3) and peptide degradation quantified by rp-HPLC. Cyclic AMP production (n=3) and insulin-releasing activity of GIP peptides were assessed in acute (20 min) studies using BRIN-BD11 cells. Acute and persistent (24 h) effects of GIP peptides (25 nmol/kg bw; ip) on plasma glucose and insulin concentrations were examined in 20-22 week old *ob/ob* mice (n=6). For sub-chronic studies, o*b/ob* mice were divided into four groups (two most potent peptides plus controls, n=6;) and received once-daily injections (17:00 h) of either saline vehicle (0.9%, w/v, NaCl), *N*-AcGIP, GIP(Lys^{37}MYR) or *N*-AcGIP(Lys^{37}MYR) (25 nmol/kg bw) over a 24-day period. Food intake, body weight, glucose and insulin levels were monitored at intervals of 2 to 4 days. Glucose tolerance (18 mmol/kg glucose) and insulin sensitivity (50 U insulin/kg) tests were performed at the end of the study. Furthermore, blood was taken for measurement of triglycerides and pancreatic tissue excised for determination of insulin content.

Results: Although GIP was rapidly degraded by DPP-IV (half-life 2.2 h), GIP analogues remained fully intact during the incubation (half-lives >24 h). All GIP analogues followed a similar pattern of concentration-dependent (10^{-12} to 10^{-6} M) stimulation of cyclic AMP production compared to native GIP (EC_{50} value 0.70 nM) but exhibited improved EC_{50} values (0.07 to 0.27 nM). Similarly, GIP analogues dose-dependently stimulated insulin secretion in BRIN-BD11 cells but with enhanced potency compared to both control (5.6 mM glucose; 2.8 to 4.0-fold; P<0.001) and GIP (1.5 to 2.1-fold; P<0.01 to P<0.001). Administration of GIP analogues to *ob/ob* mice significantly lowered plasma glucose (1.2 to 1.4-fold; P<0.05 to P<0.001), with GIP(Lys^{37}MYR), *N*-AcGIP(Lys^{37}MYR) and GIP(Lys^{37}PAL) additionally enhancing plasma insulin responses (1.2 to 1.8-fold; P<0.05 to P<0.001) compared to GIP-treated animals. Furthermore, GIP(Lys^{37}MYR) and *N*-AcGIP(Lys^{37}MYR) elicited significantly protracted glucose-lowering effect (1.2 to 1.3-fold; P<0.01 to P<0.001) when administered 24 h prior to a glucose load. Daily administration of GIP(Lys^{37}MYR) and *N*-AcGIP(Lys^{37}MYR) to *ob/ob* mice for 24 days decreased glucose (1.4 to 1.7-fold; P<0.05 to P<0.001) and significantly improved plasma insulin (1.4 to 2.2-fold; P<0.01 to P<0.001), glucose tolerance (1.3 to 1.5-fold; P<0.05 to P<0.001) and beta-cell glucose responsiveness (1.5 to 4.4-fold; P<0.01 to P<0.001). Insulin sensitivity, pancreatic insulin content and triglyceride levels were not changed

Conclusion: These data demonstrate that fatty acid derivatisation of GIP, particularly with myristic acid provides a class of stable, longer-acting forms of GIP with good biological effectiveness for further evaluation in diabetes therapy.

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787

Insulinotropic and glucose lowering effects of small molecule GLP-1-receptor agonist 6,7-dichloro-2-methylsulfonyl-3-N-*tert*-butylaminoquinoxaline

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Background and aims: Enzyme resistant GLP-1 mimetics such as exenatide and liraglutide are employed for the treatment of type 2 diabetes, but must be administered by injection. To improve therapeutic utility of the GLP-1 receptor target and avoid parenteral administration, development of small molecule receptor ligands suitable for oral administration would be an advantage. Therefore, the present study assessed the *in vitro* insulinotropic and *in vivo* glucose-lowering actions of the recently described low molecular weight selective GLP-1 receptor (GLP-1-R) agonist 6,7-dichloro-2-methylsulfonyl-3-N-*tert*-butylaminoquinoxaline (DMB). Biological activity of DMB has been compared with native GLP-1, exenatide and liraglutide.
Materials and Methods: Concentration-dependent acute (20 min) insulin-releasing activity of DMB, native GLP-1, exenatide and liraglutide (n=8) was assessed using clonal pancreatic BRIN-BD11 cells. In addition, acute effects of DMB and related GLP-1 peptides on plasma glucose concentrations were examined in normal mice (14-18 week-old) derived from the colony originally maintained at Aston University. Mice (n=8) received an intraperitoneal injection of glucose alone (18 mmol/kg body weight) or in combination with DMB (2500 nmol/kg body weight) or related GLP-1 peptides (each at 25 nmol/kg body weight). In a second series of experiments, glucose (18 mmol/kg) and DMB (2500 nmol/kg) were administered (i.p.) in the presence and absence of liraglutide, exenatide or exendin-4(9-39) (each at 25 nmol/kg).
Results: As expected, native GLP-1, liraglutide and exenatide induced respective 1.3-1.9 -fold (10^{-11} to 10^{-6} M, P<0.01 - P<0.001), 1.4-2.2 -fold (10^{-11} to 10^{-6} M, P<0.001) and 1.4-2.8 -fold (10^{-12} to 10^{-6} M, P<0.001) increases in insulin secretion from BRIN-BD11 cells. DMB also stimulated insulin secretion in a concentration-dependent manner, but with decreased potency compared to native GLP-1 and either GLP-1-R mimetic (2.3-5.0 -fold increases at concentrations of 10^{-4} M to 10^{-3} M, P<0.001). Administration of DMB in combination with glucose to normal mice significantly lowered individual plasma glucose levels (t=15 min, P<0.05) and decreased overall glucose excursion (1.4-fold; P<0.05) compared to controls (0-60 min AUC values; 365.3 ± 30.2 mmol/l.min *vs*. 501.6 ± 31.2 mmol/l.min for control). Similarly, exenatide and liraglutide significantly (P<0.001) reduced the overall glycaemic excursion when compared to controls and were significantly (P<0.001 and P<0.05; respectively) more potent than DMB. Interestingly, administration of DMB in combination with the GLP-1-R antagonist, exendin-4(9-39), did not significantly effect glucose-lowering actions (0-60 min AUC values; 365.3 ± 30.2 mmol/l.min *vs*. 361.0 ± 11.9 mmol/l.min for DMB + exendin-4(9-39)), confirming allosteric GLP-1-R binding. However, combined administration of DMB with liraglutide or exenatide did not improve therapeutic effect.
Conclusion: DMB stimulates insulin secretion and lowers blood glucose concentrations but is considerably less potent than native GLP-1 or stable peptide GLP-1-R mimetics. Low molecular weight GLP-1-R agonists may have future therapeutic value for type 2 diabetes but potency together with the issues of specificity and safety are major obstacles before the concept can be considered in humans.

788

Presence and characteristics of GLP-1 receptors in osteoblastic cells

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Background and aims: Very recently, it has been reported that GLP-1, an incretin with antidiabetic properties, apart from having direct stimulatory actions upon glucose and lipid metabolism in extrapancreatic tissues, exerts an anabolic effect on the altered bone metabolism of type 2 diabetic and insulin resistant rat models. Exendin-4 (Ex-4) and exendin-9 (Ex-9), structurally homologous with GLP-1, have proven to be agonist and antagonist, respectively, of the pancreatic GLP-1 receptor and of several of its actions in rat liver, muscle and fat. Here, we have searched for the possible presence of GLP-1 receptors, and their characteristics, in osteoblasts.
Materials and methods: MC3T3-E1 osteoblastic cells, grown ($2x10^5$ cells/well) in α-MEM supplemented with 1% penicillin/streptomycin, 2 mM glutamine and 10% FBS, were incubated, at 37ºC or 25ºC -in the same media except for the absence of FBS and presence of Trasylol (500 KIU /ml), 10 mM $MgSO_4$ and 50 mM HEPES-, with 20 fmol mono-^{125}I-GLP-1 (70 MBq/mol) as tracer, for different time periods (0-120 min), in the absence and presence of GLP-1, Ex-4 or Ex-9 (10^{-9}-10^{-6} M), and 10^{-6} GLP-2 or insulin. Degradation of the tracer -by TCA precipitation-, dissociation of ^{125}I-GLP-1-binding -by dilution-, molecular size (Mr) -by cross-linking and SDS-PAGE- and cyclic-AMP content -in the absence and presence of IBMX-, were also studied.
Results: The tracer-GLP-1 binding was time- and temperature-dependent, at 25ºC being detectable at 15 minutes incubation period (2.20±0.11% total tracer, n=4 separate experiments), reaching the maximum value after 30 to 45 minutes (overall mean: 2.65±0.07%, n=10) and decreasing thereafter (120 min: 1.81±0.20%, n=3). At 37ºC, but not at 25ºC, degradation of the ^{125}I-GLP-1 in the presence of cells occurred (TCA precipitated at 20 min: 77±1% total; 30 min: 69±1%, both n=3). The tracer specific binding (Sp B) at 25ºC during 30 min (0.33±0.04% total, n=11) was dissociable, resulting in a reduction to 37.5±2.5% Sp B, (n=5) at minute 30; Sp B was displaced by increasing concentrations of GLP-1, with a 50% inhibition dose (ID_{50}) of $3x10^{-9}$ M; GLP-2 equally displaced GLP-1-tracer Sp B; neither Ex-4 nor Ex-9 or insulin competed with the ^{125}I-GLP-1 binding. The Scatchard analysis of the unlabeled GLP-1 data revealed the presence of high affinity binding sites, with a dissociation constant (Kd) of $1.46x10^{-12}$ M, an affinity constant (Ka) of $6.87x10^{10}$ L/mol, and a binding capacity of 4 fmol/10^6 cells; low affinity binding sites were also present. Cross-linking and SDS-PAGE showed a GLP-1-bound to cell protein estimated in 68,000 Mr. GLP-1 did not apparently modify cAMP content.
Conclusion: These data indicate that the reported insulin-independent anabolic effect of GLP-1 in normal and in states associated with impaired bone metabolism could be exerted through a GLP-1 specific receptor, which is likely different from the pancreatic GLP-1 receptor.
Supported by: the Spanish Ministry of Health

789

Different modulation of dipeptidyl-peptidase IV activity between microvascular and macrovascular human endothelial cells

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Background: Glucagon-like peptide-1 (GLP-1) is a gastrointestinal hormone, mainly secreted after meals, which enhances glucose-induced insulin secretion and induces satiety. GLP-1 is rapidly inactivated by dipeptidyl peptidase IV (DPP-IV), an enzyme produced by endothelial cells in different districts and that circulates in plasma. Patients with type 2 diabetes show a reduction of active GLP-1 that could be due to impairment of secretion or its degradation or both. Some authors suggest that metformin has no direct inhibitory effect on DPPIV activity and that metformin and the other biguanides enhance GLP-1 secretion; others suggest a possible role of metformin in the inhibition of the DPPIV activity.
Materials & methods: In order to better elucidate the role of insulin sensitizers on the modulation of GLP-1 circulating levels, DPP-IV activity and mRNA expression were measured in cultured human aortic endothelial cells (HAEC) and human microvascular dermal endothelial cells (HMVEC) exposed to high glucose, Metformin and Rosiglitazone.Human aortic endothelial cells and adult dermal microvascular endothelial cells (HMVEC) were cultured in different glucose concentrations (11, or 22 mmol/L) for a week before experiments. Cells were also cultured in glucose 11 mM and mannitol 11 mM, in order to verify the effects of hyperosmolarity. DPP-IV activity was measure at 4, 12, 24, 48, 72 and 168 hours of incubation with or without Rosiglitazone (20 μM) as used by Benvenuti et al or Metformin (500 μMol/L) as used by Lindsay et al. All experiments are performed in triplicate. Results: Present data show that hyperglycaemia is capable of increasing in a significant manner, the DPP-IV activity only in microvascular endothelial cells. Rosiglitazon is able to modulate in a negative and significant manner the expression of DPPIV but not its activity in macrovascular endothelial cells while at 24 h of exposure it is able to increase significantly DPPIV activity but not its expression in microvascular endothelial cells. Metformin at 48 h only in microvascular endothelial cells is able to reduce in a significant manner (p= 0,01) the activity of DPPIV but not its expression .
Conclusion: The modulation of DPPIV is site specific.
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790

Novel GLP-1 analogues cross the blood brain barrier and enhance synaptic plasticity in the brain: a link between diabetes and Alzheimer's disease

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Background and aims: Recently, the incretin glucagon-like peptide 1 (GLP-1) has been shown to have neuroprotective properties. We therefore tested whether novel GLP-1 analogues that are resistant to protease degradation and are developed as a treatment for type 2 diabetes (eg. Liraglutide) can cross the blood brain barrier (BBB) and have effects on neuronal communication and synaptic plasticity (LTP) in the brain.
Materials and methods: Male mice were injected ip. with a range of concentrations of GLP-1, Val(8)GLP-1, or Liraglutide. The brain tissue was analysed using protein separation and purification steps and a RIA analysis kit. In the electrophysiology study, male rats were anaesthetised and electrodes lowered into the hippocampus area CA1 to record field excitatory field potentials (fEPSPs). Synaptic plasticity (LTP) was induced using a high frequency protocol (100 stimuli at 200Hz), stimulating the Schaffer collateral projections to CA1 neurons. Liraglutide, asp(7)GLP-1, Val(8)GLP-1, N-glyc-GLP-1, and Pro(9)GLP-1 were injected icv. for this test. Data were analysed using 2-way ANOVAs or t-tests.
Results: Val(8)GLP-1 and Liraglutide both crossed the BBB. Injection of 25nM or 250 nM Liraglutide ip. of either peptide resulted in a significant increase in the brain 30 min or 3 h post-injection. 25nM at 30 min: $p<0.05$; at 3h: $p<0.005$, 250nM at 30 min: $p<0.01$, at 3h: ns. Val(8)GLP-1 25nM ip. at 30 min: $p<0.05$, at 3h: ns., 250nM at 30 min: $p<0.01$, at 3H: p,0.05. In the LTP studies, all novel GLP-1 agonists tested enhanced LTP. The novel GLP-1 analogue Liraglutide injected icv. (15nmol in 5µl) enhanced LTP. A two-way repeated measures ANOVA showed a difference between the drug group and saline control ($p<0.005$) and over time ($p<0.001$). All groups n=6. Native GLP-1 injected icv. (15nmol in 5µl) also increased LTP, a 2-way ANOVA showed a difference between groups ($p<0.001$) and over time ($p<0.001$). N=8 per group. The GLP-1 analogue Val(8)GLP-1 (15nmol icv.) also increased LTP. A 2-way ANOVA showed a difference between groups ($p=0.003$) and over time ($p=0.006$) N=6 per group. The other GLP-1 analogues, Asp(7)GLP-1, N-glyc-GLP-1, and Pro(9)GLP-1 injected icv. (each 15nmol in 5µl) also increased LTP, a 2-way ANOVA showed a difference between groups ($p<0.001$ each peptide) and over time ($p<0.001$). N=6 per group. The inactive metabolite GLP1(9-36) had no effect on LTP.
Conclusion: These results demonstrate that the GLP-1 agonists Val(8)GLP-1 and Liraglutide readily cross the BBB and have pronounced facilitatory effects on LTP. Other novel GLP-1 analogues also had facilitating effects on synaptic plasticity. Since GLP-1 analogues have been shown to be neuroprotective, and impairment of neuronal transmission is one of the symptoms in mouse models of Alzheimer's disease, these novel protease-resistant incretin analogues developed to treat type 2 diabetes also show promise as a treatment of neurodegenerative diseases.
Supported by: the Alzheimer Society, UK

PS 63 Incretins - mechanisms of action 1

791

Effects of endogenous glucagon-like peptide-1 on gastric emptying in healthy subjects - relationship to glycaemia

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Background and aims: Exogenous glucagon-like peptide-1 (GLP-1), when given in pharmacological doses, attenuates postprandial glycaemic excursions, in part, by slowing gastric emptying (GE). Studies using the specific GLP-1 antagonist, exendin (9-39) amide (ex), have established that endogenous GLP-1 inhibits fasting and nutrient-stimulated antropyloroduodenal motility in humans. However, a previous study using ex(9-39) reported no effect on GE, but the latter was measured using an imprecise technique (xylose absorption). The aims of this study were to determine the effects of endogenous GLP-1 on GE and intragastric distribution of a high carbohydrate meal, as measured with scintigraphy, and to relate any changes in GE to those on postprandial glycaemia.
Materials and methods: Ten healthy subjects (8M:2F, age 48 ± 7 yr) received ex(9-39) (300pmol/kg/min), or placebo, from t=-30-180 min in a randomised, double-blind, fashion. At t=0 a mashed potato meal containing 20g glucose and 45g margarine (2600KJ), labelled with 20MBq ^{99m}Tc-sulphur colloid, was ingested, and GE measured for 180 min. To quantify GE, regions-of-interest were drawn around the total stomach, which was further divided into proximal and distal regions. The time taken for 50% of the meal to empty from the stomach (T50) was calculated. Blood was sampled for measurement of glucose.
Results: Results are shown (mean ± SEM). Ex(9-39) accelerated GE (T50 ex: 68±8 min vs placebo: 83±7 min; $P<0.01$) (figure 1A Retention of meal during infusion of ex(9-39) and placebo; *$P<0.01$), however, there was no difference in intragastric meal distribution. While there was no difference in blood glucose at baseline (at t=0min ex: 6.5±0.4 mmol/l vs placebo: 6.1±0.3 mmol/l; P=NS), ex(9-39) increased postprandial glycaemic excursions ($AUC_{0\text{-}180}$ ex: 1540±106 mmol/l/min vs placebo: 1388±90 mmol/l/min; $P<0.02$) (figure 1B glycaemic response to meal during intravenous ex(9-39) and placebo; #$P<0.02$). There was a relationship between the magnitude of the rise in blood glucose from baseline and T50, so that the rise in glycaemia (at t=60 min) was greater when GE was relatively more rapid ($r=-0.46$; $P=0.04$).
Conclusion: GLP-1 plays a physiological role to slow GE in health, which impacts on postprandial glycaemia.

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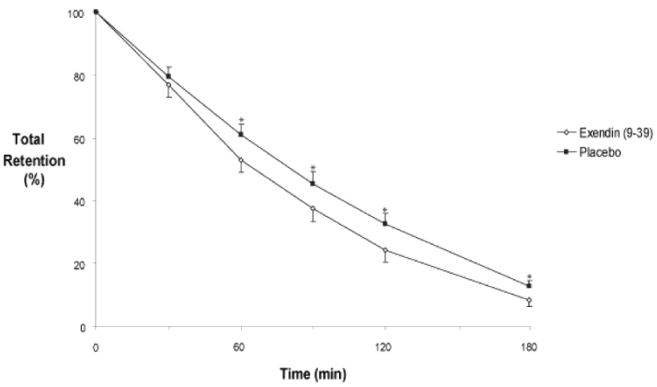

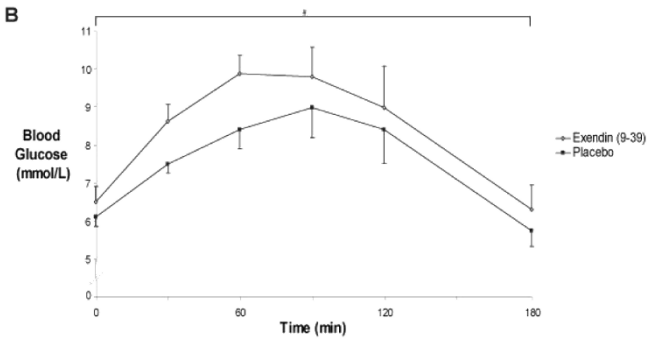

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792

Glucose-dependent insulinotropic polypeptide (GIP) does not potentiate the antidiabetic effects of glucagon-like peptide-1 (GLP-1) in hyperglycaemic patients with type 2 diabates mellitus

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Background and aims: Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) are incretin hormones from the gut mucosa. GLP-1 exerts insulinotropic action in type 2-diabetic patients, whereas GIP effects are no longer prominent in such patients. It was the purpose of the present study to examine whether exogenous GIP can alter the insulinotropic or glucagonostatic activity of exogenous GLP-1 in hyperglycaemic type 2-diabetic (T2DM) patients.
Materials and methods: Twelve patients with T2DM (9 males/3 females; age 61 ± 10 years; BMI 30.0 ± 3.7 kg/m^2; HbA_{1c} 7.3 ± 1.5 %; mean ± SD) were studied in a single-blinded manner within a 4-way cross-over design. In randomized order and after discontinuing antidiabetes therapy (insulin and/or OADs), intravenous infusions of GLP-1 [7-36 amide] (1.2 pmol·kg^{-1}·min^{-1}), GIP (4 pmol·kg^{-1}·min^{-1}), GLP-1 plus GIP, and placebo were commenced after an overnight fast and continued for 360 min. There were 1 day wash-out periods between experiments. Capillary blood glucose was measured and venous blood was drawn for total and active GIP, total and active GLP-1, insulin, C-peptide, glucagon (specific immunoassays) and free fatty acids (FFA). Statistics: repeated-measures ANOVA.
Results: With exogenous GLP-1 alone, glucose was reduced from approximately 185 mg/dl to 91 ± 4 mg/dl. Insulin secretion was stimulated (insulin, $p < 0.0001$ and C-peptide, $p < 0.0001$) and glucagon was suppressed ($p = 0.009$). With GIP alone, glucose was somewhat lowered, but not normalized; insulin and C-peptide were stimulated to a much lesser degree than with GLP-1 ($p < 0.001$). Adding GIP to GLP-1 did not further enhance the insulinotropic activity of GLP-1 (insulin, $p = 0.90$; C-peptide, $p = 0.85$). Rather, the suppression of glucagon elicited by GLP-1 was antagonized by the addition of GIP ($p = 0.008$). FFA were suppressed by GLP-1 ($p < 0.0001$) and hardly affected by GIP ($p = 0.07$).
Conclusion: The addition of GIP is not able to further amplify the insulinotropic and glucose-lowering effects of GLP-1 in T2DM. Rather, the suppression of glucagon by GLP-1 is antagonized by GIP. Upon acute exposure, GIP does not exert a prominent antidiabetic effect, whether administered alone or in combination with GLP-1.
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793

No enhancement of meal-induced GLP-1 secretion and reduced intact GLP-1 levels in Japanese patients with type 2 diabetes as well as healthy controls

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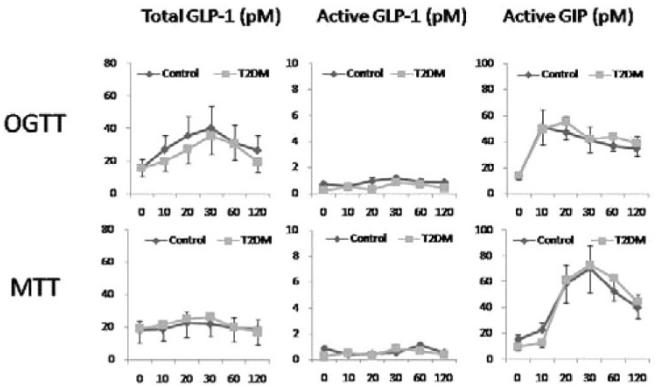
Background and aims: High levels of intact GLP-1 and GIP among Asians are rarely seen. Here, we investigate intact and total (intact plus degraded) GLP-1 and GIP during OGTT and MTT in Japanese with or without type 2 diabetes.
Materials and methods: Japanese healthy controls (controls; n=17, female 18%; age 51±12; HbA1c 5.2±1.4%; BMI 22.4±5.4) and age-matched and untreated patients with type 2 diabetes (T2DM; n=18, female 22%; age 55±13; HbA1c 7.0±0.2%; BMI 23.9±5.6; duration 3.0±0.9 years) were subjected to OGTT and MTT. Blood samples were corrected sequentially, and levels of intact and total GLP-1 and GIP were measured in addition to those of glucose, IRI and IRG.
Results: Fasting levels of total GLP-1 were 16.4±4.9 and 16.0±4.8pM, and those of intact GLP-1 were 0.8±0.2 and 0.2±0.0pM in controls and T2DM, respectively. During OGTT, total GLP-1 reached peak 30 min after glucose administration, while intact GLP-1 levels remained low and showed no significant peak. AUCs for total and intact GLP-1 were similar in the two groups. During MTT using standard Japanese meal (480kcal, carbohydrate: protein: fat=2.8:1:1), total and intact GLP-1 showed no obvious peak. AUCs for total and intact GLP-1 were also similar. Fasting levels of total GIP were 22.2±6.7 and 29.0±8.9pM, while those of intact GIP were 16.4±4.9 and 16.0±4.8pM in controls and T2DM, respectively. During OGTT, total GIP reached the peak 30 min after glucose administration, and intact GIP reached the peak as early as 10 min after glucose administration. Although total GIP levels measured higher in controls, there was no statistical significance between the two groups. AUCs for total and intact GIP in the two groups were similar. During MTT, total and intact GIP reached peak 30 min after meal ingestion in both controls and T2DM. AUCs for total and intact GIP were similar.
Conclusion: These results indicate that intact GLP-1 but not GIP levels are considerably reduced in Japanese compared to those of Caucasians reported previously, and that meal-induced enhancement of GLP-1 secretion is negligible in Japanese. These differences may underlie reduced insulin secretory capacity in Japanese.



Supported by: Japan Diabetes Foundation, Diabetes Masters Conference

794

Taspoglutide, a human once-weekly GLP-1 analogue, reduces post-challenge secretion of peptide YY and glucose-dependent insulinotropic peptide in an animal model of type 2 diabetes

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Background and aims: Taspoglutide is a novel, human once-weekly glucagon-like peptide (hGLP)-1 analogue under clinical development for the treatment of type 2 diabetes (T2D). We evaluated the chronic effect of taspoglutide on postprandial response of glucose, insulin, lipids, and intestinal polypeptides PYY and GIP following an oral glucose tolerance test (oGTT) in the ZDF rat, an animal model of T2D progression.
Materials and methods: Single application of taspoglutide (1 mg sc, formulated to mimic human exposure) or vehicle was administrated to 6-wk-old ZDF rats (n=6/group), and body weight and food intake were monitored daily for 3 wks. Glucose, insulin, total PYY, total GIP, and triglycerides were assessed at 0, 10, 30, 60, and 120 min after an oGTT (2g/kg) in 16-h fasted rats, performed 3 wks after taspoglutide administration.
Results: A significant reduction in body weight was seen during the first week after administration and was maintained until the end of the study when body weight was significantly lower by about 7% in rats treated with taspoglutide

compared with vehicle (276±2.6 vs. 298±3.7 g, p<0.005). Food intake decreased slightly during the first 5 days after taspoglutide administration, but then normalized to control values. Taspoglutide significantly reduced glucose excursion during the oGTT at 30, 60, and 120 min and $AUC_{Glu,0\text{-}120\ min}$ (-50%) without affecting insulin levels compared with vehicle. A significant decrease in GIP levels was observed 30 min after a glucose challenge, and the $AUC_{0\text{-}120\ min}$ was reduced by >30%. More importantly, a 70% decrease in the AUC for PYY was demonstrated over the 2 hours of the oGTT compared with vehicle, which might reflect a suppression of endogenous GLP-1 secretion. There were no significant differences in fasting PYY or GIP levels.
Conclusion: This study demonstrates the pharmacological effect of a GLP-1 analogue on the post-challenge response of PYY and GIP. The suppression of these intestinal peptides is important for the regulation of glucose and insulin secretion in response to a meal. These data bring new insights into the understanding of the mechanism of how taspoglutide affects incretin physiology in T2D.

795

Changes in adipokines in type 2 diabetic patients treated with exenatide versus glimipiride on metformin background - results of a prospective, randomized controlled study over 9 months

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Background and aims: In addition to the already known laboratory values with a predictive power for cardiovascular disease such as hsCRP and fibrinogen, new fat tissue hormones have been discovered in the recent years. The data presented here originate from the EURopean EXenAtide Study (EUREXA), a long-term prospective, open-label study comparing the effects of Exenatide (EXE) versus Glimipiride (GLIM) on maintainance of glycemic control. One objective of this addendum to the EUREXA study is to explore how EXE versus GLIM differentially affects the adipokine levels adiponectin, fibrinogen, leptin, PAI-1 and TNFα after 9 months of treatment in a subgroup of patients.
Materials and methods: Out of the 1039 patients randomized in the EUREXA study 35 entered the addendum after giving informed consent and 33 overweight (mean body-mass-index (BMI) 32.8±3.98kg/m²) T2D patients (15 female, 18 male) were eligible for analysis. Patients were randomized, stratified on the basis of their BMI, to receive either EXE (n=15) or GLIM (n=18) as an add-on treatment. At baseline (BL) and at endpoint (EP) after 9 months of treatment blood-samples were collected and analyzed centrally. Data were analyzed by means of descriptive statistics and predefined, exploratory treatment groups comparisons were done by either analysis of covariance or the Mann-Whitney test (last observation carried forward approach).
Results: At BL patients had a mean (SD) age of 58.1 (9.0) years and a mean diabetes duration of 5.2 (5.16) years. Mean BL weight was 96.7 kg (13.55) in the EXE-group and 98.0 kg (10.76) in the GLIM-group. While patients in the EXE-group had a LS mean [SEM] weight loss of 5.26 kg [1.03], patients gained 2.14 kg [0.94] on GLIM (p<0.001) with corresponding changes in BMI of -1.75 kg/m² [0.35] for EXE and +0.62 kg/m² [0.32] for GLIM (p<0.001). LS mean BL to EP change [SEM] in HbA1c was -0.80% [0.27] in the EXE- and -0.71% [0.24] in the GLIM-group (p=0.796) with mean (SD) BL-values of 7.5% (0.55) and 7.6% (0.72) for the EXE- and the GLIM-group, respectively. While Adiponectin showed a median [IQR] decrease of 0.6µg/ml [-2.2 to +1.6] in the EXE-group, it increased by 0.3µg/ml [-1.6 to +1.0] in the GLIM-group after 9 months of treatment (p=0.975). Leptin showed a median [IQR] decrease of 2.4µg/l [-6.0 to +0.1] to a median EP concentration of 13.2µg/l [7.5 to 26.2] under EXE, but an increase of 6.8µg/l [+1.9 to +15.3] to a median EP concentration of 27.2µg/l [14.1 to 56.1] under GLIM (p=0.004).
In the EXE-group LS mean [SEM] PAI-1 concentrations decreased by 2.84 U/ml [1.10] to a mean (SD) EP concentration of 4.8 U/ml (2.56). In the GLIM-group the corresponding LS mean change in PAI-1 was +0.53 U/ml [1.06] to a mean EP concentration of 7.9 U/ml (4.50)(p=0.039).
LS mean TNFα statistically significantly increased by 1.64 ng/l ([0.63]; p=0,016) to a mean of 9.2 ng/l (2.21) at EP and by 2.02 ng/l ([0.60]; p=0.003) to 9.9 ng/l (1.98) at EP for EXE and GLIM, respectively. Fibrinogen remained unchanged in both groups after 9 months of treatment.
Conclusion: After 9 months of treatment obese patients with T2D experienced beneficial changes in the adiponectin profile when treated with EXE versus GLIM.
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796

Sitagliptin effects on triglyceride and free fatty acid metabolism

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Background and aims: The effect of dipeptidyl peptidase-4 (DPP-4) inhibitors on lipid metabolism in type 2 diabetes is controversial, with conflicting reports in the literature. No studies of the effect of sitagliptin compared to placebo on postprandial lipemia have been published. Individuals with impaired fasting glucose (IFG) are at increased risk of cardiovascular disease. As part of a study examining the effect of the DPP-4 inhibitor sitagliptin (SITA) on IFG, we determined the effect of SITA on triglyceride (TG) and free fatty acid (FFA) metabolism using a placebo-controlled, parallel-group design.
Materials and methods: A mixed meal study was conducted in subjects with IFG (n=22) at baseline and again 8 weeks after randomization to SITA 100 mg/d or placebo (PL). In a subset of participants (n=17), tracer quantities of [1-^{13}C] oleate and a [oleyl-^{3}H] triolein-labeled lipid emulsion were infused to allow determination of oleate rate of appearance (Ra) as well as the Ra of ^{3}H oleate. Lipoprotein lipase-mediated fractional spillover of fatty acids from dietary fat was calculated from ^{3}H oleate Ra during peak chylomicronemia after ingestion of breakfast (45% carbohydrate, 40% fat, 15% protein). Blood samples were taken at baseline and at intervals for 6 hours after the meal for measurement of plasma TG concentration, ^{13}C oleate enrichment, ^{3}H oleate specific activity, ^{3}H TG and plasma FFA concentration.
Results: In both SITA and PL, there was no change from baseline to week 8 in fasting (453±39 vs. 411±40 and 435±31 vs. 414±37 µmol/L, respectively), 2-hour postprandial (39±5 vs. 44±5 and 40±6 vs. 45±9 µmol/L) or postprandial area under the curve (21±6 vs. 25±10 and 15±2 vs. 16±2 mmol•L^{-1}•6h^{-1}) plasma FFA concentrations (P=NS in all cases). Fractional spillover of ^{3}H oleate from lipolysis of [9,10-^{3}H] triolein was 29±5% in SITA (n=7) and 25±3% in PL (n=10) at week 8 (P=NS). Oleate Ra was calculated assuming steady-state conditions during the frequent sampling interval and also assuming nonsteady-state conditions for the entire postprandial period. With both approaches there was no difference in oleate Ra, comparing both SITA to PL (1.38±0.24 vs 1.03±0.03 µmol/kg/min respectively; P = 0.24) and 8 weeks of SITA to baseline (1.38±0.24 vs 1.36±0.28 µmol/kg/min respectively; P = 0.96). However, average peak (270 to 330 minutes) postprandial TG concentrations decreased from 204±17 to 178±20 mg/dL (P<0.01 comparing groups using a matched pair t-test) in the SITA arm; there was no change in the PL arm (174±16 vs 175±15 mg/dL, P = NS). SITA lowered postprandial TG to a greater extent than PL at week 8 compared to baseline (Δ = -26±7 vs +1±5 mg/dL, P<0.01).
Conclusion: SITA reduced postprandial TG concentrations by ~13% but did not alter adipose tissue lipolysis in subjects with IFG. SITA reduced postprandial lipemia despite no effect on FFA metabolism. The mechanism responsible for this effect is unknown, but could involve an effect of SITA on intrahepatic very low density lipoprotein TG synthesis or secretion, and/or on peripheral metabolism of TG by lipoprotein lipase. Further investigations to define the mechanisms and significance of this finding are required.

797

No effect of glucose-dependent insulintropic polypeptide, GIP, on triglyceride clearance in humans

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Background and aims: GIP is an incretin hormone that has been implicated in fat metabolism but the actions of GIP in humans are unclear. We examined whether exogenous GIP infused intravenously alone or in combination with a concomitant stimulation of endogenous insulin affects the plasma clearance of triglycerides (TG) in man.
Materials and methods: Ten healthy subjects were examined in a randomized, cross-over study on 4 different days: 1 day with Intralipid (IL) infusion; 1 day with IL and glucose infusion, all days with and without GIP-infusion (1.5 pmol/kg/min) for 300 min. IL was given as a bolus of 0.15 g/kg followed by continuous infusion of 0.225 g/kg/h for one hour. Glucose was infused to mimic the meal-induced glucose excursions and to investigate the combined action of insulin and GIP in lipid metabolism.
Results: Basal GIP concentrations averaged 10.83 ± 0.5 pM and increased significantly during GIP infusions to 285.1 ± 8.4 (p<0.0001). On IL + GIP day, plasma insulin concentrations increased significantly during the first 60 min

(2133 ± 188 vs. 1538 ± 156 pM x min, p=0.001) compared to IL + saline day but no difference was seen at time 60-300 min. Likewise, a significant increase in insulin was seen during the first 60 min on IL + Glucose + GIP day compared to IL + Glucose + saline day (13203 ± 1742 vs. 6263 ± 607 pM x min, p=0.001). Maximal TG concentrations were reached after one hour of infusion of IL and resulted in a concomitant plasma FFA and glycerol increase. No difference was seen in the plasma TG or glycerol clearances on all 4 experimental days (p=NS). However, the plasma FFA concentration was significantly reduced at time 60 - 300 on IL + GIP day compared to IL + saline day (p=0.007). No difference was seen in plasma FFA concentration between IL + Glucose + GIP and IL + Glucose + saline days but on both days the FFA concentration was significantly reduced at time 60-300 min when compared to IL + GIP/saline days (p<0.05).
Conclusion: In conclusion, these data suggest that GIP does not affect TG clearance in man. However, both insulin and GIP lower post-IL FFA concentration, GIP probably via stimulation of insulin secretion, increasing FFA reesterification.
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798

Changes in prandial glucagon levels after 2 years treatment with vildagliptin or glimepiride
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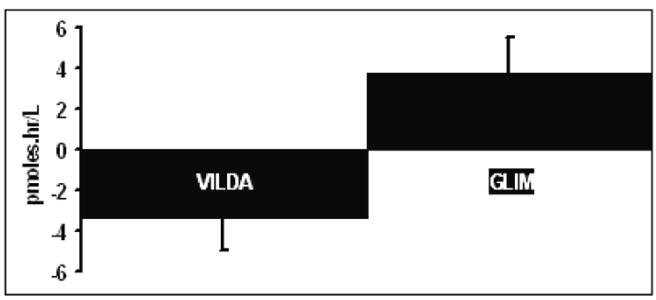
Background and aims: Previous studies indicate that the glucagon levels during meals are reduced following treatment with vildagliptin (VILDA). In order to test the hypothesis that VILDA therapy has favorable long term effects relative to glimepiride (GLIM) on prandial glucagon levels, glucagon levels were measured over 2 hours during a standard meal challenge test following either VILDA or GLIM 2-yrs treatment in combination with metformin.
Materials and methods: These measurements were made in a subset of patients at endpoint in the intent to treat population, from a study that was designed to demonstrate the 2 year efficacy and safety of add-on therapy with 50 mg bid VILDA compared to up to 6 mg qd GLIM in patients with type 2 diabetes inadequately controlled with prior metformin monotherapy (HbA1c > 6.5% and ≤ 8.5%). Insulin secretion rate was determined by deconvolution of C-peptide levels.
Results: At baseline HbA1c was ~7.3%. Prandial glucagon $AUC_{0\text{-}2h}$ at baseline was 55.6±2.3 pmol·hr/L with no difference between the groups. After up to 2 years treatment, the prandial glucagon $AUC_{0\text{-}2h}$ decreased from baseline to study endpoint by 3.4±1.6 pmol·hr/L in the VILDA group (n=137), whereas an increase by 3.8±1.7 pmol·hr/L was seen with the GLIM group (n=121); the between group difference was 7.2±2.1 pmol·hr/L (P<0.001 by ANCOVA). After the treatment, prandial glucose excursions were reduced by 0.89±0.25 mM in the VILDA group and by 0.96±0.25 mM in the GLIM group (P=0.77) and the insulin secretion rates relative to glucose were greater in the GLIM group than the VILDA group (34±0.18 pmol·hr/L for VILDA vs. 91±0.19 pmol·hr/L for GLIM, P< 0.02).
Conclusion: This study thus confirms in a large cohort that the DPP-4 inhibitor VILDA reduces prandial glucagon levels and suggests that the sulfonylurea GLIM increase them. Since glucose excursion was the same in both groups, we conclude that VILDA improves glycemia with less insulin than GLIM by more effectively reducing prandial glucagon levels.



Supported by: Novartis Pharmaceuticals

PS 64 Incretins - mechanisms of action 2

799

Metabolic and insulin resistance-related indices during pioglitazone and vildagliptin association versus glimepiride and vildagliptin association in type 2 diabetic patients
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Background and aims: The aim of our study was to verify the effects of pioglitazone (P) and vildagliptin (S) association vs glimepiride (G) and vildagliptin (V) association on metabolic and insulin resistance related-indices in type 2 diabetic patients.
Materials and methods: Ninety-one type 2 diabetic patients with uncontrolled type 2 diabetes mellitus (HbA1c > 7.5 %) were randomised to P 30 mg o.d. and V 50 mg b.i.d. or to G 2 mg t.i.d. and V 50 mg b.i.d. All type 2 diabetic patients were resulted not well controlled with diet and physical activity and P at dosage of 30 mg/day and G at dosage of 6 mg/day. The treatment period had a 9 months duration. We evaluated BMI, HbA1c, FPG, PPG, FPI, Homa index and collected plasma samples of adiponectin (ADN), resistin (R), tumor necrosis factor-alpha (TNF-α), and high-sensitivity C reactive protein (Hs-CRP) at baseline, and after 9 months. Eighty-four patients completed the study (42 in PV and 42 in GV group).
Results: Eighty-six patients completed the study (44 in PV and 42 in GV group). BMI does not change in both groups. HbA1c was decreased by 1.3±0.08 % (p< 0.01), and by 1.3±0.08 % (p< 0.01); FPG was reduced by 20±4 mg/dl (p< 0.01), and by 23±5 mg/dl (p< 0.01); PPG was decreased by 36±8 mg/dl (p< 0.01), and by 44±9 mg/dl (p< 0.01), in PV and GV group, respectively. FPI was decreased by 2.3±0.1 μU/ml (p< 0.05, p< 0.05 vs GV), and by 0.4±0.03 μU/ml (p< 0.05), and Homa index by 1.7±0.6 (p< 0.01 vs baseline, p< 0.05 vs GV), and by 1.1±0.4 (p< 0.05) in PV and GV group, respectively. ADN was increased by 0.9±0.01 μg/ml (p< 0.05 vs baseline, p< 0.05 vs GV), and by 0.2±0.002 μg/ml (ns vs baseline), in PV and GV group, respectively. Resistin was reduced by 1.1±0.1 ng/ml (p< 0.05 vs baseline, p< 0.05 vs GV), and by 0.1±0.001 ng/ml (ns vs baseline), TNF-α by 0.9±0.2 ng/ml (p< 0.05 vs baseline, p< 0.05 vs GV), and by 0.1±0.001 ng/ml (ns vs baseline), and Hs-CRP by 0.8±0.007 mg/l (p< 0.05), and by 0.7±0.005 mg/l (p< 0.05), in PV and GV group, respectively. There was a significant correlation between Homa index decrease and ADN increase (r= -0.49, p< 0.01), R decrease (r= 0.52, p< 0.01), and TNF-α decrease (r= 0.54, p< 0.01).
Conclusion: Both combinations ameliorated diabetes control, but only PV improved insulin resistance related-parameters. The ADN increase, R and TNF-α decrease seem to be related to Homa index improvement.

800

Effect of alogliptin combined with pioglitazone on beta cell function and insulin resistance in metformin-treated patients with type 2 diabetes
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Background and aims: This phase 3, multicenter, randomized, double-blind, placebo-controlled, 12-treatment arm study assessed the efficacy and safety of alogliptin (ALO) alone (12.5 or 25mg qd) or combined with pioglitazone (PIO) (15, 30, or 45mg qd) in patients with type 2 diabetes on metformin with inadequate glycemic control.
Materials and methods: Primary analyses compared PIO alone (all doses pooled; n=387) with ALO 12.5mg plus any dose of PIO (A12.5+PIO; n=390) or ALO 25mg plus any dose of PIO (A25+PIO; n=390) over 26 weeks.
Results: ALO in combination with PIO significantly improved measures of β-cell function (proinsulin:insulin ratio [PI:INS] and HOMA-B) compared to PIO alone. Least-squares mean changes from baseline in PI:INS and HOMA-B were significantly different (*P*<.001) for A12.5+PIO (-0.087 and 18.2) and A25+PIO (-0.076 and 22.2) vs PIO alone (-0.027 and 5.1) at Week 26. HOMA-IR, a measure of insulin resistance, decreased in all treatment groups; however, there was no significant difference between either A12.5+PIO (-2.2) or A25+PIO (-1.7) and PIO alone (-1.6) at Week 26. Combination treatment

with ALO and PIO was well tolerated; the number of patients experiencing ≥1 adverse event was similar across all treatment groups. Hypoglycemia was rare, occurring in 2.1% of patients in the PIO group, 1.0% in the A12.5+PIO group, and 1.5% in the A25+PIO group.
Conclusion: In conclusion, ALO/PIO combination therapy significantly improved β-cell function compared to PIO alone as evident by a decrease in PI: INS ratio and increase in HOMA-B. Comparable improvements in insulin sensitivity were seen with all treatment groups. These results suggest ALO and PIO improve glycemic control in type 2 diabetes mellitus by complementary mechanisms of action, enhancing β-cell function and improving insulin resistance, respectively.
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801

Liraglutide but not vildagliptin restores normoglycaemia in *Psammomys obesus*

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Background and aims: *Psammomys obesus*, or the sand rat, is a model of human type 2 diabetes because the development of diabetes resembles what is seen in humans, i.e. initial hyperinsulinemia concomitant with obesity and later hypoinsulinemia and hyperglycemia. We studied the effects of the GLP-1 analog liraglutide and the DPPIV inhibitor vildagliptin in sand rats.
Materials and methods: One group of animals was maintained on low energy diet and seven groups were fed high energy diet (HED) that induced diabetes over a four week period. The diabetic animals were treated for 0, 6 or 14 days with vehicle, 0.2 mg/kg liraglutide twice daily subcutaneously or 30 mg/kg vildagliptin twice daily orally. This dose was demonstrated to decrease DPP4 activity to below 50% 18 hrs after the last dosing. Beta-cell mass (BCM) and proliferation- and apoptosis frequencies were determined using stereological point counting on sections stained for insulin, Nkx6.1, ki67 and tunel activity.
Results: Liraglutide significantly reduced blood glucose (6 day vehicle:13.9 ± 1.8 mM vs. liraglutide:3.1 ± 0.2; 14 day vehicle:16.5 ± 1.2 vs. liraglutide:9.4 ± 2.1). Blood glucose was normalized in all 13 animals treated with liraglutide for 6 days and in 11 of 17 animals after 14 days treatment compared to none in any of the two control groups (n=12 and 14, respectively). HED increased BCM and treatment with liraglutide did not change this (LE:28.3 ± 4.5 mg/kg vs. 0 day:107.5 ± 28.7). There were no significant changes in proliferation frequency after treatment with liraglutide, but there was a tendency for decreased apoptosis frequency in the normalized, liraglutide-treated animals (6 days vehicle:0.013 ± 0.004% vs. liraglutide:0.006 ± 0.003; 14 days vehicle:0.022 ± 0.008 vs. liraglutide:0.009 ± 0.003). Pancreatic insulin content at 6 and 14 days was significantly higher in the normalized animals (P<0.05). Vildagliptin was not able to reduce blood glucose (6 days vildagliptin:16.4 ± 1.8 mM; 14 days vildagliptin: 19.3 ± 1.1), did not alter the HED induced increase in BCM, nor did it increase pancreatic insulin content.
Conclusion: These results suggest that liraglutide improved diabetes in sand rats partly by improving the function of the remaining islets. Vildagliptin did not improve the glycemic state in sand rats.
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802

Dipeptidyl peptidase 4 expression and activity in rodent models of type 1 and 2 diabetes

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Background and aims: Glucagon-like peptide 1 (GLP-1) is a gastrointestinal hormone, mainly secreted after meals, which enhances glucose-induced insulin secretion and induces satiety. The increase in plasma GLP-1 levels after mixed meal ingestion is deteriorated in patients with type 2 diabetes, in part, leading to impaired insulin secretion. Secreted GLP-1 is rapidly inactivated in vivo, with the cleavage of an N-terminal dipeptide. Because this reaction is catalyzed by dipeptidyl peptidase 4 (DPP4), plasma DPP4 activity is thought to be one of regulators of circulating active GLP-1. DPP4 activity is much higher in kidneys than in other tissues. Among cells, thymocytes in chief have high DPP4 activity, followed by endothelial cells. DPP4 mRNA expression and enzyme activity have been shown to be up-regulated by high glucose level in human renal glomerular endothelial cells in vitro. In vivo, small studies in patients with type 1 and type 2 diabetes have also suggested that chronic hyperglycemia induces an increase in plasma DPP4 activity. To confirm contribution of diabetes to DPP4 production, we studied DPP4 expression and activity in rodent models of type 1 and type 2 diabetes.
Materials and methods: We used streptozocin (STZ)-induced diabetic mice (C57/BL6) and 10-week-old db/db mice. Vehicle-administered mice and db/+ mice were used as controls, respectively. STZ diabetic mice were induced at 8 weeks old by intraperitoneal administrations of STZ (180 mg/kg weight). After 2 weeks of the inductions, the mice were investigated. DPP4 activity was determined using synthetic substrate, and DPP4 mRNA levels in kidney were measured with real-time RT-PCR.
Results: STZ mice were less in body weight (STZ 17.5±2.1 g, control 20.3±0.9 g, n=8 each, p<0.05) and had higher plasma glucose levels than control mice (STZ 537±173 mg/dl, control 146±12 mg/dl, p<0.05). Seven of eight STZ mice had plasma insulin levels below the measurable limit (0.1 ng/ml). Plasma DPP4 activity in STZ mice was increased about 1.7 times of that in control mice, but not statistically significant (p=0.07). There was no difference in DPP4 mRNA levels in kidneys between STZ mice and controls. Body weights of db/db mice were increased (db/db 38.0±1.3 g, db/+ 26.1±0.8 g, n=6 each, p<0.05). Both plasma glucose levels (db/db 556±51 mg/dl, db/+ 186±39 mg/dl, p<0.05) and insulin levels (db/db 11.7±4.3 ng/ml, db/+ 1.6±0.8 ng/ml, p<0.05) of db/db mice were increased. Plasma DPP4 activity in db/db mice was significantly increased (2.2 times) compared with that in db/+ mice. DPP4 mRNA expression in kidney was also up-regulated in db/db mice.
Conclusion: Our results suggested that hyperglycemia increases plasma DPP4 activity but not DPP4 mRNA in kidneys. Plasma insulin level and body weight seemed to be unrelated with plasma DPP4 activity. Because the increase in plasma DPP4 activity can contribute to the reduction in circulating GLP-1 and to the further hyperglycemia in type 2 diabetic patients with poor metabolic control, increased DPP4 activity due to hyperglycemia may account for the mechanism of glucotoxicity. In contrast, DPP4 mRNA levels in kidneys may not be regulated by plasma glucose levels.

803

The efficacy of exendin(9-39)amide as a GLP-1 receptor antagonist in human

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Background and aims: GLP-1 is a gut born hormone postprandially released from L-cells of the lower intestine. It stimulates insulin release glucose-dependently by action on specific receptors on the pancreatic B-cell. In addition, GLP-1 may play a role as regulator of gastric motility, secretion and of satiety. In humans, the specific GLP-1 receptor antagonist exendin(9-39) (Ex-9) was shown to reduce the GLP-1 stimulated insulin secretion by approximately 85%. However, human research in vivo on the GLP-1 physiology requires a more complete inhibition of GLP-1 action. Therefore, we aimed to fully characterize the dose-response characteristic of Ex-9.
Materials and methods: 6 healthy subjects underwent 6 experiments in random order. In each experiment, blood glucose was adjusted to 8 mmol/l for 180 min (glucose clamp). On five days, GLP-1 was IV infused from 0-90 min at 0.3 pmol/kg/min to establish physiological postprandial plasma levels, and from 90-180 min at 0.9 pmol/kg/min to induce supraphysiological plasma levels. Saline or Ex-9 at 300, 600, 900 or 1200 pmol/kg/min were IV infused as a background infusion. A sixth day with an infusion of saline only served as control. Blood glucose concentration, glucose infusion rate (GIR) and plasma levels of insulin were evaluated during the last 45 min of each of the two periods.
Results: Ex-9 was well tolerated without any side-effects. During all experiments blood glucose concentration was held constant at 143.3±0.2 mg/dl. During both doses of GLP-1, the insulin inhibition with increasing doses of Ex-9 followed an exponential pattern similar to a receptor binding curve (R^2=0.938 and 0.891, low and high dose of GLP-1). The dose range of Ex-9 was sufficient to cover the whole receptor-mediated insulinotropic capacity of GLP-1 with an ID50 of 98 and 60 pmol/kg/min for the low and high dose of GLP-1, respectively.
Conclusion: Ex-9 is well tolerated and inhibits dose-dependently the insulinotropic action of a low and high dose of synthetic GLP-1 in human. With Ex-9, it is possible to eliminate the effects of GLP-1. Ex-9 at a dose of 900

pmol/kg/min is necessary to inhibit the GLP-1 action by at least 95%. This may allow the analysis of the effects of endogenous GLP in vivo more quantitatively.

Table: Glucose infusion rate (GIR) and plasma insulin concentrations

	Saline+ Saline	GLP-1+ Saline	GLP-1+ Ex-300	GLP-1+ Ex-600	GLP-1+ Ex-900	GLP-1+ Ex-1200
GLP-1 at LOW DOSE						
GIR (mg/kg/min)	4.0±0.5	13.2±1.2*	5.0±0.4*	3.9±0.3	4.3±0.3	4.3±0.7
Insulin mU/l/10 min	21±3.4	125±23*	33±6.0*	29±5.5	27±3.9	22±2.3
% insulin stimulation	-	100%	11±3.8%	4.8±6.3%	2.9±5.6%	-0.2±4%
GLP-1 at HIGH DOSE						
GIR (mg/kg/min)	7.8±0.8	20.3±1.3*	14.9±0.8*	11.0±0.8	11.6±0.5*	10.2±1.5
Insulin mU/l/10 min	29±5.3	680±143*	116±30*	67±12	56±8	43±7
% insulin stimulation	-	100%	13±1.8%	6.1±1.1%	4.9±1.5%	2.7±1.2%

Mean±SEM during the last 45min of each infusion period, *:P<0.05 vs saline+saline (ANOVA)

Supported by: MSD

804

Higher serum dipeptidyl peptidase-4 activity and insulin resistance index in non-alcoholic fatty liver disease than in type 2 diabetes without liver disease

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Background and aims: Dipeptidyl peptidase-4 inhibitors were introduced in the treatment of type 2 diabetes mellitus and increased serum DPP-4 activity (sDPP-4) was reported in chronic liver diseases. We studied the sDPP-4 and the insulin resistance index (HOMA2-IR) in patients with type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD) and in healthy controls (CNTRL).
Materials and methods: sDPP-4 activity was measured by kinetic assay in 39 NAFLD (F/M:19/20, age: 47.42 yrs (95%CI: 43.83-51) and 86 T2DM (F/M:50/36, age: 62.8yrs (95%CI: 60.46-62.14) pateints and 23 (F/M:12/11, age:35.33yrs (95%CI: 28.76-41.91) healthy individuals. In order to generate non-overlapping groups we excluded those patients from the T2DM group who were clinically with liver disease (abnormal liver tests within 1 year or abnormal liver ultrasound morphology). All patients with NAFLD from the hepatology outpatient unit -except the 9 previously known diabetic individuals- underwent 75g CH OGTT. Insulin resistance index (HOMA2-IR) was calculated by the computational method. Kolmogorov-Smirnov-test, T-test, One-way ANOVA and Pearson correlation were used.
Results: Patients in the 2DM (BMI: 29.27 kg/m2; abdominal circumference M/F: 107.6/109.9 cm), and the NAFLD groups (BMI: 30.36 kg/m2; abdominal circumference M/F: 103.7/110.6 cm) were with similar degree of obesity. 75g CH OGTT in 39 NAFLD pts: 24 normal glucose tolerance (NGT), 4 impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) and 11 type 2 diabetes. HOMA2-IR step by step increase: CNTRL: 1.98; T2DM (liver disease excluded): 2.58; NAFLD-NGT: 3.2 ($p<0.05$ vs CNTRL); NAFLD-IFG/IGT/T2DM: 3.82 ($p<0.01$ vs CNTRL, $p<0.05$ vs T2DM) (Figure1). sDPP-4 activity was higher in the 39 NAFLD pts (mean:32.49U/L, 95%CI:29.57-35.4) than in controls (26.08U/L,95%CI: 24.29-27.87 $p<0.01$) or in the T2DM group (24.16U/L,95%CI: 22.54-25.77 $p<0.001$). Correlations were detected among sDPP-4 and ALT ($r=0.4637$,$p=0.0038$) and gGT ($r=0.4991$,$p=0.0017$) activities and HOMA2-IR ($r= 0.5295$,$p=0.0026$) in NAFLD and among the HOMA2-IR and ALT ($r=0.4340$,$p=0.0147$) and gGT ($r=0.4128$,$p=0.0210$) within the NAFLD group.
Conclusion: In contrast to NAFLD, the serum DPP-4 enzyme activity was not increased in patients with T2DM without liver disease, where the inhibitor therapy is approved. sDPP-4 activity correlated with liver tests in NAFLD supporting that the excess is of hepatic origin. Based on the association of liver tests and HOMA2-IR and on the higher HOMA2-IR values in NAFLD than in the similarly obese T2DM pateints without liver disease we concluded that liver disease should contribute to the development of insulin resistance.

Correlation between the serum DPP-4 enzymatic activity and the CD26 expression on CD3+ lymphocytes in T1DM

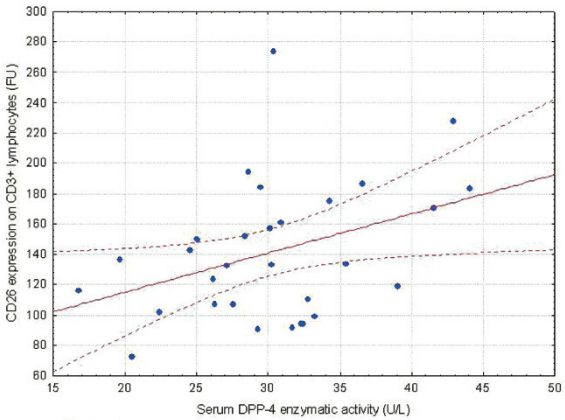

Figure 1.

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805

Prolonged GLP-1 and Ex-4 treatment on fat Glut-4 expression in insulin-resistant state

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Background and aims: It is known that GLP-1 has direct stimulatory effects, through specific receptors, upon the respective main metabolism of liver, muscle and fat. Exendin-4 (Ex-4) although being a peptide of non-mammalian nature, shares with GLP-1 a 52% structural homology and its glucoregulatory actions in normal and diabetic states; in fat tissue, both GLP-1 and Ex-4 stimulate glucose transport and also lipogenesis and lipolysis in a kinase-dependent manner. Here we studied the effect of prolonged treatment with GLP-1 and Ex-4 upon Glut-4 expression in fat tissue of insulin-resistant (IR) rats, compared to normal (N).
Materials and methods: IR model was induced in male Wistar rats by chronic feeding -8 weeks- with standard chow combined with D-fructose (20% in the drinking water). IR (n=5-8/group) and N (n=5-13/group) rats were 3-days treated with saline (control), GLP-1 (0.86 nmol/kg/h) or Ex-4 (0.1 nmol/kg/h); blood samples were taken before and by the end of the treatment for plasma glucose and insulin (RIA) measurements; Glut-4 expression -protein, by Western blot, and mRNA, by RT-PCR- was studied in the epididimal fat pads.
Results: Glut-4 mRNA in IR-control was lower ($p<0.001$) than normal (0.38±0.04 times N-control) while the glucotransporter protein value was not altered. In N, and as previously observed, GLP-1 treatment reduced both, Glut-4 mRNA (0.20±0.07 times N-control, $p<0.001$) and protein (42±5% N-control, $p<0.001$); in IR, GLP-1, while failing to modify the Glut-4 mRNA, induced though an increase ($p<0.02$) in the protein level (126±5% IR-control). In N, Ex-4 treatment reduced the Glut-4 mRNA (0.69±0.13 times N-control, $p<0.05$) without modifying the protein value; yet, in IR, Ex-4 induced an increase in the Glut-4 mRNA level (2.1±0.5 times IR-control, $p<0.05$) which was not accompanied by changes in the glucotransporter protein value (93±6% IR-control).
Conclusion: GLP-1 and Ex-4 have beneficial effects in the altered fat Glut-4 expression in insulin-resistant rats, GLP-1 by acting at the translational and Ex-4 at the transcriptional level. These effects could account for the previously observed improvement in fat glucose transport in this insulin-resistant state.
Supported by: the Spanish Ministry of Health

806

Beta cell stimulation by saxagliptin in patients with type 2 diabetes

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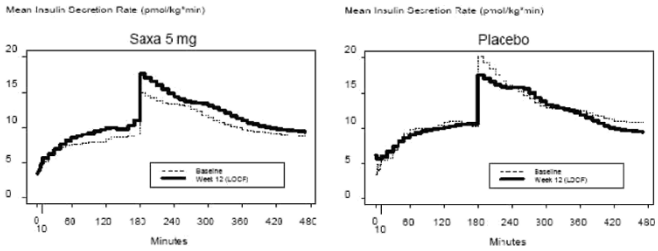
Background and aims: Saxagliptin (SAXA) is a potent, selective DPP-4 inhibitor, specifically designed for extended inhibition of the DPP-4 enzyme. DPP-4 inactivates incretins that stimulate glucose-dependent insulin secretion and inhibit glucagon secretion. A proposed MOA of SAXA involves protecting incretins from DPP-4 degradation, thus improving beta-cell response. This randomized, parallel-group, double-blind, placebo (PBO)-controlled study (CV181-041) assessed SAXA's effect on beta-cell function by intravenous hyperglycemic clamp (IV HC) in patients with type 2 diabetes (T2D).
Materials and methods: Patients were assessed at baseline (BL) and wk 12 in the fasting state (0-180min, IV HC) and after stimulating incretin secretion by orally ingesting 75g glucose (180-480min, IV-oral HC). HC infusions were adjusted to maintain plasma glucose at 280mg/dL. Insulin secretion was calculated by C-peptide deconvolution. Primary endpoint was %Δ from BL in total insulin secretion (%Δ insulin) during IV-oral HC (180-480min). Secondary endpoint was %Δ insulin during IV HC (120-180min). Patients were drug-naïve with T2D aged 43-69yrs with BL A1C range 5.9%-8.1%. Twenty patients received SAXA 5mg od; 16 received PBO.
Results: After 12 wks, SAXA significantly increased %Δ insulin from BL during IV-oral HC (adj% difference of 18.5% vs PBO, p=.035). In the fasting state during IV HC SAXA significantly increased %Δ insulin from BL (adj% difference of 27.9% vs PBO, p=.020). At wk 12 insulin secretion increased from BL with SAXA but not with PBO (Fig). Glucagon AUC during IV-oral HC also improved from BL with SAXA (adj% difference of -21.8% vs PBO, p=.031). SAXA was generally safe and well-tolerated.
Conclusion: SAXA improved pancreatic beta-cell function in the postprandial and fasting states and decreased postprandial glucagon concentration.



Supported by: BMS and AZ

PS 65 Nutrition - experimental

807

Gender-specific response of uncoupling protein 3 gene in the newborn mice to moderate supplementation of maternal diet by n-3 long-chain polyunsaturated fatty acids

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Background and aims: Perinatal development is affected by long-chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFA) transported into foetus from maternal circulation and ingested by newborns with milk during lactation. Aim of this work was to characterize the effects of a moderate n-3 LC-PUFA supplementation of mothers' diet on (i) the content of eicosanoic (EPA) and docosahexaenoic (DHA) acid in milk and liver of the offspring, (ii) body weight gain of the newborns, and (iii) expression of genes of energy metabolism in the muscle of the newborns, also with regard to possible gender-specific differences.
Materials and methods: Adult C57BL/6N female mice were randomly assigned to standard (ST) diet containing 6 % (wt/wt) fat, or ST diet with 5% of dietary fat replaced by EPA/DHA concentrate EPAX1050 TG (EPAX AS, Aalesund, Norway; ST-FO diet). Mice and their pups remained on experimental diets during pregnancy and lactation. After weaning at 31 d, pups of both dietary groups were randomly assigned either ST or high-fat (HF; 35% fat wt/wt) diet. Mice of all four subgroups (ST-HF, ST-FO-HF, ST-ST and ST-FO-ST; n=8) were maintained on the ST or HF diet for 2 weeks. Milk consumption and fatty acid composition, as well as liver fatty acid composition were evaluated at 10 d after birth, while muscle gene expression was characterized at 2 weeks after initiation of ST or HF feeding.
Results: Milk consumption was not affected by n-3 LC-PUFA intake. Milk EPA concentration was marginally elevated (0.19±0.02 vs. 0.26±0.01 Mol%; $p<0.05$), while milk DHA concentration was elevated 10-fold (0.09±0.01 vs. 0.88±0.05 Mol%; $p<0.05$), due to dietary supplementation by n-3 LC-PUFA. Also liver concentrations of both EPA and DHA were increased due to supplementation, but the increase was similar for both fatty acids (2.6- and 1.4-fold for EPA and DHA, respectively). These results are in agreement with a high metabolism of n-3 LC-PUFA in the liver. HF diet induced higher body weight gain compared with ST diet, but n-3 LC-PUFA intake during gestation and lactation had no effect. Among several genes studied, uncoupling protein 3 gene (*Ucp3*) showed by far the most significant induction due to HF diet, namely in gastrocnemius muscle of male mice. A similar pattern of changes in *Ucp3* expression was observed in soleus but not in gastrocnemius muscle of female mice, where the interaction with n-3 LC-PUFA was only observed.
Conclusion: Our results indicate muscle type- and gender-specific differences in the effects of n-3 LC-PUFA on expression of genes of energy metabolism in murine muscle during early postnatal development, namely in response to elevated DHA intake during lactation. Possible lasting metabolic consequences of these effects will be studied.
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808

Supplementation with conjugated linoleic acid causes isomer-dependent anti inflammatory effects in human monocytes

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Background and aims: Diabetes mellitus is associated with inflammatory endothelial activation and production of proinflammatory cytokines. Various beneficial of conjugated linoleic acids (CLAs) effects including decreasing atherosclerosis, diabetes and inflammation were described in vivo and in vitro. In humans evidence is currently conflicting. Aim of the presented study was to investigate effects of CLA supplementation on gene expression in monocytes and to elucidate the mechanisms involved.
Study design and methods: In a randomized, double blind, cross-over study, 34 healthy males (45-65y), consumed 4.25g preparation per day of either ref-

erence oil (linoleic acid) or cis-9, trans-11 CLA isomer or trans-10,cis-12 CLA isomer or a 1:1 mix of both isomers for 4 weeks. Intervention periods were separated by 4 weeks wash out. After each intervention monocytes were separated, genome wide expression analysis using microarrays was performed, and several regulated genes of interest were verified by a real-time PCR.
Results: 42 genes were verified by real-time PCR out of those 17 genes could be confirmed as regulated significantly after at least one intervention. The strongest change compared to reference oil was found after consuming trans-10, cis-12 CLA proinflammatory genes like IL-8 (-3.1 fold change (fc)), IL-1b (-2.6 fc) and COX2 (-1.48 fc) were significantly lower expressed. After 1:1 mix intervention the effect was similar (IL-1β 1.6 fc; IL-8 -1.3 fc; COX2 -1.3 fc). Both preparations regulated a number of additional genes. Whereas after cis9, tans11 CLA administration only a few genes were affected and changes were weaker (IL-8 -1.6 fc; IL-1b -1.6 fc; all other not significantly regulated).
Conclusion: These data suggest that CLA achieves isomer-specific anti-inflammatory effects and that the potency of a 1:1 CLA mixture of both isomers does not equal the added up effect of its single isomers. The strongest antiinflammatory effects in monocytes were found after tans-10, cis-12 CLA supplementation.
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809

Effects of choline/methionine-deficient diet upon hepatic carbohydrate metabolism and bioenergetics in perifused liver cells or isolated mitochondria from fatty livers
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Background and aims: Nonalcoholic steatohepatitis (NASH) is an important form of liver disease that may progress to cirrhosis and liver failure. Feeding rodent a lipogenic methionine-choline deficient diet (MCDD) is a frequently employed non-physiological nutritional model that leads to hepatic injuries similar to those seen in human steatosis. Nevertheless, the mechanisms underlying this major pathogenesis remain still unknown at the energy metabolism level. Using, for the first time, the liver cell perifusion system from fatty livers, the purpose of this study was to explore the alterations in both glucose utilization and mitochondrial bioenergetics induced in MCDD Wistar rats.
Materials and methods: Rats (200-250 g) were separated in two different groups and received either a standard diet or the high fat-MCDD provided in small pellets during 4 weeks. Hepatocytes, isolated from the livers of starved animals by the collagenase method, were perifused at 37°C with Krebs-bicarbonate-calcium medium saturated with O_2/CO_2, then titrated with increasing amounts of dihydroxyacetone (DHA) as gluconeogenic substrate to get seven consecutive steady states. For each of them, the net rates of glucose ($J_{Glucose}$) or lactate-plus-pyruvate (J_{L+P}) production were assessed in the cellular perifusate. We further studied the mitochondrial consequences of fat accumulation in this model by determining oxidative phosphorylation and production of reactive oxygen species (ROS) in isolated mitochondria.
Results: In DHA-perifused liver cells, MCDD significantly reduced (-52%, $p<0.001$) global metabolism of this carbohydrate source (J_{2G+L+P}), as compared with control diet. This response was largely explained by a strong inhibition of both gluconeogenesis ($J_{Glucose}$) and glycolysis (J_{L+P}). Furthermore, a decrease in L/P ratio, which reflects a more oxidized cytosolic NADH/NAD^+, was also evidenced in MCDD rats. On the other hand, liver mitochondria from these treated rats exhibited a higher oxygen consumption rate (+ 25%) in presence of succinate as energetic substrate, but these MCDD organelles had the same respiratory index than the control mitochondria. Interestingly enough, a low ROS formation was found in MCDD animals.
Conclusion: Our data show that MCD diet causes a net inhibition of the DHA global metabolism, but it also triggers a response mechanism on the bioenergetic level, likely designed to overcome, at least partially, the induced steatosis. We could suggest this dietary model as a tool for some pharmacological approach of the fatty liver diseases management.

810

Restoration of impaired intestinal permeability and changes in the gut cytokine profile are mechanisms in type 1 diabetes prevention by a hydrolysed casein diet
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Background and aims: Diabetogenic triggers from the food and gut micro flora can induce the autoimmune cascade leading to type 1 diabetes (T1D). However, in order to induce the autoimmune process, these diabetogenic antigens have to pass the intestinal barrier. T1D patients and animal models for T1D show an impaired intestinal barrier function. In this study we investigated whether prevention of T1D by the hydrolysed casein (HC) diet is related to restoration of this impaired intestinal barier function.
Materials and methods: To this end, Diabetes Prone (DP)-BB rats were given the HC-diet from weaning on and followed for the development of T1D. At fixed time points feces, gut tissue and blood was collected. Composition of the bacterial flora was established in the feces by qPCR. From the gut tissue RNA was isolated and expression of tight-junction (TJ) related proteins such as Myo9B, claudins and occludin and the cytokine profile was analysed. Serum zonulin levels (protein that regulates TJ function) were measured by ELISA. The transepithelial electrical resistance (TEER; indicator of intestinal permeability) was measured on ileum samples in vitro by snap well assay. In addition, BB-DP rats were subjected to a Lactulose Mannitol (LA/MA) test to measure intestinal permeability in vivo.
Results: BB-DP rats showed increased serum zonulin levels and a reduced TEER as compared to diabetes resistant (DR) rats. Serum zonulin levels were decreased in DP BB rats receiving the HC-diet. In addition the TEER was increased in BB-DP rats on the HC-diet. Moreover, the rats on the HC-diet showed a decreased Lactulose Mannitol ratio (which indicates a low intestinal permeability) as compared to the BB-DP rats on the standard diet. The HC-diet caused a reduction of the Myo9B expression, an increase of claudin-1 and a reduced claudin-2 expression in the ileum. Moreover, the HC diet caused an increase in IL-10 and TGF-beta expression. The percentage of rats responding with a decrease of their intestinal permeability (increase in TEER and reduction of LA/MA ratio) after feeding the HC-diet correlated with the rate of protection against T1D development. Intriguingly, the change in TEER of the ileum was negatively correlated with the bacteroides levels in the gut. This further strengthens our previous observations that high bacteroides levels are negatively correlated with T1D development (Brugman et al., Diabetologia 2006).
Conclusion: Prevention of T1D by feeding BB-DP rats an HC-diet, correlated with the restoration of the intestinal barrier function. Modification of the gut micro flora, direct effects on the tight junctions, changed cytokine profile and reduced zonulin production might be important mechanisms for this effect. Interfering with intestinal permeability via modification of the gut microflora and tight junctions might lead to novel therapies in the prevention of T1D.
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811

Ethanol consumption patterns determine the effect of ethanol on insulin sensitivity in high-fat diet-fed rats: role of adiponectin and AMPK α
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Background and aims: Protective and detrimental effects of ethanol on insulin sensitivity have been widely reported. This may be due to the different ethanol dosage and the variations in consumption patterns. Here we investigated the effect of two moderate ethanol consumption patterns on insulin sensitivity in high-fat (HF) diet-fed rat and explore the possible mechanisms mediated by adiponectin and AMP-activate protein kinase (AMPK).
Materials and methods: Total 48 male wistar rats were randomly divided into four groups and received either normal diet (group C) or high-fat diet

(group HF, HF+E1, and HF+E2). Rats in group HF+E1 received edible ethanol twice daily by gastric tube, while animals in group HF+E2 drank ethanol solution instead of tap water and the total daily ethanol dosage in both groups was the same (5 g/kg/d, a moderate ethanol dosage). After treated for 22 weeks, fasting blood samples and epididymal and perirenal fat pads were obtained. Gene transcription and protein expression were tested by RT-PCR and western blotting, respectively.

Results: As shown in table 1, ethanol administration lessened the weight gain effect of HF diet. Coincident with body weights (BWs), the epididymal and perirenal fat masses in group HF+E1 and HF+E2 were smaller than that in group HF. Elevated fasting glucose level and fasting insulin concentration in group HF were reduced by ethanol addition. Accordingly, evaluated by HOMA-IR, insulin resistance was found in HF diet-fed rats, but was then ameliorated after ethanol administration. Twice daily ethanol administration pattern was observed better than continuously drinking pattern in improving adverse effect of HF diet.

Table 1: Characterization of the Rats in 4 groups

	C	HF	HF+E1	HF+E2
BWs (g) Initial	220.3±13.1	219.5±15	224.6±19.5	221±13.9
BWs (g) Final	477.7±34.5	540.7±58.4**	495.3±43$^{\#}$	515.8±45.2
Epididymal fat mass (% of BW)	0.9±0.3	1.3±0.3*	1.0±0.5	1.2±0.3
Perirenal fat mass (% of BW)	1.3±0.5	2.3±0.5**	1.6±0.7$^{\#\#}$	2.2±0.8
Fasting glucose level (mmol/L)	4.1±1.7	5.2±1.0	4.8±1.4	5.2±1.3
Fasting insulin concentration (mIU/L)	20.6±5.2	26.4±5.6*	21.2±3.4$^{\#}$	25.1±4.3
HOMA-IR	3.7±1.2	6.5±2.3**	4.6±1.9$^{\#}$	5.9±2.1
Serum ethanol concentration (mg/dl)	0	0	10.8±4.4	4.0±1.1
Serum adiponectin concentration (μg/ml)	25.8±3.2	16.5±3.7*	22.7±6.5$^{\#\#}$	17.7±5.2
Adiponectin contents in epididymal adipose tissue (μg)	88.8±20.3	47.3±18.9**	82.6±19$^{\#\#}$	55.8±17.1

*P<0.05, **P<0.01 vs. group C, $^{\#}$P<0.05, $^{\#\#}$P<0.01 vs group HF.

Ethanol consumption increased adiponectin concentration in both serum and epididymal adipose tissue in the setting of HF diet, such an effect was more obvious in group HF+E1 than in group HF+E2. As we know, adiponectin is an activator for AMPK. In concert with the changes of adiponectin, HF diet alone reduced the ratio of activated-AMPK α to Total-AMPKα to 39.08% of that in group C ($P< 0.01$ *vs.* C) and twice ethanol consumption and continuously ethanol drinking recovered the ratio to 88.9% ($P< 0.01$ *vs.* HF) and 45.8% ($P> 0.05$ *vs.* HF) of that in group C. In parallel with the changes of AMPK activation, both GLUT4 mRNA and protein expression were significantly impaired in group HF ($P< 0.01$ *vs.* C), and were then recovered to nearly normal levels in group HF+E1 ($P< 0.01$ *vs.* HF), but only recovered to 60.1- and 68% of normal levels in group HF+E2 ($P> 0.05$ *vs.* HF).

Conclusion: Moderate ethanol consumption might ameliorate the adverse effect of HF diet on insulin sensitivity in rats, twice administration pattern was much better than continuously drinking pattern. The mechanism for the protective effect of ethanol might be mediated by increased serum and adipose tissue adiponectin levels, subsequently improved activation of AMPK α, then GLUT4 expression in adipose tissue.

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812

Acute effect of oral administration of dietary products enriched in antioxidants on post-prandial endothelial function in apparently healthy adults

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Background and aims: Oxidative stress has been advocated as a major cause for cardiovascular disease and low plasma antioxidant's concentrations have been associated with endothelial dysfunction (ED), the first step to atherosclerosis. Since food ingestion induces a temporary burst in oxidative stress and acutely impairs endothelial function (EF), it is conceivable that the long-term development of atherosclerosis might be at least partially attributed to repeated exposures to post-meal ED. Although experimental data show that the co-administration of antioxidants to an oral glucose tolerance test improves post-prandial ED, it is still unclear whether the supplementation of regular food with antioxidants is associated with an improved post-prandial EF profile.

Materials and methods: To address this issue, we conducted a placebo-controlled cross-over intervention study on 31 apparently healthy adults (13F/18M, mean age = 65 ± 4 years) who underwent to the single administration of a breakfast snack supplemented with antioxidants or a placebo snack, in a randomized order, with a week of wash out between the two tests. The antioxidant-enriched snack was composed by two products -a beverage (green tea or berries juice) and a cereal bar- supplemented with vitamin E (12 mg per serving of beverage and 15 mg per serving of bar), vitamin C (90 mg per beverage and 80 mg per bar) and cathechins (35 mg per serving of both). Placebo snacks were prepared to be identical to the active ones in terms of appearance and organoleptic properties. All subjects came in the morning, after overnight fasting, and underwent to a Flow Mediated Dilatation (FMD) test fasting, 2, and 3 hours after snack ingestion. Blood was drawn with the same time-course to evaluate changes in plasma antioxidant concentrations.

Results: We observed a significant and sustained decline in EF in the placebo meal 2 and 3 hours after snack ingestion (maximal post-ischemic vasodilation during FMD test decreased from 6.4±3.5% at baseline to 4.9±2.6% and 4.9±2.8% respectively; p<0.05 for both), whereas the assumption of the antioxidant-enriched snack was associated with a significant improvement (p<0.05) of FMD values ranging from 5.4±3.3% at baseline to 6.6±3.4% after 2 and 6.5±3.5% after 3 hours. In addition, we observed the appearance of measurable concentrations of epigallocathechin gallate at 2 and 3 hours (29.8±22.1 and 27.8±24.8 nMol/L, respectively) after the assumption of the antioxidant-enriched meal and a significant increase in vitamin C plasma levels at 2 and 3 hours (p<0.01 for both). No significant variation was identified in vitamin E plasma concentrations.

Conclusion: In a population of apparently healthy subjects, the supplementation of a mixed meal with antioxidants is able to reverse the temporary decline in endothelial function usually observed in the post-prandial phase, inducing an acute increase in endothelial-dependent vasodilation. These data suggest that functional foods supplemented with antioxidants (like snacks and beverages) could have a favourable effect on post-prandial EF, raising the speculation that their introduction in a regular diet might produce positive long term effect on atherosclerosis development.

813

Impact of the oral glucose spray, liquid sugars or dextrose tablets on the evolution of plasma glucose concentration in healthy persons

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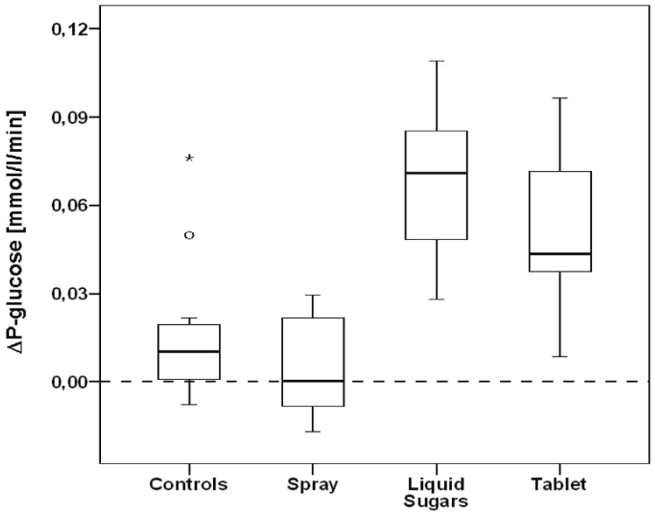
Background: In order to combat hypoglycaemia in persons with diabetes, oral glucose spray was believed to offer a sophisticated solution of this complication. However, clear references to the effects of oral glucose spray on plasma glucose concentration (PG) are missing.

Aims: The purpose of this prospective controlled trial was to assess the efficacy of three commercially available glucose products on the evolution of PG: (1) the oral glucose spray, (2) liquid sugar jelly, (3) dextrose tablet.

Methods: Sixteen healthy volunteers aged 21.8 ± 0.78 y (mean ± SE), BMI 23.5 ± 0.84 kg/m^2, tested their PG over the course of three sets of 4 sessions (S) each: S_0-control fasting, S_1 with oral administration of 10 glucose spray-doses (5 doses on the mucosa of each cheek, as indicated by the manufacturer, without swallowing), i.e., 0.84 g of glucose at time 0; S_2 with consumption of 1 sachet (13 ml) of the liquid sugar (ca. 5.2 g glucose, 5.2 g fructose, 5.2 g sucrose); S_3 with chewing and consumption of 1 dextrose tablet (6 g). Each S was performed between 6 and 9 h a. m., after fasting for the previous 6 hours. PG was tested in finger-prick capillary blood using personal glucometer Linus, Wellion, Agamatrix, Inc., Salem, NH, at start, at 5, 10, 15, 20 and 30 min. During all tests, the subjects remained seated, and did not engage in any physical activity. Statistical software SPSS v. 15, SPSS, Inc, Chicago, IL, was used. Means of 3 sessions in each of 16 subjects were analyzed.
Results: Wilcoxon-signed-rank-test revealed no differences between the change of the mean PG at start vs. 5-minute intervals neither in control- nor in intervention sessions. For the 30-minute time period after ingestion, analysis of regression coefficients demonstrated increase of PG after consumption of a sachet of liquid sugar (0.068 mmol/l/min, p = 0.001) which prevails over a single dextrose tablet (0.052 mmol/l/min, p = 0.002) but does not appear after oral glucose spray (0.005 mmol/l/min, p = 0.215), as compared to control session (see boxgraphs in Figure for details).
Conclusion: Liquid sugar jelly and dextrose tablets are effective means to increase PG within 10 minutes after ingestion. Oral glucose spray resulted in no increase of PG in the course of 30 minutes after its use. So, oral glucose spray cannot be recommended to combat hypoglycaemia.



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814

Differential effects of protein quality on postprandial lipaemia in response to a fat-rich meal in type 2 diabetes: comparision of whey, casein, gluten and cod protein

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Background and aims: Enhanced and prolonged postprandial triglyceride responses involve increased cardiovascular risk in type 2 diabetes (T2DM). Dietary fat and carbohydrates profoundly influence postprandial hypertriglyceridemia whereas little information exists about the effect of proteins. The aim of this study was to compare the effects of the proteins casein, whey, cod, and gluten on postprandial lipid and incretin responses to a high-fat meal in T2DM.
Materials and methods: Cross-over study of twelve type 2 diabetics. Blood samples were collected over 8 h after ingestion of a test meal containing 100g butter and 45g carbohydrate in combination with 45g of either casein (Cas-meal); whey (Whe-meal); cod (Cod-meal); or gluten (Glu-meal). We measured plasma levels of triglyceride, retinyl palmitate (RP), free fatty acids (FFA), insulin, glucose, glucagon, glucagon-like peptide1 (GLP-1), and glucose-dependent insulinotropic peptide (GIP). For statistical analysis were used One way repeated measurement ANOVA. $p<0.05$ are considered as statistical significant.
Results: The incremental area under the curve for triglyceride was significantly lower after the Whe-meal than after the other meals (p=0.008). The RP response was lower after Whe-meal than after Cas- and Cod-meal in the chylomicron-rich fraction (p=0.019) and higher after Whe-meal than after Cod- and Glu-meal in the chylomicron-poor fraction (p=0.003). FFA was most pronouncedly suppressed after whey ($p<0.001$). The glucose response was lower after the Whe-meal than after the other meals (p=0.015), whereas no significant differences were found in insulin, glucagon, GLP-1, and GIP responses.
Conclusion: The data suggest that as a supplement to a fat-rich meal in T2DM subjects, whey protein seems to outperform other proteins in terms of postprandial lipemia improvement possibly owing to formation of fewer chylomicrons or increased clearance of chylomicrons.
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PS 66 Nutrition

815

The effet of vitamin D status on insulin action and secretion in subjects with the metabolic syndrome

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Background and aims: Accumulated evidence suggests that low levels of vitamin D is associated with impaired glucose metabolism and an increased risk of developing type 2 diabetes. However, very few studies have examined the role of vitamin D status for β-cell function with appropriate methods. We have evaluated the relationship between serum 25-hydroxyvitamin D_3 (25-OHvitD_3) and insulin action and secretion in subjects with the metabolic syndrome (MetS).
Materials and methods: 25-OHvitD_3 was measured with HPLC-MS in baseline samples from 446 subjects with MetS recruited for the LIPGENE dietary intervention study. Subjects (45% males) were from eight European centres, mean age was 54.6 ± 8.9 years and mean BMI 32.4 ± 4.2 kg/m^2. Insulin sensitivity (Si), acute insulin response to glucose (AIR_g) and disposition index (DI) were assessed by frequent sampling insulin modified intravenous glucose tolerance test (FSIVGTT) and MINMOD Millenium software. The HOMA-calculator version 2.2 was used to calculate HOMA IR and HOMA β. Non-parametric Kruskall-Wallis test was used to compare groups and correlations were calculated using Spearman's rank method. Data are presented as median (inter quartile range).
Results: The median 25-OHvitD_3 concentration was 51.3 (32.7) nmol/L, and only 91 (20.4%) had a serum level of 25-OHvitD_3 considered to be sufficient (≥75 nmol/L). 25-OHvitD_3 levels were significantly negatively associated with BMI (r=-0.23, p<0.001) and parameters of insulin secretion (HOMA β) and insulin resistance (HOMA IR) estimated from fasting levels of insulin and glucose (r=-0.15, p=0.001 and r=-0.14, p=0.003, respectively). The IVGTT derived parameters S_i and DI did not correlate with 25-OHvitD_3 status (r=0.06, p=0.21 and r=-0.059, p=0.24).

Median (IQR) levels of BMI and parameters of insulin action and secretion across tertiles of vit D

25-OH-vit D_3 (nmol/L)	**< 41.9**	**42-63.1**	**> 63.1**	**Kruskal-Wallis p**
n	149	149	148	
BMI (kg/m²)	33.1 (5.2)	32.4 (4.9)	31.1 (6.4)	< 0.001
Si	2.4 (2.0)	2.5 (1.7)	2.7 (1.9)	0.23
AIR	319 (377)	276 (258)	255 (360)	0.33
DI	762 (861)	697 (996)	725 (883)	0.40
HOMA β (%)	89.5 (48)	83.7 (38)	78.7 (43)	0.02
HOMA IR	1.5 (1.1)	1.4 (0.9)	1.3 (1.0)	0.02

Conclusion: Although associations between serum levels of 25-OHvitD_3 and insulin secretion and action were found when assessed by HOMA-methods, we were not able to confirm these relationships when we used FSIVGTT in a large sample of subjects with MetS.
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816

Effects of the dietary omega-3 polyunsaturated fatty acids on atherosclerosis progression and cardio-metabolic parameters in metabolic syndrome patients

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Background and aims: To test the functional effects of a diet containing omega-3 PUFA supplements vs. baseline diet recommended to patients with metabolic syndrome.
Materials and methods: A total of 156 patients with metabolic syndrome (MS) according to IDF criteria, aged 61±5.4 years, without clinical evidence of atherosclerosis were allocated to 2 groups, matched by sex and age: group A (76 patients) - diet according to ESC/EASD recommendations and individual needs; group B (80 patients) - the same diet + capsules of fish oil (1,0 g eicosapentanoic acid, 1,0 g docosahexanoic acid and 0,1 g α-tocopherol acetate). Body fat mass (BFM) and body fat percent (%BF) were measured by bioimpedance analysis (BIA) using InBody 3.0 Analyzer. Fasting plasma glucose, HbA1c, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, plasma insulin, adiponectin and leptin were measured according to standard procedures. Insulin resistance was measured using HOMA-IR index. Oxidative stress was assessed using FormOx systems monitor on a blood drop. The progression of atherosclerosis was determined by measuring intima-media thickness (IMT) at commune carotid artery (ACC). The duration of the study was 6 months.
Results: Baseline characteristics were similar between groups. After 6 months, omega-3 supplements determined a significant improvement of metabolic parameters, decrease of oxidative stress (Table 1) and a statistically significant increase in adiponectin levels (from 9.46 ± 2.76 to 10.86 ± 2.68). Mean BMI, mean %BF, mean BFM and mean waist-to-hip ratio (WHR) were significantly lower in group B vs. group A (BMI- 31.12 kg/m^2 vs 29.1; %BF - 30.48 vs 27.48; BFM - 29.42 kg vs 26.78; WHR - 1.07 vs 1.02). BMI was statistically correlated with BFM (p<0.0001) and %BF (p<0.0001). Intima-media thickness was significantly decreased in group B (IMT in left ACC - 0.610 ± 0.06 vs. 0.621 ± 0.071 mm p=0.002; IMT in right ACC - 0.593 ± 0.074 vs. 0.612 ± 0.068) and was correlated with %BF (p<0.001), WHR (p=0.016), leptin values (p<0.001), adiponectin values (p<0.05) and leptin/adiponectin ratio (p<0.001).
Conclusion: Omega-3 PUFA enriched diets bring metabolic parameters closer to target values, thus lowering cardiovascular risk of MS patients. Also, IMT values and oxidative stress are decreased, underlying the role of omega-3 in the delay of atherosclerosis progression and endothelial cells damage.

Table 1

Parameters	**Group A - diet**	**Group B - diet + omega-3**	**P value**
Total cholesterol (mg/dl)	213 ± 20.5	198 ± 18.0	P<0.002
HDL-cholesterol (mg/dl)	46 ± 14	55 ± 12	P<0.05
Triglycerides (mg/dl)	143 ± 71	132 ± 58	P=0.002
HOMA-IR	4.62 ± 3.2	4.57 ± 2.4	P=0.016
Fasting Plasma Glucose (mg/dl)	121 ± 18	110 ± 14	P<0.0001
FormOx (Fort units)	319 ± 94	268 ± 76	P<0.0001

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817

Does a low-fat vegetarian diet improve insulin resistance and beta cell function in individuals with type 2 diabetes?

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Background and aims: Diabetes prevalence is relatively low among individuals consuming plant-based and vegetarian diets, and clinical trials using such diets have shown greater effect on weight loss, greater reduction of LDL cholesterol, HbA1c and diabetes medication than conventional diabetic diet.

The aim of our study was to evaluate the effect of low-fat vegetarian diet compared to conventional diabetic diet with similar caloric restriction on insulin resistance, β-cell function, body weight and resting energy expenditure in individuals with type 2 diabetes after a 3-month-diet-intervention.
Materials and methods: Open, parallel randomized study. 70 individuals with type 2 diabetes were randomly assigned to either experimental group (EG) following a low-fat vegetarian diet or the control group (CG) following conventional diabetic diet with similar caloric restriction (-500 kcal/day). We measured all common anthropometric and laboratory parameters; glucose tolerance and insulin secretion was evaluated after standard meal stimulation; we assessed insulin resistance by a 3-hour hyperinsulinemic isoglycemic clamp; resting energy expenditure was measured by indirect calorimetry. All the procedures were done at start (0) and after 12 weeks.
Results: Average weight loss was greater in the EG than in the CG (6.41±4.23 vs. 3.47±4.07 kg; p<0.01). Diabetes medication was reduced according to the study protocol in 15 subjects from the EG (42.86%) vs. in 2 subjects from the CG (6.45%). We noticed an increase in post-load plasma C-peptide concentrations in the EG (p<0.05), whereas changes in stimulated C-peptide in the CG were not significant. We observed a trend to a greater reduction of plasma HbA1c, cholesterol, free fatty acids and both fasting and post-load glucose in the EG, but the differences were not statistically significant. Insulin resistance decreased (p<0.01) similarly in both groups. Respiration coefficient decreased (p<0.01) and fasting fat oxidation increased (p<0.01) similarly in both groups.
Conclusion: Our results indicate that low-fat vegetarian diet leads more effectively to reduction of diabetes medication and improvement of β-cell function than the conventional diabetic diet and it could be a more convenient alternative in treatment of type 2 diabetes.
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818

Association between regularity and frequency of meals and metabolic parameters in type 2 diabetic patients
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Background and aims: Medical Nutritional therapy is a key component for preventing diabetes, managing existing diabetes, and preventing the development of diabetic complications. The aim of this study was to assess the association between dietary habits and metabolic parameters in Korean type 2 diabetic patients who live in a different culture and have different dietary habits and patterns from those of western countries.
Materials and methods: 1,251 diabetic patients from the Korean National Diabetes Program (KNDP) cohort who completed a dietary habit questionnaire and clinical evaluation from March, 2006 to November, 2007 were included in this study. Dietary intake was assessed using a 24 hour dietary recall method and anthropometric data and biochemical data were collected.
Results: The mean age of the patients were 54.0±10.1 years, duration of diabetes 5.0±5.9 years, body mass index 25.1±3.1 kg/m^2, HbA1c 7.2±1.2%. 56.3% of the patients were male and 6.5% of the patients were on insulin therapy. 87% of the patients had meals 3times per day and 13% of the patients had meals 1 or 2 times per day. Patients who had meals 3 times per day were older (54.6±9.8 vs 50.5±10.9kg, p<0.001), had a lower BMI (25.0±3.0 vs 26.0±3.4 kg/m^2, p<0.001) and higher intake of fibers (26.4±10.0 vs 23.6±9.7 g/day, p=0.001) and higher intake of calories (1764.8±454.4 vs 1647.7±588.8 kcal/day, p=0.015) compared with those who had meals 1 or 2 times per day. Three times per day frequency of meals was associated with lower BMI after adjustment for age and sex (p<0.001). Assessing the regularity of time of meals, 63% of the patients kept a meal schedule that was regular most of the time (≥5days per week) and 37% of the patients had a more irregular meal schedule (regular time of meals < 5 times per week). Patients who's meal schedule was regular most of the time were older (55.2±9.9 vs 51.98±10.0 years, p<0.001), had a lower BMI (24.8±2.9 vs 25.6±3.3 kg/m^2, p<0.001), a lower waist circumference (87.2±7.8 vs 88.3±8.4 cm, p=0.02), and a lower fasting glucose level (134.5±36.8 vs 139.4±41.4 mg/dl) compared with those who's meal time was more irregular (regular time of meal <5times per week). Regularity of meal time schedule was associated with lower BMI (p<0.001) but not lower fasting glucose (p=0.06) after adjustment for age and sex.
Conclusion: Meal frequency of 3 times per day and more regular meal schedules was associated with decreased BMI but not glycemic control. Dietary habits seem to be an important factor for maintaining lower body weight in type 2 diabetic patients.
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819

Satiating effects of milk but not sugar- or artificial-sweetened soft drinks and water
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Background and aims: Energy intakes from drinks may not be compensated for by proper satiety sensation. Consequently weight gain, obesity and type 2 diabetes may occur as a result of the excessive energy consumed. We aim to provide evidence linking drinks to insufficient satiety and excessive energy intake as well as elucidating the mechanisms underlying this association, as far as they can be explained by variations in concentrations of the appetite-regulating hormones Ghrelin, PYY, GIP and PP.
Materials and methods: The present study is a cross-over, intervention clinical trial including 24 overweight (BMI: 31.4 +/- 2.9kg/m^2) subjects (12men and 12woman). Baseline blood-samples were collected after an overnight fast and a 500ml drink (sugar-sweetened beverage (250 kcal/1 MJ), aspartam-sweetened beverage, semi-skimmed milk (240 kcal/0.9 MJ) or water) was served. Blood-samples and satiety score using a visual analogue scales (VAS) were collected and filled in every 30 minutes. After four hours an ad libitum meal was served and the energy intake calculated.
Results: Compared to water milk causes 94% higher satiety VAS score (dAUC = 64.8mm (95% CI: 15.0;114.6), P= 0.008) and 122% higher fullness VAS score (dAUC = 62.2mm (95% CI:10.9;113.5), P= 0.04) whereas the effects of a sugar-sweetened drink were no different than water. Ghrelin concentration is significant lower after consumption of milk than after consumption of water (0.76fmol/ml (95% CI:0.61;0.95), P=0.008), and there is a tendency (not significant) towards ghrelin concentration being less decreased by the soft drinks preloads compared to milk (artificial-sweetened: P=0.2, sugar-sweetened: P=0.9). However between the four preload drinks there were no significant differences in energy intake. Although the ad libitum energy intake was 4654±383 kJ after milk and 4924 ±481 kJ after the sugar-sweetened beverage.
Conclusion: So far our study supports the notion that milk is more satiating than equicaloric sugar-sweetened soft drink, aspartam-sweetened soft drink and water. The association between milk and increased satiety is reflected in subjective appetite sensation. However no differences in energy intakes between the four preloads support the notion that energy in drinks may cause higher total energy intake.
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820

Changes of plasma free amino acids in type 2 diabetes
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Background and aims: Dietary components are strongly associated with type 2 diabetes. High fat diet and high carbohydrate diet have been intensively investigated, and been found to contribute to the high prevalence of type 2 diabetes. Another major diet component, proteins, has drawn more and more attention recently. Some studies indicated that high protein diet (HPD), especially high meat protein intake, may also contribute the increased risk of type 2 diabetes, while many other studies didn't discover any positive association. On the other hand, old and new evidence indicated that plasma free amino acids (AA) are associated with both plasma insulin level and insulin sensitivity. To understand what happened to plasma free AAs in type 2 diabetic patients may give some interesting clues to the discrepancy among high protein diet or AAs studies. Given the fact that the data about the profile of plasma free AAs in type 2 diabetic and normal individuals was very limited, the primary aim of this study was to investigate the alterations of plasma free AAs in type 2 diabetic patients so as to provide some valuable information for further clinical high protein diet and AAs researches.

Materials and methods: Our study was a cross-sectional case-control study. Both T2DM patients and non-diabetic controls were from a part of an ongoing cross-section survey in China, designed to investigate the prevalence of type 2 diabetes and metabolic syndrome among Chinese populations. These participants were of Yi population, an old minor nationality in China. A total of 132 type 2 diabetic patient and 137 age, sex, cigarette and alcohol use matched controls were included in this study. All of them had no history of severe disease such as pulmonary, heart, liver and kidney diseases, and they were included before AAs measurement. Dichotomous data were described as percent (%). Continual data with normal distribution or near normal distribution was described as mean ±S.D., others were described as median and quartiles and logarithm transformed before statistical analysis. Categorical variables were compared by chi square tests. Continuous variables were evaluated by Student's t tests. Correlations between analyzed by Pearson test. All reported P values were two tailed, and those $\alpha<0.05$ were considered statistically significant

Results: 1. Levels of total plasma free amino acids (TAA), essential amino acids(EAA) and branched chain amino acids (BCAA) in type 2 diabetic patients were higher than those in controls (P=0.000 for all). Non-essential amino acids (NEAA)' level was also high in type 2 diabetic patients but with marginal significant difference (P=0.044). Statistic results of individual AAs showed that plasma alanine, valine, leucine, isoleucine, phenylalanine, tyrosine, methionine, glutamate and lysine were higher in type 2 diabetes than in control (P<0.01 for all) , while plasma glycine, glutamine were lower in type 2 diabetes than in control (P=0.003 and P=0.034 respectively) 2. TAA, especially EAA and BCAA, and individual AAs, alanine, valine, leucine, isoleucine and glutamate were positively associated with BMI, waist, GGT, ALT, while, glutamine was negatively related to GGT, and glycine negatively related to ferritin.

Conclusion: There were significant differences in plasma amino acids between type 2 diabetic patients and controls. Some AAs, especially BCAA and glutamate were correlated with identified type 2 diabetes risk factors. Whether abnormal metabolism of AAs contributed to increased risk of type 2 diabetes is very necessary to be further investigated by prospective studies.

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821

Diet induced 2-year weight loss initiates a cluster of inter-correlated changes in lipids, inflammation and insulin resistance, more profound among diabetics than in non-diabetics; results from the DIRECT study

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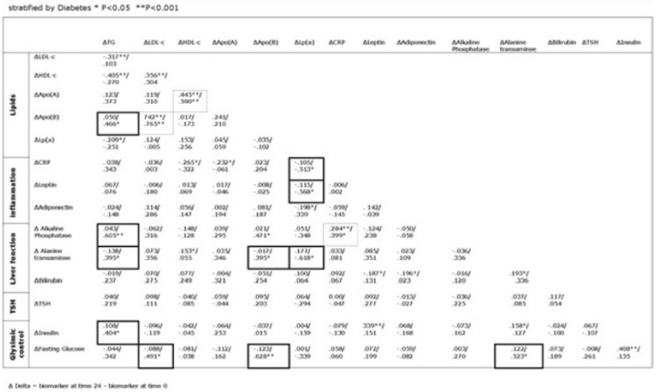
Background: Data regarding inter-correlations of *changes* of biomarkers induced by long term dietary interventions and the differential effect among patients with type 2 diabetes (T2D) and non diabetics is sparse.

Methods: 322 Participants of the Dietary Intervention Randomized Controlled Trial (DIRECT) were randomly assigned to one of three dietary strategies over 2 years. We compared the intra- correlations of *changes* in lipids, markers of inflammation, liver function tests, glycemic control and TSH during the 2 year weight loss trial among non-diabetic controls (n=277) and patients with T2D (n=45).

Results: Mean weight change was -3.49 kg among patients with T2D and -4.10kg among non-diabetics (p=0.50). In absolute values, the improvement in HDL-c was higher among diabetics that among non-diabetics (+9.41 vs. + 6.57mg/dL, p<0.05). Correlations adjusted for Age, sex and 2-year change in BMI revealed than beyond the common inter-correlations found between biomarkers in both groups [such as significant correlations between the 2 year decrease in triglycerides and the 2 year increase in both LDL-c, HDL-c and Lp(a) levels (p<0.05 for all)], we found specific correlations among the T2D patients only: A 2-year decrease in fasting glucose was significantly associated with a decrease in LDL-c, Apo(B), and alanine transaminase (p<0.05 for all). Among T2D patients only, a decrease in triglyceride levels was significantly associated with a decrease in fasting insulin, Apo(B), alanine transaminase, and alkaline phosphatase (p<0.05 for all). Surprisingly, a decrease in Lp(a) levels was significantly correlated with an increase of hs-CRP, leptin and alanine transaminase (p<0.05 for all). However, a 2-year decrease in waist circumference was positively associated with a decrease in TSH levels (p<0.05).

Conclusion: A cluster of biomarkers change during 2-years of a weight-loss dietary intervention, which are more inter-correlated among patients with T2D diabetes as compared to non-diabetic obese controls, underscoring the ability to particularly improve insulin resistance, inflammation and lipids by dietary intervention in this population.

Table 1: Changes after 2 years of dietary intervention: Intra -correlations (r) between *changes* (delta) in biomarkers; adjusted for age, sex and ΔBMI, stratified by Diabetes * P<0.05 **P<0.001



Δ Delta = biomarker at time 24 – biomarker at time 0

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822

Food composition database harmonization for between-country comparisons of dietary data in the TEDDY Study

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Background and aims: Between-country comparison of diet sets special requirements and challenges on national food composition databases (FCDBs) used for analyses. The aim of this study was to evaluate the comparability of FCDBs used in Finland, Germany, Sweden, and the U.S. - countries participating in The Environmental Determinants of Diabetes in the Young Study (TEDDY).

Materials and methods: TEDDY is a prospective multi-center, multi-national study, in which approximately 8000 children with increased genetic susceptibility to type 1 diabetes will be followed across six study centers in Europe and the U.S. The participants are monitored for islet autoantibodies and diabetes until the age of 15 years. The study aims at examining the associations between islet autoimmunity and various environmental exposures, e.g. diet. The first dietary assessment is carried out by 24-hr recall at the age of 3 months and after that by 3-day food record every 3 months until the child is 12 months old and then every six months. In order to produce comparable results the national FCDBs have to contain mutually comparable food composition data. Systematic comparison (definition, unit of measurement, method of analysis) of energy, protein, fats, carbohydrates, cholesterol, fiber, 12 vitamins and 8 minerals was carried out among the FCDBs.

Results: The original version of the nutrient calculation program used with German FCDB (LEBTAB) did not take yield and retention factors into account in data processing. They will be added to the LEBTAB food composition database as part of the harmonization efforts. Nitrogen values were added to the Swedish and U.S. databases. Total fat, cholesterol, vitamin A (retinol equivalents and beta-carotene), thiamin, riboflavin, niacin, pyridoxine, vitamin B12, calcium, phosphorus, iron, potassium, magnesium, and zinc are comparable between the databases. Carbohydrates, sugars, fatty acids, vitamin D, vitamin K, vitamin C, pantothenic acid, manganese, copper, and fiber are comparable or can be converted comparable only across three databases. Vitamin E is comparable after Finland and Germany have subtracted tocotrienols from their values. After recalculation of protein from nitrogen (Sweden and USA), and recalculation of energy these values will be comparable across the countries. Starch and folate are not comparable.

Conclusion: According to this comprehensive review, most of the nutrients are comparable or can be converted to be comparable among the four FCDBs.

Supported by: NIDDK, NIAID, NICHD, NIEHS, JDRF, CDC

823

Effects of protein on gastric emptying and the glycaemic, insulin and gastrointestinal hormone responses to a carbohydrate meal in type 2 diabetes

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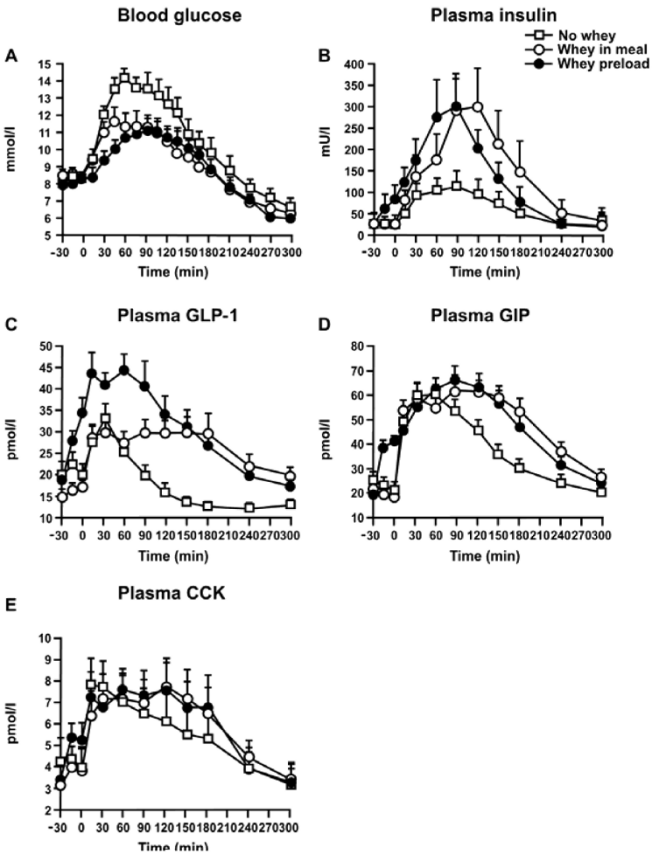
Background and aims: Strategies that slow gastric emptying and stimulate incretin hormones can potentially attenuate postprandial glycaemic excursions. We evaluated whether a whey protein "preload" could slow emptying of a subsequent meal, stimulate glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP) and insulin release, and thereby reduce blood glucose.
Materials and methods: 8 diet-controlled type 2 diabetic patients were studied on three days each in random order. On each day, subjects ingested beef flavoured soup 30 min before a mashed potato meal; either 55 g whey protein was added to the soup ("whey preload"), or to the potato ("whey in meal"), or no whey was given ("no whey"). Data were given as mean ± SEM.
Results: Gastric emptying was slowest after "whey preload" (half emptying time: 87.3 ± 5.4 min), and slower after "whey in meal" (53.0 ± 8.3 min) than "no whey" (39.0 ± 6.2 min; P < 0.0005). The incremental area under the blood glucose curve was substantially less after "whey preload" (363.7 ± 64.5 mmol.min/l, P < 0.005) and "whey in meal" (406.3 ± 85.9 mmol.min/l, P < 0.005) than "no whey" (734.9 ± 98.9 mmol.min/l), and the peak glucose level was lower after "whey preload" (11.3 ± 0.5 mmol/l) and "whey in meal" (11.7 ± 0.6 mmol/l) than "no whey" (14.3 ± 0.5 mmol/l; P = 0.0001). Cholecystokinin, GIP and insulin concentrations were higher on both whey days than after "no whey", whereas GLP-1 was greatest after "whey preload" (P < 0.05 for each).
Conclusion: Whey protein consumed before a carbohydrate meal in type 2 diabetes stimulates insulin and incretin hormone secretion, and slows gastric emptying, leading to a marked attenuation of the postprandial glycaemic response. This observation has substantial implications for dietary management of type 2 diabetes.



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PS 67 Prediction and prevention of type 2 diabetes mellitus - 1

824

Impact of a brief lifestyle advice intervention on physical activity

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Background and aims: To determine if a brief lifestyle advice intervention could increase physical activity in adults at increased risk of cardiovascular disease (CVD).
A lack of regular physical activity may contribute to the early onset and progression of CVD. Moderate and high levels of physical activity are associated with a 2-21% reduction in CVD and all-cause mortality.
Materials and methods: Participants were randomised in a 2x2 factorial design to receive or not receive a personalised 10-year CVD risk estimate and, simultaneously randomised to receive or not receive a brief lifestyle advice intervention. We report here the brief lifestyle advice intervention arm. The primary outcome was the change in physical activity measured by an accelerometer, with 80% power at the 5% level of significance to detect a difference of 30,000 total accelerometer counts per day between groups (approximately equivalent to 10 minutes of brisk walking). A total of 215 individuals without known CVD but at increased CVD risk (defined as a Framingham-derived 10-year CVD risk >20%) were recruited from 4 general practices in Oxfordshire, UK. Of 194 eligible participants, 98 were randomised to receive a brief lifestyle intervention informed by self-regulation theory and aimed at motivating behaviours designed to reduce CVD risk. The other 98 received no intervention. The brief lifestyle intervention involved the setting of goals and steering behaviour towards them. It took the form of a 15 minute computerised presentation focusing on increasing physical activity and fruit and vegetable consumption and smoking cessation (if necessary). The physical activity goal was 30 minutes of brisk walking per day. Accelerometer, anthropometric and biochemical information was collected before and one month after randomisation.
Results: Participants had median (IQR) age 62.3 (54.9, 66.1) years, with 67% men, 19% having known diabetes and 19% current smokers. At baseline, mean (SD) accelerometer counts per day (x1000) were 297 (110), blood pressure 140/81 (18/11) mmHg, LDL cholesterol 3.1 (0.9) mmol/L, HDL cholesterol 1.23 mmol/L (0.29) in men and 1.53 mmol/L (0.37) in women and plasma vitamin C 61.8 (21.2) μmol/L. Triglycerides were median (IQR) 1.4 mmol/L (1.0, 1.9), body mass index 28 kg/m^2 (26, 31), serum cotinine in smokers 50 (49, 62) ng/ml and estimated 10-year CVD risk 48% (34, 60) in men and 31% (22, 43) in women. Baseline accelerometer counts were higher than expected equating to 77% achieving 30 minutes or more of moderate intensity activity per day. In the 185 (95%) participants attending follow-up, no significant within or between group differences were seen in accelerometer counts, body mass index, blood pressure, LDL or HDL cholesterol, plasma vitamin C or estimated 10-year CVD risk. In the group randomised to receive the brief lifestyle advice intervention, compared to control, there was a reduction in waist circumference in men (2.6%, p=0.006) but not in women, 10.2% reduction in triglycerides (p=0.02) and 7.9% reduction in serum cotinine in smokers (p=0.028).
Conclusion: A brief lifestyle advice intervention in adults at increased CVD risk did not alter physical activity but achieved small improvements in some CVD risk factor levels. The lack of physical activity response may reflect the large number of participants already at target at entry. Our findings suggest that a theory-based brief lifestyle advice intervention alone is insufficient to increase physical activity.

825

Implementation and effectiveness of the first community lifestyle intervention program to prevent type 2 diabetes in Greece. The DEPLAN Study

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Background and aims: To report the experience and the initial results of implementing the first community-based lifestyle intervention program to prevent Type 2 diabetes mellitus (T2DM) in a sample of the general population in Athens, Greece

Materials and methods: The FINDRISC questionnaire was distributed to a total of 3,240 people in the city and the suburbs of Athens in order to detect high-risk individuals for the development of T2DM. A total of 191 persons entered the intervention program (bi-monthly sessions with a dietician) and 125 of them returned for a repeat oral glucose tolerance test (oGTT) one year later.
Results: Participants lost on average 1.0±4.7 kg (1.1% of initial body weight, p=0.022). Higher adherence to the intervention sessions resulted in greater weight loss. Persons with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) at baseline lost more weight than the normal glucose tolerance (NGT) ones (1.5±4.8 vs. -0.02±4.5 kg). The percentage of people with any type of glucose abnormality (IFG/IGT) decreased with the intervention (68% at baseline vs. 53.6% one year later, p=0.009). A total of 5.6% developed DM (2.5% of NGT, 7.1% of IFG/IGT participants). Multivariate logistic regression analysis revealed that the probability of deterioration of the glycemic status was associated only with the amount of weight lost (inversely) during the intervention (β=-0.11, OR=0.89, p=0.045).
Conclusion: The implementation of a lifestyle intervention program to prevent T2DM in the community is practical and feasible.
Supported by: European Communities, Directorate C - Public Health, grant agreement No. 2004310

826

One risk questionnaire to identify persons at risk for cardiovascular disease and type 2 diabetes - the Hoorn Study
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Background and aims: The common aim of risk prediction of cardiovascular disease (CVD) and type 2 diabetes (T2DM) is identification of high-risk groups that would benefit from early treatment. Since the strategy for prevention of these so-called cardio-metabolic diseases largely overlaps, we investigated whether it was possible to develop one tool that predicts risk for both CVD and T2DM.
Methods: The study population consisted of 1258 participants, aged 50-75 years, from the Hoorn Study who were free of prevalent CVD or known T2DM at baseline (1989). All participants had an oral glucose tolerance test at the follow-up examination in 1996, and complete follow-up information on CVD morbidity and mortality until 1996. The outcome to be predicted was a composite end-point of CVD morbidity and mortality (ICD-codes 390-459), sudden death (ICD-code 798) or T2DM, as measured by an oral glucose tolerance test. Logistic regression analysis with backward selection of predictors (demographics, medical history, dietary and lifestyle habits) was used to develop the model. Discriminative value of the models was assessed by the area under the receiver operating characteristic curve. Model validation was done in a subsample of the study population with follow-up duration until 2000.
Results: During a follow-up period of 6.4 (0.5) years, 375 (29.8%) out of 1258 persons developed the composite end-point. For the risk questionnaire, age, sex, waist circumference, use of anti-hypertensive medication, history of gestational diabetes, parent or sibling with T2DM, shortness of breath during walking, current smoking and parent or sibling with myocardial infarction or stroke were selected as predictors. Discriminative value of the risk questionnaire was 0.74 for the composite end-point. The separate outcome measures were equally well predicted (discriminative value was 0.70, 0.76 and 0.74 for CVD morbidity, CVD mortality and T2DM, respectively). Nearly similar discriminative values were found in the validation dataset, so internal validation was good. In the development dataset, a cut-off value at the optimum sensitivity and specificity captured 71% of the incident cases (sensitivity). Furthermore, 68% of the persons who did not develop the outcome were indeed in the low-risk category (specificity). According to the questionnaire, 44% of this elderly population had a high risk. Persons in this high-risk group had a risk of 47% to develop CVD and/or T2DM within 6 years (positive predictive value).
Conclusion: Risk of cardiovascular disease and type 2 diabetes can be predicted by one single risk questionnaire to identify individuals at high risk, which is useful for public health purposes.
Supported by: the Dutch Diabetes Research Foundation

827

Leicestershire self assessment score for impaired glucose regulation for use in a multi ethnic setting
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Background and aims: One in five of those screened in the ADDITION-Leicester population based screening study had some form of Impaired Glucose Regulation (T2DM or impaired fasting glucose (IFG) or impaired glucose tolerance (IGT)) (IGR). Modelling studies have shown that early detection of IGR and appropriate intervention can decrease morbidity and early mortality from cardiovascular disease. Self assessment scores identify those at high risk and are particularly useful in populations where response rates to screening programmes are low. To date no self assessment scores have been developed and validated for multi ethnic populations.
Materials and methods: We used data on 6,390 subjects aged 40-75 from the ADDITION-Leicester screening study from a multi ethnic UK setting (76% White European, 22% South Asian, 3% other). All participants were given a 75g Oral Glucose Tolerance Test. We developed logistic regression models for predicting IGR (IFG/IGT/T2DM) using data from self reported questionnaires. Using the best fitting model we developed the Leicester Self Assessment Score. Initial analysis was carried out using complete cases only, then missing data indicators were used as a check for robustness of the modelling. We externally validated the tool using data from 3,298 subjects aged 40-75 screened as part of a second screening study.
Results: The final model includes age, ethnicity (White European versus other - predominantly South Asian), sex, 1st degree family history, antihypertensive therapy or history of hypertension, waist circumference and body mass index. The score ranges from 0 to 47. Validating this model using the STAR data gives an area under the ROC curve of 71% (95% CI 68% to 74%). Using a cut point of 16 to predict those at high risk of IGR had a sensitivity of 82%, specificity of 47% and correctly classified 53% of cases.
Conclusion: The Leicester Self Assessment Score can be used to reliably identify those at high risk of IGR in multi ethnic populations. The score is simple (7 questions) and non invasive.
Supported by: Diabetes UK

828

Automated detection of high risk for impaired glucose regulation and type 2 diabetes mellitus, using primary care electronic data, in a multi-ethnic UK community setting
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Background and aims: Screening for cardiovascular risk factors is now acknowledged to be a high priority, and much research continues to be carried out on the most effective and efficient risk scores. To complement this work, it would be valuable to have an accepted and reliable risk score for type 2 diabetes (T2DM) and impaired glucose regulation (IGR) which use only data routinely recorded on computerised primary care databases. Tools using routine data would be invaluable to determine people at high risk for further investigation. The aim of this study was to construct a primary care computer risk score for use in a multi-ethnic population with substantial (30%) south Asian ethnic minority.
Materials and methods: A random sample of people aged 35-70 years from 24 general practices in Leicestershire was invited for a 75g Oral Glucose Tolerance Test (OGTT), as part of ongoing screening studies in Leicester, UK. Demographic and anthropometric characteristics were recorded using standard operating procedures. The variables we considered for inclusion were - age, gender, BMI, ethnicity (as the proportion of south Asians in the individual's general practice), family history (of Type 1 or Type 2 diabetes mellitus, with separate terms for any first degree relatives, and for only relatives of second or degree or more distant), smoking status (never, ex-smoker or current smoker), history of high blood pressure (as indicated by prescription of anti-hypertensive medication), and whether the BMI had been recorded in the practice data base (as a possible proxy for level of deprivation). We could not assume that ethnicity or any socioeconomic indicator would be available for individuals. Logistic regression modelling was used to identify sets of variables independently predictive of T2DM/IGR; interaction terms were used

to assess the values of complex combinations of predictors, and fractional polynomial terms were used to examine potential nonlinear relationships.
Results: 6,203 individuals participated. 5,086 individuals (82%) had all relevant data recorded and were included in the analysis, of these 149 (2.9%) were diagnosed at OGTT with screen-detected T2DM, and an additional 633 (12.4%) were diagnosed with IGR; the age range was 35-70 years; mean age was 55.3 (SD 8.7), 47.2% were male and 25.7% were of south Asian ethnicity. The strategy of logistic modelling resulted in a risk score incorporating age, gender, average ethnicity in the practice, body mass index, family history of T2DM (any degree), and previous hypertension. Internal validation with a cut-point identifying the top 10% individuals by risk gave 44% sensitivity and 80% specificity for T2DM/IGR; similar validation on an external data set gave sensitivity around 51% and specificity around 76%.
Conclusion: We have developed a practical low cost risk score for identification of prevalent T2DM and IGR in primary care. This tool can be used for screening people at high risk for appropriate interventions.
Supported by: Diabetes UK

829

Risk and predictors of deterioration of glucose metabolism in subjects with and without CV complications - a prospective observational study
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Background and aims: Abnormal glucose metabolism is more prevalent among subjects with, than without cardiovascular (CV) complications. However, it is not known whether rate of glycaemic deterioration is different or more associated to certain parameters of inflammation or the insulin-like growth factor (IGF) system. In a 4-yr prospective observational study, we examined risk for, and rate of, glycaemic deterioration in subjects without diabetes mellitus (DM) with and without CV complications and explored its association of traditional risk factors and markers of inflammation (high sensitivity C reactive protein (CRP), soluble tumor necrosis factor receptor type I (sTNF-RI), transforming growth factor (TGF)-β1, Interleukin (IL)-10) and parameters in the IGF system (IGF-1, IGF binding protein (BP)-1 and IGFBP-3).
Materials and methods: We studied 72 subjects (31 female, 66 years, BMI 24.6±3.6 kg/m^2) with a previous CV complication (42% coronary artery-, 28% cerebrovascular- and 30% peripheral arterial disease) and 61 (38 female, 60 years, BMI 25.1±4.5 kg/m^2) without. At both time points a 75 g oral glucose tolerance test, classified according to the WHO criteria (DM, impaired glucose tolerance (IGT) or impaired fasting glycaemia (IFG)) was conducted. Inflammatory and IGF-system parameters were analysed in fasting serum samples.
Results: Respectively, 14 (23%) subjects without and 29 (40%) with a CV complication deteriorated in glycaemic status (i.e. normal to IFG/IGT/DM or IFG/IGT to DM) yielding DM incidence rates from normal or IFG/IGT respectively of 1.06 and 1.77 versus 1.67 and 5.65 cases pr 100 patient year. Odds ratio (OR) for glycaemic deterioration was 2.24 (95% CI 1.03 - 4.88). The magnitude of the OR (2.05 - 2.38) did not change when adjusted for baseline factors (age, weight, homeostasis-insulin resistance [HOMA-IR], insulin, TGF-β1, CRP, sTNF-R1, IL-10, IGF-1, IGF-BP1, IGF-BP-3) or longitudinal parameters (degree of physical activity or daily sleep hours). Only parameters of the IGF system were significantly associated with glycaemic deterioration (table 1), which was further underscored with elevated OR and 95% CI for progression of highest vs lowest quartile adjusted for CV-complications for low levels of IGFBP-1 and elevated HOMA-IR and insulin. This was also the case for weight (3.95 (1.32, 11.80)), but not age (1.12 (0.35, 3.60)).
Conclusion: Subjects with CV complications have a 2-fold higher risk for deterioration of glucose metabolism and should receive regular assessment of glycaemic status. Our findings also support that low levels of IGFBP-1 may play a role in the deterioration of glycaemic status. These findings may have implications for future strategies aiming at identifying individuals at high risk for DM for prevention.

830

Serial measurement of fasting and postload glucose and HbA_{1c} in subjects who did and did not develop diabetes during a 10-year period in the Hoorn Study
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Background and aims: Three markers of hyperglycemia are currently used for diagnosis and management of diabetes: fasting glucose, postload glucose -2 hours after a 75 g oral glucose tolerance test, and HbA1c. It is not known whether any of these three markers changes earlier, or more rapidly, during the development of diabetes. Therefore we compared fasting and postload glucose levels and HbA1c in people who did and did not develop diabetes during a 10-year period.

Deterioriation of glucose metabolism according to basline inflammatory and IGF system parameters.

	Overall cohort	No progression *(NGT,IFG, IGT at both timepoints)*	Progression to IFG from NGT	Progression to IGT from NGT or IFG	Progression to DM from NGT, IFG or IGT	OR for progression (4th vs 1st)	
						OR (95% CI)	*Quartile 1st and 4th cut- off*
IL-10 (pg/ml)	1.4 (0.5, 2.3)	1.5 (0.3, 2.8)	0.7 (0.6, 0.8)	3.0 (-2.4, 8.4)	0.6 (0.6, 0.7)	0.92 (0.29, 2.93)	≤0.5, >0.7
sTNF-R1 (pg/ml)	875 (815, 934)	876 (793, 958)	936 (784, 1089)	882 (719, 1045)	749 (638, 860)	0.86 (0.25, 2.90)	≤689, >985
IGFBP1* (ug/ml)	49.8 (42.1, 57.5)	57.5 (46.1, 68.9)	47.7 (34.5, 61.0)	39.6 (22.9, 56.3)	20.9 (10.8, 31.0)	6.25 (1.79, 21.74)§	≤22.8, > 63.2
IGFBP3 (ug/ml)	1.6 (1.5, 1.7)	1.6 (1.5, 1.7)	1.6 (1.4, 1.8)	1.5 (1.2, 1.7)	1.6 (1.2, 1.9)	0.81 (0.27, 2.44)	≤1.3, >1.9
TGF-β1 (ng/ml)	6.6 (6.0, 7.4)	7.0 (6.0, 8.1)	6.9 (4.8, 9.0)	5.3 (3.8, 6.8)	4.7 (2.6, 6.8)	0.63 (0.20, 2.13)	≤3.7, >8.5
CRP (mg/l)	2.6 (1.9, 3.3)	2.8 (1.8, 3.7)	2.7 (0.9, 4.4)	3.0 (0.9, 5.2)	1.1 (0.6, 1.6)	0.87 (0.26, 2.88)	≤0.7, >2.9
IGF-1 (ng/ml)	72.8 (67.3, 78.4)	74.0 (66.8, 81.1)	64.5 (54.0, 74.9)	70.5 (46.4, 94.6)	84.2 (61.1, 107.3)	0.58 (0.19, 1.83)	≤52.0, >91.3
Insulin* (pmol/l)	90 (79, 102)	81 (68, 94)	87 (74, 101)	91 (60, 122)	144 (69, 219)	4.42 (1.26, 15.6)	≤60, >101
HOMA-IR*	3.9 (3.4, 4.5)	3.5 (2.9, 4.0)	3.7 (3.1, 4.0)	4.2 (2.7, 5.6)	6.7 (3.1, 10.3)	4.36 (1.22, 15.59)	≤2.6, >4.2

Data given as mean (95% CI) or OR (95% CI)
*: $p < 0.05$ (ANOVA), §: 1st vs 4th quartile.
Abbreviations: NGT - normal glucose tolerance.

Materials and methods: The Hoorn Study is a population-based cohort study of diabetes, which started with 2484 men and women, aged 50-75 at baseline in 1989. Follow-up examinations were performed in 1996-98, and in 2000, in all subjects with fasting glucose ≥ 6.1 mmol/l or postload glucose ≥ 7.8 mmol/l in 1996-98 and a sample of those with normal glucose metabolism. In 2000, 564 subjects without diabetes at baseline participated. Diabetes was defined by fasting glucose ≥ 7.0, postload glucose ≥ 7.8 mmol/l, or HbA1c ≥7 % or use or glucose lowering medication.
Results: A total of 92 subjects had developed diabetes within 10 year. The mean fasting and postload glucose and HbA1c levels at the 3 examinations according to diabetes status in 2000 are shown in the table. All three markers were significantly increased at all time-points in those who developed diabetes, with slightly lower levels of significance for HbA1c differences. Of those who had diabetes in 2000, 56% already had developed diabetes as defined by abnormal glucose levels at the first follow-up measurement in 1996-1998, and only 8 % still had normal glucose fasting and postload glucose levels. In contrast only 3 % had an HbA1c ≥ 7 %, 6 % HbA1c ≥ 6.5 % and 49 % of those with diabetes in 2000 had HbA1c < 6% at the first follow-up. At the baseline examination in 1989, 63 % of those who developed diabetes in the next 10 year already had impaired fasting glucose or impaired glucose tolerances, but only 20 % had HbA1c ≥ 6%.
Conclusion: Fasting and postload glucose levels increase more rapidly than HbA1c during the process of developing diabetes. This may have consequences for decisions on the preferred method in diagnostic testing for diabetes.

Mean (SD) of hyperglycemia markers in people with and without diabetes after 10 year

period:	1989		1996-1998		2000	
	no DM 2000	DM 2000	no DM 2000	DM 2000	no DM 2000	DM 2000
Fasting glucose (mmol/l)	5.31 (0.49)	5.87 (0.52)	5.83 (0.66)	6.92 (1.00)	5.68 (0.52)	7.34 (1.62)
Postload glucose (mmol/l)	5.15 (1.53)	7.28 (2.06)	5.95 (1.81)	9.41 (3.29)	6.57 (1.81)	11.47 (2.70)
HbA1c (%)	5.27 (0.45)	5.55 (0.53)	5.40 (0.47)	5.94 (0.62)	5.76 (0.41)	6.49 (0.72)

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831

Prediction of rapid and slow progression from IFG or IGT to diabetes in a high-risk screening programme. The ADDITION study, DK.
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Background and aims: Only few baseline risk factors predicted progression to diabetes in a group identified with impaired fasting glycaemia (IFG) and impaired glucose tolerance (IGT) in a high-risk screening programme. The aim was to see whether different predictors were associated with "rapid" and "slow" progression to diabetes in this population.
Materials and methods: Patients aged 40-69 years in general practices received a diabetes risk score questionnaire. Random blood glucose (RBG) and HbA1c were measured followed by fasting blood glucose (FBG) and 2-hour blood glucose (2hBG) after an oral glucose tolerance test (OGTT). At baseline 1821 individuals were identified with IFG or IGT (WHO 2006 definition). In 1510 individuals follow-up data are available with an OGTT performed after about 1 and 3 years. Conversion to diabetes within 0 to 1.5 years was defined as "rapid" progression; conversion within 1.6 to 3.5 years as "slow" progression. Progression rates were calculated per 100 person-years using a regression model for interval censored follow-up data. Baseline predictors for progression to diabetes for "rapid" and "slow" progression were identified by backward elimination including: age, sex, BMI, known hypertension, family history of diabetes, physical inactivity, RBG, HbA1c, FBG, 2hBG, waist, systolic BP, total cholesterol, triacylglycerol, myocardial infarction and smoking status.
Results: There were 442 diabetes cases. For the IFG and IGT groups the progression rates in the first 1.5 years were 16.0 (95% CI: 13.1-19.0) and 23.8 (20.7-26.9), and in the following 1.6 - 3.5 years the progression rates were 6.1 (4.0-8.2) and 7.2 (5.2-9.3). Baseline predictors for "rapid" progression were in the IFG group: BMI (per kg/m^2, rate ratio [RR] 1.05 [1.01-1.10]), physical inactivity (yes vs no, RR 1.99 [1.02-3.89]), FBG (per 0.5 mmol/l, RR 4.33 [2.90-6.45]) and triacylglycerol (per twofold increase, RR 2.32 [1.42-3.77]), and in the IGT group: HbA1c (per 0.5%, RR 1.35 [95% CI 1.10-1.65]), FBG (per 0.5 mmol/l, RR 1.69 [1.40-2.05]), and 2hBG (per 0.5 mmol/l, RR 1.30 [1.20-1.42]). Baseline predictors for "slow" progression were in the IFG group: HbA1c (per 0.5 %, RR 1.58 [1.09-2.29]), FBG (per 0.5 mmol/l, RR 2.01 [1.25-3.22]), systolic BP (per mmHg, RR 1.02 [1.00-1.03]) and total cholesterol (per mmol/l, RR 1.42 [1.05-1.93]), and in the IGT group: sex (female vs male, RR 0.61 [0.40-0.93]), physical inactivity (yes vs no, RR 1.93 [1.00-3.73]), FBG (per 0.5 mmol/l, RR 1.56 [1.26-1.96]) and 2hBG (per 0.5 mmol/l, RR 1.22 [1.10-1.35]).
Conclusion: The cumulative risk of diabetes in this study was high. The decline in progression rates after the first year indicates that some individuals progress more rapidly than others. It has been suggested that prediction of "rapid" versus "slow" progression to diabetes would make sense with regard to risk assessment. In the present analyses, baseline predictors differed between "rapid" versus "slow" progression, yet high levels of blood glucose were significant predictors in all situations and to some extend physical inactivity. Prediction of progression to diabetes in high-risk individuals with IFG or IGT may seem meaningless with regard to risk assessment and intervention.
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832

Body mass index, physical inactivity and type 2 diabetes - are these associations explained by genetic selection? Results from the Swedish twin register
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Background and aims: Overweight and lack of physical activity are the most well-known environmental risk factors for type 2 diabetes. However, twin studies indicate that both high BMI and physical inactivity have a strong genetic component. If the same genes that are associated with type 2 diabetes also promote high BMI and inactivity, part of these associations could be due to genetic factors rather than a causal relationship. The aim of this study was to investigate to what extent genetic factors explain the association between BMI, physical inactivity and type 2 diabetes using twin data.
Materials and methods: Data are based on twins born 1926-1958 of the Swedish Twin Register who answered a postal questionnaire in 1973. Self-reported information on diabetes was collected by telephone interviews between 1998 and 2000. We used regression models to investigate the risk of type 2 diabetes associated with BMI and physical inactivity among all twins, without taking genetic factors into account and with control for genetic factors in co-twin control analyses of diabetes-discordant dizygotic (DZ) (N=480) and monozygotic (MZ) (n=253) twin pairs.
Results: Among 25,531 twins, 844 cases of type 2 diabetes were identified at follow-up (3.4%). In the cohort analysis, in which twins are analyzed as independent individuals, the odds ratio (OR) of type 2 diabetes was 1.27 (95% CI=1.24-1.29) for 1 unit increase in BMI and 1.24 (95% CI=1.07-1.44) for physical inactivity (low vs. moderate or high activity). When we compared twins with diabetes to their healthy co-twins, the OR of diabetes in DZ twins was 1.20 (95%CI=1.12-1.27) per BMI unit and 1.19 (95% CI=0.86-1.65) for physical inactivity. Corresponding analyses in MZ twins indicated that BMI was associated with an increased risk of diabetes (OR=1.26, 95% CI=1.12-1.42) but no association could be demonstrated between physical inactivity and type 2 diabetes (OR=0.83, 95% CI=0.51-1.36).
Conclusion: The results indicate that the association between BMI and type 2 diabetes is not due to genetic factors and thus supports a causal link between BMI and type 2 diabetes. However, the weak association between physical inactivity and type 2 diabetes seen in MZ twins is compatible with a contribution of genetic factors to this association.
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PS 68 Prediction and prevention of type 2 diabetes mellitus - 2

833

Predicting the effects of lifestyle or pharmacological intervention on progression of type 2 diabetes: evaluation of a novel mathematical model against results of the DPP

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Background and aims: Mathematical models have recently been used to evaluate the risk of development of, and the progression of, type 2 diabetes over an individual's lifetime. Models attempt to coherently integrate commonly accepted physiological mechanisms in order to predict the long-range behavior of disease processes, where long-term clinical observations may be lacking. A fundamental question, preliminary to the practical use of such models, concerns their validation against known medium-term disease progression data.
Materials and methods: We have developed a mathematical model for the long-term development of diabetes (the 'DiabProg' model). The DiabProg model mathematically represents phenomena such as progressive worsening of insulin resistance, the effects of glucose toxicity, and changes in beta-cell net replication/apoptosis rates. Parameter values for the model have been derived from a careful assessment of the literature. It is anticipated that the model can be used to test hypotheses about the natural history of the disease and about the effects of therapies. A valid disease and therapeutic model can also be used to inform clinical trial design. For example, decisions about study population, sample size, and treatment duration can be based on model simulations and predicted interactions between drug mechanisms and disease progression.
Results: In the present work, the DiabProg model has been used to simulate the course of glucose homeostasis and diabetes incidence in the Diabetes Prevention Program (DPP). A population of virtual subjects was 'selected' to resemble the DPP study population based on key entry criteria for that study. We then examined changes in glucodynamics over several years in subjects randomized to the same therapies used in the DPP (placebo, lifestyle modification, metformin, or troglitazone). The model is shown to replicate very well the results of the placebo and lifestyle arms of the DPP clinical trial, in particular reproducing closely the observed cumulative incidence of diabetes at years 1 through 4 of study, as well as the observed overall diabetes incidence while in study (simulated incidence of 11.3 cases/100 person years on Placebo and 5.1 cases/100 person years with Life Style intervention vs. observed incidences of 11.0 and 4.8, respectively) The ability of DiabProg to predict changes in insulinemia, glycemia, and diabetes incidence in the troglitazone and metformin arms of the DPP will also be presented.
Conclusion: The results obtained so far indicate that the DiabProg model, a mathematical representation of the most relevant and generally accepted mechanisms in the pathogenesis of T2DM, is capable of predicting medically important diabetes progression endpoints with good fidelity, and has a good potential to be used for in silico clinical trial evaluation and design.
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834

Early identification of type 2 diabetes in primary care medical offices in Austria. A multicentre randomized study

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Background and aims: As in many western countries also in Austria type 2 diabetes (DM2) is the most frequent disease to cause end stage renal disease. Prevalence of DM2 increases, thereby representing a serious problem that has to be recognized by all physicians. The early identification of patients with DM2 and initiation of adequate treatment may be crucial to public health budgets. Diabetic complications account for a high burden to both patients and reimbursement systems that pay for its management. Current guidelines recommend diagnostic tools not really feasible within the setting of a general practitioners office. A simple diagnostic parameter is desirable, independent of fasting state and performable quickly.
Materials and methods: We, therefore, have studied HbA1c as a diagnostic tool to identify patients with DM2 in 9 offices of family medicine. The gold standard for comparison was an oral glucose tolerance test (OGTT) with 75g of glucose. During 8 months 3724 patients were screened. History and/or medical records revealed known DM2 in 19.73 % and impaired glucose tolerance (IGT) in 15.81 %. Due to therapy with corticosteroids 3.2 %, and because of active infectious disease 7.16 % of patients had to be excluded. The inclusion criterion of age ≥ 40 years was fulfilled in 2805 of the screened persons. Finally, 573 agreed to participate in the study and filled in a questionnaire. Among these 231 were selected randomly to have extensive blood tests and urine analyses.
Results: Using OGTT 10 patients with de novo DM2 were identified (blood glucose at 2 hours ≥200 mg/dl. In these patients the mean HbA1c was 5.77 ± 0.64 % and differed statistically significant from study participants with normal OGTT who had a mean HbA1c of 5.07 ± 0.37 % (p=0.0001). Patients with IGT (blood glucose at 2 hours >140 and <200 mg/dl; n = 26) had a mean HbA1c of 5.49 ± 0.54 %. ROC analyses using HbA1c of 5.2% as a threshold revealed sensitivity and specificity of 0.72 each. A specificity of 100 %, i.e. having no DM2 or IGT, is given at a HbA1c value of ≤4.6 %, and a sensitivity of 100 %, i.e. manifest DM2, is given at a HbA1c value of ≥6.6 %. Univariate analyses of No-DM2 versus IGT + DM2 showed that a significant impact on diagnosis was seen of higher age (p=0.0001), higher body mass index (p=0.0133), higher systolic blood pressure (p=0.0015), higher total cholesterol (p=0.0462) and presence of proteinuria (p=0.0017). Using stepwise multivariate logistic regression analysis age (p=0.0007), body mass index (p=0.0118) and proteinuria (p=0.0365) were included in the model to significantly influence the diagnosis of No-DM2 versus IGT + DM2.
Conclusion: We conclude that measurement of HbA1c is a reliable diagnostic parameter to diagnose DM2 irrespective of the fasting state and thus day time with only one blood sample to be drawn. Our analyses show that the pretest probability to identify patients with IGT or DM2 increases with increasing age, body mass index, higher systolic blood pressure and presence of proteinuria.

835

Predictors of cardiometabolic control in intensified, multi-factorial treated, screen detected type 2 diabetics; results of ADDITION the Netherlands study

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Background and aims: To assess determinants of optimal cardiovascular control after four years of intensified multi-factorial treatment in patients with screen-detected type 2 diabetes.
Materials and methods: This study is embedded in the Dutch part of the ADDITION Study (Anglo-Danish-Dutch Study of Intensified Treatment in People with Screen-detected Diabetes in Primary Care), that studies the effects of intensified multi-factorial treatment compared with usual care on cardiovascular morbidity and mortality in screen-detected type 2 diabetes patients. Data of patients from the intensified arm of the ADDITION the Netherlands were retrieved at baseline and after four years of follow-up. The patients were treated to the following targets: 1) HbA1c% < 6.5 % mmol/L 2) cholesterol level < 3.5 mmol/L and 3) blood pressure < 120/80 mm Hg. Non-optimal cardiovascular control after four years of follow-up was defined as reaching zero or one goals. Optimal cardiovascular control was defined as reaching two or three goals. Univariate and multivariate logistic regression analyses were performed in order to assess which factors were related to cardiovascular control and to what extent.
Results: A total of 206 patients from the intensified arm were analysed. 14.1% (n=29) reached none of the targets, 48.1% (n=99) reached one target, 29.6% (n=61) reached two targets and 8.3% (n=17) reached three targets. Therefore 62.1% (n=128) had non-optimal cardiometabolic control and 37.9% (n=78) achieved optimal cardiometabolic control. On univariate analysis the following characteristics were associated with the achievement of optimal cardiometabolic control (P<0.2): alcohol use at baseline, systolic blood pressure at baseline and a decrease in BMI between baseline and four years of follow-up. On subsequent multivariate analysis only a decrease in BMI between baseline and four years of follow-up was an independent predictor for optimal cardiometabolic control (p=0.042).
Conclusion: Weight loss is an independent predictor for achieving optimal cardiometabolic control in patients with screen detected type 2 diabetes.
Supported by: NovoNordisk

836

FINDRISC (Finnish Diabetes Risk Score): a useful tool as a predictor of both diabetes risk and cardiovascular risk in an Italian population

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Background and aims: Risk scores based on phenotypic characteristics have been developed in several populations to identify individuals at risk of developing or having undiagnosed diabetes (T2D). FINDRISC is currently the best available tool for use in daily practice in Caucasian populations. FINDRISC seems also a reasonable predictor of coronary heart disease, stroke, and total mortality.
Materials and methods: FINDRISC, 75-g oral glucose load, and major cardiovascular risk factors together with the HeartScore (10-year cardiovascular mortality estimation) were assessed in 1051 Caucasian nondiabetic subjects (435 men, 41%; 616 women, 59%) attending opportunistic diabetes screening at the Regional Centre for Diabetes and Metabolic Disease in Tuscany, Italy. The study, part of a larger European project (DEPLAN) was aimed to assess: 1. distribution of main risk factors for T2D; 2. prevalence of high risk subjects; 3. the predictive power of FINDRISC for prediabetes and undiagnosed T2D; 4. relationship between FINDRISC, cardiovascular risk factors and HeartScore.
Results: Men (M) and women (W) differed by age (51±11 vs 49±12 years, p<0.0001) and smoking habits (no-/ex/current-smokers: M 36/42/22%; W 57/21/22%, p<0.0001). There was no difference for family history of diabetes (M 64%, W 70%), personal history of high glucose values (M 34%, W 28%), fruit and vegetable consumption (M 83%, W 86%), antihypertensive drug use (M 28%, WD 26%). Physical inactivity was more frequent among women (74% vs 66%). Distributions of BMI (30 kg/m2: M 23/48/29%, W 27/30/43%, p<0.0001) and waist circumference (102/88 cm: M 24/34/42%, W 8/16/76%, p<0.0001) were less favorable in W, while distribution for age strata (<45, 45-54, 55-64, ≥65 years: M 26/27/35/12%, W 37/25/27/11%, p=0.003) less favorable in M. The distribution of classes of risk for diabetes (FINDRISC: low, score <10; modest, 10-14; moderate, 15-19; high, ≥20) was superimposable in M (27/37/27/9%) and W (20/42/27/11%, X2=6.36, p=0.17). Prevalence of subjects with isolated impaired fasting glucose (IFG: M 15.2%, W 11.3%), impaired glucose tolerance (IGT±IFG: M 31.3%, W 29.0%) and undiagnosed T2D (M 17.2%, W 6.5%) was higher in men (X2=40.74, p<0.0001). The performance of FINDRISC in detecting undiagnosed T2D or T2D+IFG+IGT has been evaluated employing score ≥15 as the discriminant threshold. In the whole cohort, sensitivity, specificity and positive predictive value resulted 57%, 64%, 16% for diabetes and 52%, 77%, 72%, for diabetes+IFG+IGT; in M, 54%, 66% e 24% for T2D and 48%, 79% e 79% for T2D+IFG+IGT; in W 63%, 63%, 11% for diabetes and 56%, 76% e 67% for T2D+IFG+IGT. The increase in FINDRISC score was associated with progressive increase in systolic and diastolic BP (p<0.0001), non-HDL cholesterol (p<0.001), triglycerides (p=0.01), reduction of HDL (p<0.0001). The 10-year mortality risk for cardiovascular events (HeartScore) in subjects with IFG (1.7%), IGT (1.7%) and undiagnosed diabetes (2.4%) was higher than in subjects with normal glucose tolerance (0.7%; p<0.0001), and increasing FINDRISC score (<10: HeartScore 0.9%; 10-14: 1.0%; 15-19: 1.9%; ≥20: 2.1% (p<0.0001).
Conclusion: The FINDRISC seems to be useful to estimate future risk of diabetes and to detect undiagnosed diabetes and prediabetes in the Italian population. Furthermore, the FINDRISC can also identify individuals at high risk for cardiovascular disease and mortality.
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837

Elevated serum levels of transforming growth factor-beta1 (TGFbeta1) precede the development of type 2 diabetes: MONICA/KORA Augsburg case-cohort study, 1984-2002

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Background and aims: Subclinical inflammation represents an important mechanism in the development of insulin resistance and beta-cell dysfunction, but it is unknown whether elevation of circulating anti-inflammatory immune mediators predicts a lower risk for type 2 diabetes. Therefore, the aim of this study was to assess the association between transforming growth factor-β1 (TGF-β1), a mainly immunosuppressive regulator of the immune system, with incident type 2 diabetes.
Materials and methods: We measured serum levels of TGF-β1 by ELISA in 460 individuals with and in 1474 individuals without incident type 2 diabetes during a follow-up period (± SD) of 10.9±4.7 years in a prospective case-cohort study within the population-based MONICA/KORA Augsburg cohort.
Results: In Cox proportional hazards models, elevated TGF-β1 concentrations were associated with higher risk for type 2 diabetes in the age, sex and survey-adjusted model (HR (95% CI) for increasing TGF-β1 tertiles: 1.0; 1.08 (0.83; 1.42); 1.41 (1.08-1.83); p for trend = 0.012). Adjustment for BMI, metabolic factors (systolic blood pressure, ratio of total cholesterol to HDL cholesterol, parental history of diabetes) and lifestyle factors (smoking status, alcohol consumption, physical activity) had virtually no impact on the effect size. Additional analyses included tests for interaction with age, sex and obesity. These analyses indicated that the association between high TGF-β1 levels and increased risk of type 2 diabetes may be stronger in younger participants (<55 years; p for interaction = 0.033) and in obese participants (p for interaction = 0.013), whereas no sex differences were found.
Conclusion: Elevated serum concentrations of TGF-β1 indicate an increased risk for type 2 diabetes that is independent of multiple potential confounders. This association may be caused by upregulation of TGF-β1 in response to as yet unidentified metabolic or immunological disturbances before the clinical manifestation of type 2 diabetes.
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838

Diabetes, pre-diabetes and associated risks on Minnesota code-indicated major ECG abnormality among Chinese: a cross-sectional diabetic study in Fujian province, southeast China

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Background and aims: This study 1) investigated the incidence of diabetes mellitus (DM), impaired glucose regulation (IGR) and related metabolic disorders(overweight, obesity and hypertension) in a population 20-74 years in Fujian China and 2) explored the relationship between glucose metabolism and Minnesota code-indicated major abnormal electrocardiogram (MA-ECG).
Materials and methods: Of 3,960 people selected from urban and rural areas from July 2007 to May 2008 by multi-stage stratified sampling, 3,298 completed and 3,208 of 20-74 years old eventually analyzed (including physical measurements, blood biochemical analysis, oral glucose tolerance test and 12-lead resting ECG).
Results: According to WHO diagnostic criteria, prevalence of DM and IGR were 9.51% (male, 10.08%; female, 9.14%) and 14.40% (male, 14.48%; female, 14.35%), respectively. Fully 53.44% were newly diagnosed DM patients. Based on the 2000 China census, the age-standardized prevalence of DM was 7.19% (male, 7.74%; female, 6.61%), and 11.96 % for IGR (male, 12.35%; female, 11.56%). Age-standardized prevalence of DM and IGR in urban areas (7.74% and 12.97%, respectively) was slightly higher than in rural areas (6.67%, 10.86%) but not statistically significant. Prevalence of overweight, obesity and hypertension were 25.50%, 3.52% and 28.52%, respectively (age-sex standardized rates: 23.69%, 3.02 % and 22.45 %). After adjusting for other risk factors, DM and IGT remained independent risk factors for MA-ECG. Non-diabetic subjects with increased 30 min post glucose level showed higher risk of MA-ECG after adjusting for other factors, especially for those NGT with 30min PG≥7.8mmol/l[OR:1.371(1.055-1.780)].
Conclusion: Prevalence of DM, IGR following with other metabolic disorders has increased dramatically in the last decade in China, especially in rural areas, with many undiagnosed cases of DM. Even slightly raised glucose levels indicate early cardiovascular events.
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839

Trajectories of HbA_{1c} prior to the diagnosis of diabetes. The Inter99 study

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Background and aims: In view of the increasing emphasis on HbA_{1c} as a diagnostic tool for diabetes, it is important to understand the natural history of HbA_{1c} changes prior to disease onset. Our aim was to compare the trajectory of HbA_{1c} in the years prior to the diagnosis of diabetes to the trajectory for a control group free of diabetes during the study, based on repeated HbA_{1c} measurements.
Materials and methods: We studied 4,635 individuals from the population-based Inter99 study without diabetes at baseline who had at least one follow-up examination. The participants had a median of 2 HbA_{1c} measurements each (range: 2-4) giving a total of 12,833 measurement points. The study population was divided into incident diabetes cases (86% diagnosed through a 75g oral glucose tolerance test) and controls who did not develop diabetes during the observation period. We used a mixed effect model with an added spline term for incident cases.
Results: During a median observation period of 5.3 years (range: 1.0-6.3 years), 231 individuals developed diabetes. HbA_{1c} levels were 5.8% five years prior to the diagnosis/the last examination in both cases and controls. In cases, HbA_{1c} levels increased on average by 0.05% per year in the first half of the observation time. The rate of increase accelerated to an average of 0.08% per year in the second half of the observation time, reaching 6.2% at the time of diagnosis. In controls, the estimated HbA_{1c} level remained unchanged at 5.8% during the observation period.
Conclusion: We found that the level of average glycaemia, as measured by HbA_{1c}, starts to increase at least five years prior to the diagnosis of diabetes, and the rate of increase accelerates prior to diagnosis. In contrast, HbA_{1c} levels in those who did not develop diabetes remain unchanged. Our findings indicate that HbA_{1c} can be used to track pathophysiological changes that precede the diagnosis of diabetes.
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840

Population-based screening for type 2 diabetes in Denmark, the Netherlands and the United Kingdom: uptake and prevalence in the ADDITION study

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Background and aims: The Anglo-Danish-Dutch Study of Intensive Treatment in People with Screen-detected Diabetes in Primary Care (ADDITION) evaluates whether screening for undiagnosed type 2 diabetes is feasible and whether subsequent intensified treatment is beneficial. The present study describes the different screening strategies applied in ADDITION.
Materials and methods: Diabetes was defined according to WHO 1999 criteria. We describe the different strategies used to identify those at high risk of undiagnosed diabetes, the overall attendance rates (screening uptake), and the number of screen-detected type 2 diabetes patients identified in each country.
Results: In Denmark, the Danish Diabetes Risk Score was a) mailed to eligible patients aged 40-70 years, or b) completed by patients visiting their general practitioner (step 1). Those at high risk were invited to attend a screening procedure including a random blood glucose (RBG) test and HbA_{1c} test (step 2), a fasting blood glucose (FBG) test (step 3) and an oral glucose tolerance test (OGTT; step 4). In the Netherlands, the Hoorn Diabetes Risk Score was mailed to eligible patients aged 50-70 years (step 1). Eligible individuals were invited to attend a four-step procedure consisting of RGB (step 2), FBG (step 3) and OGTT (step 4), or a three-step procedure consisting of FBG (step 2) followed by OGTT (step 3). In Cambridge, routine electronic data were searched to identify individuals aged 40-70 years at high risk of undiagnosed type 2 diabetes using the Cambridge Diabetes Risk Score (step 1). Those at high risk were invited to attend a screening for which steps 2-4 were identical to those used in Denmark. In Leicester, a sample from eligible people aged 40-70 years (step 1) were invited to attend an OGGT (step 2). Numbers of target populations, invited individuals, individuals diagnosed with type 2 diabetes, and screen-detected diabetes prevalence are shown in the table. Data on the use of OGTTs in the different steps and screening strategies will be presented at the EASD conference.
Conclusion: The results suggest that population-based screening for type 2 diabetes is challenging but feasible. Although the response rates may be confounded by centre-specific covariates such as ethnicity, culture, social class, invited age groups, and different strategies, the different yields of screen-detected diabetes only range from 0.42% to 1.09%.

Results of different screening strategies

	DK - mail distributed	DK - opportunistic	NL - four-step	NL - three-step	Cambridge	Leicester	Total
General practices (n)	149	33	41	38	49	24	334
Target population (n)	132,408	30,777	29,251	27,727	135,825	47,806	403,794
Step 1							
Invited to complete risk questionnaire (n)	125,437	8,579	29,251	27,727	-	-	
Invited for blood tests (n)	-	-	-	-	33,539	23,060	
Next steps							
Attending blood tests (n)	23,447	4,584	11,028	6,855	24,654	5,601	76,169
Number confirmed type 2 diabetes (n)	1,369	262	285	301	867	201	3,285
As % of target population	1.03	0.85	0.97	1.09	0.64	0.42	0.78
As % of individuals attending blood tests	5.84	5.72	2.58	4.39	3.52	3.50	4.30

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841

A school-based physical activity program increases fitness and decreases adiposity and cardiovascular risk factors in primary school children: a cluster-randomized trial

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Introduction: Childhood obesity and physical inactivity are increasing dramatically worldwide with detrimental effects on the prevalence of diabetes as well as on cardiovascular and psychological health. Schools provide an ideal setting for preventive interventions. We therefore conducted a randomised controlled trial to determine whether a school-based physical activity (PA) program during a full school-year improves physical fitness and cardiovascular risk factors in young schoolchildren.
Methods: Twenty-eight classes were randomly allocated in a single blinded manner to the intervention (16 classes, n=297) and control (12 classes,

n=205) groups. The intervention consisted of a multi-component PA intervention program including daily physical education. Primary outcomes included body fat (skinfold thickness), aerobic fitness (shuttle run test), PA (accelerometry), and quality of life (questionnaires). Secondary outcomes included a cardiovascular risk factor score including all components of the metabolic syndrome.
Results: Compared with controls, children in the intervention group showed statistically significant decreases in body mass index z-scores (adjusted difference -0.09; 95%-percent confidence interval [CI], -0.18 to -0.003), sum of four skinfolds (adjusted difference -2·10mm; 95%-CI -3·48 to -0·90), and significant improvements in aerobic fitness z-score (adjusted difference 0·22; 95%-CI, 0·01 to 0·42) and moderate-vigorous PA at school (adjusted difference 14; 95%-CI, 5 to 23). There was also a significant improvement of the cardiovascular risk score (adjusted difference -0.18; 95%-CI, -0.30 to -0.05, intervention: n=227 and control: n=103). Quality of life did not change.
Conclusion: Our findings show that a stringent school-based PA intervention is effective in improving physical fitness and in reducing cardiovascular risk factors in children.
Supported by: Swiss Federal Office of Sports (FOSPO), Swiss National Science Foundation (SNF)

PS 69 Risk factors for type 2 diabetes mellitus

842

Low serum levels of insulin-like growth factor binding protein-2 predict type 2 diabetes
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Background and aims: Obesity is a major risk factor for type 2 diabetes (T2D). Insulin-like growth factor binding protein-2 (IGFBP-2) and adiponectin are both secreted by adipose tissue. Studies have shown that adiponectin as well as another binding protein, IGFBP-1, are inversely associated with incident T2D. The aim of the present study was to evaluate circulating levels IGFBP-2 in the prediction of T2D, and in comparison to adiponectin and IGFBP-1.
Materials and methods: Individuals with normal OGTT at baseline who developed abnormal glucose regulation at follow-up 8-10 years later (199 women and 278 men) were selected from a prospective cohort study comprising middle-aged Swedish men and women. T2D was detected in 60 women and 107 men. Age-matched controls were selected among those having normal glucose regulation at both examinations, 210 women and 290 men. Logistic regression analysis was performed, estimating odds ratio (OR) and 95% confidence interval (CI). Associations between biomarkers and T2D were evaluated and areas under receiver-operating-characteristic (AUC-ROC) curves were assessed.
Results: Levels of IGFBP-2, as well as levels of adiponectin and IGFBP-1, were at baseline significantly lower in individuals developing T2D as compared to controls. In women, the lowest quartile of IGFBP-2 conferred an OR for T2D of 18.5 (95%CI: 6.4-53.7), while the ORs for the lowest quartile of adiponectin and IGFBP-1 were 18.1 (6.7-48.9) and 16.7 (5.8-48.2), respectively. In men corresponding OR was 8.1 (4.0-16.5) for IGFBP-2, 3.6 (1.9-7.0) for adiponectin and 18.2 (8.1-40.9) for IGFBP-1. The associations remained significant also after controlling for BMI. ROC-AUC was 0.78, 0.80 and 0.79 for IGFBP-2, adiponectin and IGFBP-1 respectively, as compared to 0.80 for BMI in women. In men, corresponding ROC-AUCs were 0.71, 0.64, 0.78 and 0.70, respectively. In multivariate analysis, including IGFBP-2, adiponectin and IGFBP-1, they were all significantly and independently associated to T2D in women, whereas in men only IGFBP-1 remained significantly associated.
Conclusion: Low baseline levels of IGFBP-2 are, similar to adiponectin and IGFBP-1, associated with increased risk of incident T2D, suggesting IGFBP-2 as a marker for the development of T2D.
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843

Plasma procalcitonin is related to obesity and obesity-related development of type 2 diabetes mellitus
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Background and aims: A cumulating body of evidences suggests that inflammation plays a role in the development of type 2 diabetes mellitus (DM2). Recent studies suggest that adipose tissue produces procalcitonin (PCT), a pro-inflammatory hormokine previously thought to be confined to sepsis and bacterial infections, under non-infectious conditions. We aimed to investigate whether plasma PCT concentration is associated with obesity and whether it predicts development of DM2 in apparently healthy non-diabetic population.
Materials and methods: Data were from the Prevention of Renal and Vascular End Stage Disease (PREVEND) study, an ongoing, community-based cohort of 8592 subjects in the Netherlands, that started in 1997. Plasma PCT was assessed in baseline samples using an ultra-sensitive immunoluminometric assay (BRAHMS PCT sensitive LIA). Subjects with DM at baseline and subjects with plasma PCT concentrations indicative of potential presence

of infectious conditions (>0.10 ng/mL) were excluded. Development of DM2 was defined according to ADA criteria after a median (interquartile range) follow-up of 4.2 (4.0- 4.5) years in 5084 eligible subjects.
Results: A total of 159 participants (3.1%) developed DM2. Subjects who developed DM2 vs. subjects who did not develop DM2 had higher baseline BMI (29.6±4.6 vs. 25.8±4.0 kg/m^2, p <0.0001) and were older (55.7±10.5 vs. 47.8±11.8 years, p <0.0001). Baseline plasma PCT was positively associated with age (r=0.25, p < 0.0001) and BMI (r=0.24, p < 0.0001). It was also higher in men than in women (0.018 [0.015-0.022] vs. 0.014 [0.012-0.017] ng/mL, p < 0.0001). In a univariate model, the odds ratio (OR) for development of DM2 was 1.68 (95%CI 1.38-2.04, p < 0.0001) per doubling of PCT. After adjustment for age and sex the OR was 1.47 (1.17-1.85, p = 0.001). Adjustment for BMI markedly attenuated the OR to 1.19 (0.93-1.53, p = 0.18).
Conclusion: In the non-infectious range (<0.10 ng/mL), PCT is related to age, BMI and sex, and predicts type 2 DM independent of age and sex. However, the predictive value of PCT disappears after adjustment for the association of PCT with BMI. Based on this finding, we hypothesize that excessive exposure to PCT is involved in the pathogenesis of obesity-related diabetogenesis.
Supported by: the Center for Translational Molecular Medicine (CTMM)

844

A quantitative change in core tissue microbiome is associated with type 2 diabetes
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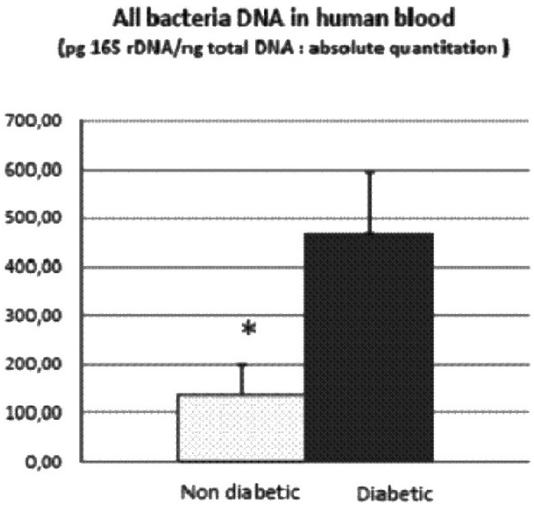
Background and aims: Although the role of gut microbiome on weight intake has been established, the role of gut and tissue microbiome on the onset of type 2 diabetes is poorly understood. We explored in the present study the changes in intestine, blood and tissue microbiome related with the onset of diabetes.
Materials and methods: First, we assessed using DGGE analysis profile the changes in intestine, blood and mesenteric adipose tissue (MAT) microbiome in diabetic mice fed a high fat diet. Then, we explored the role of leptin and of CD14 receptor: a key receptor of innate immunity on bacterial translocation. Finally we assessed in humans the relevance of these experimental data in a cross sectional population based sample.
Results: Distinct intestinal bacterial 16s rDNA sequences determined using DGGE analysis profiles were observed in diabetic mice fed a high-fat diet when compared with mice fed a normal chow. Conversely to the large diversity observed in the intestine, in blood only a few bacterial 16s rDNA bands were found. Importantly, the blood and MAT bacterial DNA concentrations were higher in diabetic animals than in controls. Arguing in favor of an intestinal origin of the increased bacterial DNA concentration in diabetic state we showed in four week HFD fed diabetic mice that after a single oral administration of fluorescent E Coli more fluorescent bacteria were present in the intestinal mucosa and MAT. Then, we explored the role of leptin and of the lipopolysaccharide receptor CD14 pathway on bacterial translocation and its consequences on the onset of diabetes. Blood and MAT bacterial DNA concentration were significantly higher in the leptin deficient mice. This was reversed when the mutant mice were also CD14 deficient (ob/ob CD14 KO). Importantly, the glucose tolerance test was improved in CD14 KO mice and in ob/ob CD14 KO mice as compared with ob/ob mice. Then, we assessed the clinical relevance of these data. We found in a population based-sample (n=311) that bacterial 16S rDNA blood concentrations are positively and independently associated with diabetes and high fasting glucose.
Conclusion: We find that a quantitative change in tissue microbiome is associated with type 2 diabetes. This change is driven by leptin and CD14 pathways.



Supported by: Délégation à la recherche clinique CHU de Toulouse

845

Short sleep duration is a potential risk factor for newly-diagnosed type 2 diabetes in Taiwanese
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Background and aims: Short sleep duration is independently associated with an increased risk of obesity, coronary heart disease, symptomatic diabetes and mortality risk. However, there are few studies investigate the relationship between sleep duration and newly-diagnostic diabetes/prediabetes, esp. no study from Asia. The aim of this study is to examine the potential effect of short sleep duration on the risk of newly diagnosed Type 2 Diabetes in Taiwanese.
Materials and methods: All subjects received a health examination in our hospital from 2006-2007 in Tainan, Taiwan were invited. After excluding subjects who had a history of diabetes or taking hypnotic drug, a total of 3686 subjects (62.7% men) were recruited for analysis. A 75-g OGTT was applied and diabetes/prediabetes (impaired fasting glucose and impaired glucose tolerance) were defined according to the American Diabetes Association diagnostic criteria. The number of hours of sleep was assessed through self-administered questionnaire. Subjects were classified into three groups: short sleeper (≤ 5 hours), normal sleeper (6-7 hours) and long sleeper (≥ 8 hours)
Results: Comparing to subjects with normal glucose tolerance, subjects with newly diagnosed diabetes and prediabetes were older, and had a higher percentage of obesity, family history of diabetes, cigarette smoking habit, alcohol drinking habit, but lower education levels, lower percentage of coffee drinking and regular exercise habit and shorter sleep hours. In multi-nominal regression analysis using normal sleeper as a reference, after adjusting for age, sex, education level, family history of diabetes, BMI, cigarette smoking, alcohol drinking, tea drinking, coffee drinking, regular exercise habit, the odds ratios for newly-diagnostic diabetes and prediabetes were 1.47(1.03-2.08) and 1.16(0.94-1.43) for short sleeper, respectively.
Conclusion: Short sleeper, but not long sleeper, had a higher risk of developing newly diagnosed diabetes in Taiwanese population.

846

The association of serum triglyceride changes over 4 years of period with type 2 diabetes risk in Korean adults
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Background: Hypertriglyceridemia is one of the common features of Type 2 diabetes mellitus, and is caused from dyslipidemia due to insulin resistance, one of the main mechanism of the disease. We investigated the association of

the changes in triglyceride (TG) concentrations in 4 years of foll-up and type 2 diabetes in apparently healthy Korean adults.
Methods: Serum TG levels at baseline (time 1) and 4 years later (time 2) were measured in 23,147 subjects (mean age 42.5 years, males 66%) with TG<500mg/dL. Subjects with diabetes mellitus at baseline, were excluded. We separated the subjects into 3 groups according to TG concentrations at baseline and 4 years later; low, intermediate,high.
Results: After 4 year, 315 cases of diabetes occurred in 23,147 subjects. Baseline age,BMI,LDL-C, exercise and smoking status correlated positively with future development of diabetes. HDL-C levels negatively correlated with the development of diabetes. Subjects who were in the lowest tertile of TG concentration at time 1 and progressed to the hightest tertile over 4 years (low-high), exhibited a hazard ratio (HR) of 5.50 (95% CI 2.14-14.12) for diabetes risk compaired with those remaining in the lowest tertile at both time points (reference group:low-low). Whereas subjects which belong to high-high group had a HR for diabetes of 7.23 (95% CI 3.80-13.72), those whose TG level decreased to the lowest tertile(high-low & intermediate-low) did't exhibit significant risk statistically.Alterations in TG levels during 4 years of follow-up were associated with BMI,HDL-C, LDL-C and hypertension status. In multivariate model with age, BMI,HDL-C and LDL-C and hypertension in the model, TG changes and hypertension were the sigificant determenants for future diabetes risk.
Conclusion: The increased or persistently high serum TG concentrations in 4 years of period were the independent predictors for type 2 diabetes in Korean adult.

847

Association of compensatory beta cell dysfunction and obesity and plasma nonesterified fatty acid concentration in young Korean men with normal glucose tolerance

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Background and aims: Obesity is known to increase the risk of the development of type 2 diabetes mellitus(T2DM), and the prolonged elevation of nonesterified fatty acid(NEFA) is also known to be an important factor attributed to progressive β-cell dysfunction in T2DM. But association of obesity and NEFA with β-cell function in young healthy people with normal glucose tolerance(NGT) is not well elucidated as yet. This study thus compared compensatory β cell function according to obesity in Korean young men with NGT.
Materials and methods: Standard 75g OGTT was performed in 362 healthy young men. Obesity was classified based upon the Asia-Pacific obesity criteria(Normal weight:18.5≤BMI<23, Overweight:23≤BMI<25, Obese: BMI≥25). The Whole Body Insulin sensitivity Index(WBISI) and $HOMA_{IR}$, Insulinogenic Index(IGI), and Disposition Index(DI = IGIxWBISI) and NEFA were measured.
Results: NGT was 260(mean age 25.6±3.3 years). Compensatory β-cell function (DI) was higher among overweight group than in the normal group (8.86 in overweight vs 7.04 in normal, P=0.055). But in the obese group, Disposition Index was significantly lower than the overweight group (6.46 in obese vs 8.86 in overweight, P=0.045). Regression analysis between BMI and Disposition Index showed a nonlinear relationship in spite of normal glucose tolerance. NEFA concentration was higher in the obese group compared with overweight and normal group, and NEFA had a negative linear regression relationship with Disposition Index (p=0.015). Also, after multiple regression analysis, NEFA showed a negative linear relationship with Disposition Index (p=0.017). In stratified analysis for NEFA concentration, the subject with NEFA more than 400 uEq/L showed significantly lower Disposition Index than subject with NEFA less than 200 uEq/L (p=0.009). And also, normal weight subject and obese subjects with NEFA more than 400 uEq/L had a low Disposition Index (5.84 in normal weight and 5.79 in obese, respectively).
Conclusion: This study suggests that obesity is associated with the decrease of compensatory ß-cell function in Korean young men with normal glucose tolerance. In particular, regardless of general obesity, metabolic obesity, which is status with elevated NEFA, may account for compensatory ß-cell dysfunction in the early stage of the natural history of type 2 diabetes.
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848

Effect of metabolic syndrome and altered glucose tolerance on diastolic cardiac function and intima-media thickness

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Background and aims: Metabolic syndrome (MS) and type 2 diabetes are both associated with high risk for cardiovascular diseases (CVD). However the respective role of MS and altered glucose tolerance on vascular damage is under debate. We investigated whether MS affects early markers of vascular injuries induced by altered glucose tolerance.
Materials and methods: 132 subjects, divided in two groups by the presence of MS according to ATP-III criteria, underwent to an OGTT. Intima-media thickness (IMT) was assessed by carotid ultrasound. Augmentation and Augmentation Index (Aug and Aug I), surrogate markers of arterial stiffness, and subendocardial viability ratio (SEVR), index of diastolic cardiac function, were assessed by applanation tonometry.
Results: Gender, age, BMI, waist circumference, total and LDL cholesterol were not significantly different among subjects with (n=64) or without MS (n=68). Subjects with MS had significantly higher levels of triglycerides, fasting glucose and blood pressure and lower levels of HDL-cholesterol. Maximal IMT was significantly higher in MS than in non MS group [1.35 mm (CI 1.21-1.51) vs 1.05 mm (CI 0.96-1.16), p=0.001]. Aug and Aug I were higher, but not significantly, in MS subjects (13.4±17.5 vs 8.4±5.5 mmHg, p=ns and 25±14.4 vs 24±13.3 %, p=ns, respectively). SEVR was significantly reduced in MS compared to non MS (149±26.9 vs 159.9±31.7 mmHg, p=0.03). After OGTT, the subjects were divided in normotolerants (NT) and with altered glucose tolerance (AGT, blood glucose at 120' > 140 mg/dl). IMT was similar in AGT subjects with MS [1.37 mm (CI 1.21-1.55)] and without MS [1.38 mm (CI 1.11-1.70)], p=ns. In contrast, IMT was significantly higher in NT subjects with MS [1.32 mm (CI 1.05-1.65)] as compared with NT without MS [(0.91 mm (0.8-0.98), p<0.001]. SEVR was significantly reduced in AGT patients with MS (148±28 mmHg) compared to AGT without MS (163±30 mmHg, p=0.05) and in NT with MS (148±23 mmHg) compared to NT without MS (159±32 mmHg, p<0.05). Logistic regression analysis showed that both altered glucose tolerance and MS were independently associated with high risk of increased IMT (OR=2.326, p=0.03 and OR=2.771, p=0.03, respectively).
Conclusion: Diastolic cardiac function is impaired in MS independently by the presence of altered glucose tolerance. In contrast, IMT was increased both in altered glucose tolerance and MS.

849

Skeletal muscle microvascular exchange capacity is associated with hyperglycaemia in non-diabetic subjects with central obesity

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Background and aims: Poor glycaemic control is associated with increased risk of microvascular disease but the relationship between glycated haemoglobin levels (HbA1c) and microvascular function in skeletal muscle has not been described. We tested the association between HbA1c and a measure of microvascular exchange capacity (Kf) in skeletal muscle controlling for fatness, insulin sensitivity, physical activity and fitness in people at risk of developing type 2 diabetes.
Materials and methods: Microvascular function was measured in 28 women and 19 men (mean (SD) age 51 (9) years) with central obesity who did not have diabetes. We estimated insulin sensitivity (M/I), visceral and total fatness, fitness (VO2 max), physical activity energy expenditure (Metabolic Equivalents of TaskS (METS)), and skeletal muscle microvascular exchange capacity (Kf).
Results: In regression modelling, age, sex and fasting plasma glucose accounted for 30.5% of the variance in HbA1c (r^2=0.31, p=0.001). Adding Kf to this model explained an additional 26.5% of the variance in HbA1c (r^2=0.57, p=0.0001 and Kf was strongly and independently associated with HbA1c (standardised B coefficient -0.45 (95%CI -0.19, -0.06), p=0.001). The independent association between Kf with HbA1c was unchanged by adding M/I or visceral fat to the model and neither were independently associated with HbA1c.

Conclusion: We found a strong negative independent association between a measure of skeletal muscle microvascular exchange capacity (Kf) and HbA1c that explained almost as much of the variance in HbA1c as fasting plasma glucose. The association between Kf and HbA1c was independent of insulin sensitivity, fatness, fitness and physical activity.
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850

Do serum potassium levels influence the metabolic effects of combined RAS blockade with hydrochlorothiazide in obese hypertensive individuals?

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Background and aims: Traditionally, a reduction in serum potassium (K^+) has been implicated as the prime mechanism by which diuretic therapy increases the risk for the development of diabetes mellitus. This 16-week randomized, multi-center, study compared valsartan and hydrochlorothiazide (V/HCTZ) to amlodipine/HCTZ (A/HCTZ) on glucose metabolism in obese hypertensives. We evaluated the metabolic response in patients (pts) who had a low (≤3.9mmol/L) or normal K^+ (>3.9mmol/L) level at the end of the study in the two groups.
Materials and methods: 412 obese (BMI=35±7 kg/m^2; BW=98±23 kg), hypertensives (seated BP=159±8/94±8 mmHg) (aged 56±9 years, 66% females) entered double-blind treatment with V/HCTZ 160/12.5 mg or HCTZ 12.5 mg after a 4-week washout period. Pts were force-titrated to V/HCTZ 320/25 mg or A/HCTZ 10/25 mg, respectively. At week 16, changes were measured from baseline in fasting and 2-hour postprandial glucose and insulin after an oral glucose tolerance test.
Results: At Week 16, fasting glucose increased with A/HCTZ in both low and normal K^+ groups, with significantly greater increases vs. V/HCTZ ($p<0.05$) in the low K^+ group (Table). Significantly greater increase in postprandial glucose at 2 hours was also observed with A/HCTZ vs. V/HCTZ ($p< 0.05$) in both K^+ stratified groups.
Conclusion: The metabolic response to HCTZ was not different in patients with low K^+ compared to those pts with normal K^+. In the V/HCTZ group the addition of valsartan reduced the postprandial glucose level in the low and normal K^+ groups through a greater and earlier postprandial insulin response. Thus the mitigating effect of valsartan on HCTZ induced hyperglycemia is not influenced by serum potassium levels.

Parameter	Low K^+ (≤ 3.9mmol/L) EOS#		Normal K^+ (> 3.9mmol/L) EOS#	
	A/HCTZ	V/HCTZ	A/HCTZ	V/HCTZ
N (%)	123 (63)	92 (49)	71 (37)	96 (51)
K^+ at baseline (mmol/L)	4.22	4.12	4.44	4.36
K^+ - EOS (mmol/L)	3.55*	3.67	4.31	4.3
Fasting glucose at baseline (mmol/L)	5.51	5.45	5.49	5.45
Fasting glucose - EOS (mmol/L)	5.73*	5.39	5.68	5.52
Postprandial glucose (Δ baseline) at 2h (mmol/L)	1.1*	0.1	1.0*	0.1
Fasting insulin at baseline (pmol/L)	138.9	121.5	141.7	149.3
Fasting insulin - EOS (pmol/L)	163.2	153.5	165.3	168.8
Postprandial insulin (Δ baseline) at 2h (pmol/L)	211.1	99.3	168.8	230.2

*p< 0.05 vs V/HCTZ
#Stratified by low and normal potassium values obtained at the end of study (EOS = week 16)

Dr. Deedwania served as consultant and participated in speaker's bureau activities for Novartis Pharmaceuticals Corporation.

851

eZscan, a new non-invasive technology, has the same sensitivity to detect abnormalities in insulin resistance subjects as the 45' OGTT glucose values

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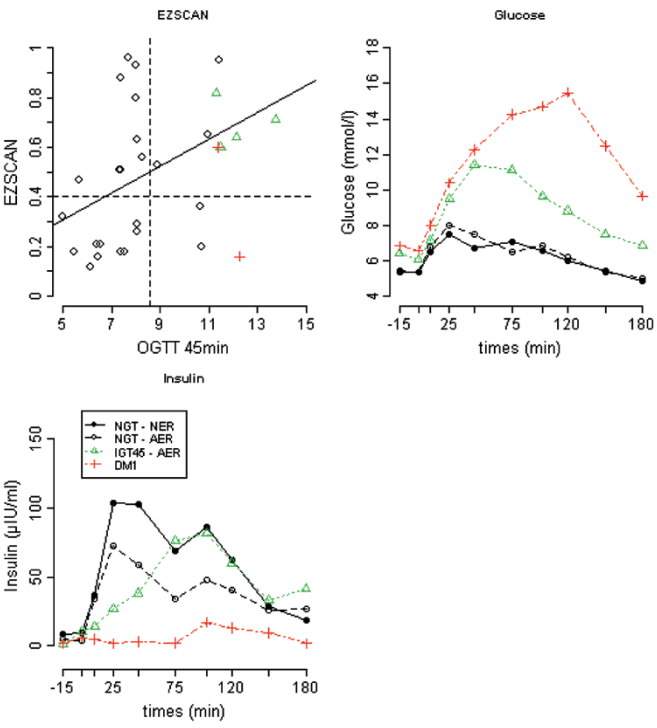
Background and aims: The eZscan is a new patented technology which uses low level DC voltage inducing reverse iontophoresis, together with chronoamperometry to evaluate the functions of tissues in specific locations of the body. The whole test takes only 3 minutes, does not require patient preparation and does not cause inconveniences for the subjects. The aim was to determine the ability of eZscan to detect abnormalities in carbohydrate metabolism as compared to a 45 minute OGTT.
Materials and methods: A cross-sectional study was done in Chennai, India with subjects who were not known to have any abnormalities in carbohydrate metabolism. A frequent sampling OGTT with measurements of glucose and insulin levels at 10 time points was done. Testing with the eZscan was done. A reading of >40% was considered positive on the eZscan Insulin Resistance scale. The criterion was used for the cut-off values of the 45 min OGTT (8.60 mmol/l).
Results: A total of 29 subjects were assessed. Age was 41±11 yrs, BMI 28±6, M/F ratio 1, $OGTT_{45mins}$ (mmol/l) 8.65 ±2.33, and $OGTT_{2hours}$ (mmol/l) 7.21±2.22. Of the 6 subjects who were found to have abnormal $OGTT_{45mins}$ results (Fig 1 A), 4 had IGT and 2 had DM. Five of these cases tested positive with the eZscan (83% sensitivity), while one case (23 yrs old Type I DM) was not detected by the eZscan. There was a good correlation between the results of the eZscan as compared to the $OGTT_{45mins}$ (r=0.55, p=0.02) (Fig 1 A). Graphical plots of the time profile of glucose (Fig 1 B) and insulin (Fig 1 C) levels show that the eZscan measurements (NER=normal eZscan Reading and AER=abnormal eZscan Reading) correlate better with presence of insulin resistance rather than insulin deficiency (comparison of IGT45-AER and NGT-NER/AER curves).
Conclusion: The eZscan is a promising tool which is able to detect the presence of early carbohydrate metabolism dysfunctions in insulin resistance subjects.



PS 70 Insulin sensitisers 1

852

Pioglitazone and Rosiglitazone have significantly different effects on lipid metabolism in mouse cultured liver explants

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Background and aims: Pioglitazone (PioGTZ) and rosiglitazone (RosiGTZ) are widely used as oral antidiabetic agents for treatment of type 2 diabetes. While these medications exert similar effects on blood glucose, clinical studies indicate that PioGTZ has a more pronounced beneficial effect on lipid parameters than RosiGTZ. This prompted us to set up a study designed to test whether PioGTZ, compared to RosiGTZ, could exert direct effects on lipid liver metabolism in relation with plasma triglycerides and HDL-Cholesterol.
Materials and methods: Thin liver slices were incubated 21h either with RosiGTZ (1 µM) or PioGTZ (7.5 µM) or vehicle. Intracellular lipid content, lipid uptake and secretion and the expression of several genes involved in lipid metabolism were studied.
Results: Both glitazones slightly reduced HMG-CoA reductase mRNA levels at the same degree but only PioGTZ reduced intracellular cholesterol content, suggesting an alteration of cholesterol uptake rather than an inhibition of cholesterol biosynthesis. This concept was supported by the reduction of SR-BI expression, hepatic lipase activity and HDL-cholesterol uptake in PioGTZ-treated liver explants. Conversely, hepatic lipase mRNA levels were increased 3.5-fold. RosiGTZ induced acetyl-CoA carboxylase and fatty acid synthase gene expression and increased apoB secretion suggesting a stimulation of lipogenesis. FAT/CD36 and acyl-CoA oxidase mRNA levels were modified neither by PioGTZ nor by RosiGTZ suggesting that PPARγ and PPARα activations were not required for direct action of glitazones on liver explants. Besides, PioGTZ appeared to be a more potent activator of AMPK than RosiGTZ.
Conclusion: PioGTZ and RosiGTZ exert specific direct effects on liver independently of PPARγ or PPARα activation. RosiGTZ promotes lipogenesis and increases lipid content in hepatic cells, but PioGTZ does not. These differences could explain the significant improvement in plasma lipids observed in diabetic patients treated with PioGTZ, but not with RosiGTZ.

853

BNP elevation following 12-week pioglitazone treatment in patients with type 2 diabetes

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Background and aims: Some studies show that thiazolidinediones (TZD) and Alpha-glucosidaseinhibitors (AGI) decrease incidence of coronary artery disease among diabetic patients. The aim of this study was to see the differences in cardiac function between TZD and AGI treatment among mild type 2 diabetic patients.
Materials and methods: After obtaining informed consent, eighty-nine Japanese type 2 diabetic patients were enrolled and randomized into
the two treatment groups, TZD and AGI group.Pioglitazone was administrated in TZD group (n=50) and miglitol in AGI group (n=39) for 12 weeks. We evaluated fasting plasma glucose (FPG),1,5-anhydroglucitol (1,5AG), HbA_1C and brain natriuretic peptide (BNP),which was considered as a surrogate marker of congestive heart failure,at baseline (before administration), 4 weeks, 8 weeks and 12 week s after treating TZD or AGI.
Results: A significant improvement of 1,5AG was shown in the AGI treatment group (+5.20±3.70 µg/ml) compared with TZD group (+2.10±0.87µg/ml). HbA_1C reduced significantly at 12 weeks (-0.39±0.28%) in AGI group,whereas -0.59±0.65% in TZD group. The reduction of FPG was -10.0±21.5 mg/dl in AGI group and -25.6±36.8 mg/dl in TZD group, respectively. We observed a tendency of BNP reduction in AGI group and significantly increase in TZD group at 12 weeks (-2.76±15.0 pg/ml vs +9.15±17.5 pg/ml, two-way layout ANOVA, $p<0.05$). When we performed subanalysis in patients with BNP more than 18.5 pg/ml at baseline, 37% of patients in AGI group remained within normal range of BNP (<18.5 pg/ml) at 12 weeks,while none of TZD group showed normal BNP (Fisher analysis, $p<0.05$). In patients with BNP less than 18.5 pg/ml at baseline, 5% of AGI group showed abnormal BNP (>18.5) at 12 weeks, whereas 30% of TZD group abnormal (Fisher analysis, $p<0.05$).
Conclusion: The possibility of unfavorable BNP change should be taken into account when administrating TZD on mild type 2 diabetic patients.

854

Effect of pioglitazone and metformin fixed-dose combination on glycaemic control in untreated patients

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Background and aims: The fixed-dose combination (FDC) of pioglitazone (PIO) and metformin (MET) uses complementary mechanisms to improve glycemic control in type 2 diabetes mellitus (T2DM). PIO reduces insulin resistance via activation of peroxisome proliferator-activated receptor γ. Metformin improves glycemic control primarily by suppressing hepatic glucose production. This study evaluated the effects of initial therapy with FDC PIO/MET compared with the effects of each drug alone on efficacy and safety in a randomized, double-blind, parallel-group controlled trial in T2DM patients.
Materials and methods: These patients were on no treatment with antidiabetes medications for 12 weeks and had a hemoglobin A1c (A1c) ≥7.5 but ≤10. The primary endpoint was change from Baseline in A1c of PIO/MET FDC (N=201) compared with PIO (N=189) or MET (N=210) monotherapy after 24 weeks of treatment. Fasting plasma glucose (FPG) was evaluated as a secondary endpoint.
Results: A significant decrease in A1c from Baseline was observed in all 3 study groups at Week 24 ($P<0.0001$ compared with Baseline of respective group). A1c reduction was -1.83% for FDC from Baseline 8.89 vs -0.96% and -0.99% for PIO monotherapy from Baseline 8.69, and MET from Baseline 8.65, respectively, demonstrating the nearly additive effects of the FDC relative to PIO and MET alone ($P<0.0001$ compared with FDC). Results for FPG were consistent in direction and degree in each group with A1c changes. FPG reduction was -39.9 mg/dL from Baseline (177.5 mg/dL) for FDC vs -22.2 and -24.8 mg/dL for PIO (Baseline 170.8 mg/dL) and MET (Baseline 170.5 mg/dL) monotherapy, respectively ($P<0.0001$ compared with Baseline of respective group). Significant differences were also observed between FDC and each monotherapy ($P<0.002$). Endpoint responder (A1c ≤7%) rates were higher in PIO/MET FDC (63.8%) than for PIO (46.3%) and MET (38.9%) monotherapy. There were no significant differences in hypoglycemic event rate (1.0%, 0.5%, and 1.4% for FDC, PIO, MET, respectively), and total treatment-emergent adverse events were similar among all 3 study groups, with 50.7% for PIO/MET FDC and 52.1% and 53.1% for PIO and MET monotherapy, respectively.
Conclusion: PIO/MET FDC showed significantly improved glycemic control compared with monotherapy in T2DM patients as first-line therapy without an increase in hypoglycemia.
Supported by: Takeda Global Research & Development Center, Inc.

855

PIOace-Study: pioglitazone, but not ramipril improves thrombocyte function and reduces low grade inflammation in non-diabetic patients with increased cardiovascular risk

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Background and aims: Non-diabetic patients with isolated vascular insulin resistance suffer from reduced nitric oxide production in the endothelial cells. This may lead to hypertension, impaired endothelial function and a higher tendency for thrombocyte aggregation. The aim of this analysis was to investigate the effects of pioglitazone (PIO), ramipril (RAM) or their combination (PIRA) on low grade inflammation and thrombocyte function in hypertensive patients with increased cardiovascular risk.

Materials and methods: This placebo-controlled, double-blind, multicentre, randomized, parallel study was performed with 149 non-diabetic patients (72 male, 77 female, age: 60±9 yrs., BMI: 30.4±4.7 kg/m², duration of hypertension: 9±8 yrs.). Patients were treated with 15/30 mg PIO (dose titration), 2.5/5 mg RAM or a combination thereof for 12 weeks. Efficacy parameters for this analysis were hsCRP, Plasminogen-activator-inhibitor I (PAI-I), thromboxane B2 (TXB2, degradation product of TXA2), and endothelin-1. In addition, transforming growth factor 1ß (TGF1ß, reduces inflammation and activation of macrophages) and 6-keto-prostaglandin-F1α (6K-PGF, degradation product of the vaso-dilatator prostacyclin) were determined in a subpopulation of 35 patients at a single site.
Results: A pronounced and general improvement of biomarkers indicating reductions of chronic systemic inflammation and thrombocyte activation was observed by (co)treatment with PIO only. There was a reduction in hsCRP. Baseline and endpoint values are provided in the table. RAM alone had a positive impact on the 6k-PGF values.

Baseline and endpoint values of the obseration parameters (mean±SD)

parameter	week	PIO (n=52)	RAM (n=44)	PIRA (n=53)
hsCRP [mg/l]	0	3.54±2.54	2.90±2.26	2.98±2.15
	12	2.65±2.02*	3.47±2.62	2.50±1.98*
TXB2 [ng/ml]	0	2.92±3.14	2.08±2.07	2.81±3.13
	12	2.32±2.50	2.53±2.93	2.45±2.86
PAI-I [ng/ml]	0	57.9±20.1	62.3±19.3	54.2±19.0
	12	48.0±21.6*	62.7±20.8	47.2±19.4*
TGF1ß [ng/ml]	0	15.8±5.7	12.6±3.4	12.0±3.2
	12	18.0±10.0*	12.7±4.3	12.0±2.9
6k-PGF [ng/ml]	0	2.2±1.6	1.8±1.7	1.3±1.2
	12	1.2±0.9*	3.2±3.7*	1.1±1.0

*:p<0.05 vs.baseline

Conclusion: Activation of PPARγ by pioglitazone had an anti-inflammatory and platelet aggregation inhibiting effect in a three month treatment with PIO in non-diabetic hypertensive patients as shown by a mean decrease of hs-CRP, PAI-I and the anti-inflammatory cytokine TGF1ß, and a slight non-significant decrease in TXB2 and endothelin-1. ACE inhibition by ramipril showed an increase in prostacyclin concentrations. These findings may help to understand the results from outcome trials regarding cardiovascular risk reduction performed with both drugs.
Supported by: Takeda Pharma

856

Thiazolidinediones, but not sitagliptin, exacerbate ovariectomy-induced bone loss in rats

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Recent reports have established that thiazolidinediones (TZDs) increase risk of bone loss and fracture in non-osteoporotic fracture sites in patients with type 2 diabetes. Sitagliptin (Sita) represents a new class oral antihyperglycemic agents and is a potent, selective and once daily inhibitor of dipeptidyl peptidase-4 (DPP-4). We compared the effects of rosiglitazone (Rosi), pioglitazone (Pio), and Sita on ovariectomy (OVX)-induced bone loss in virgin female non-diabetic Sprague-Dawley rats (group size = ~11). Rats (18 weeks old) were OVXd and immediately treated once daily by oral gavage for three months with vehicle (Veh), Sita (100 and 300 mg/kg; both dose groups achieved ≥ 80% inhibition of plasma DPP-4), Rosi (5 and 30 mg/kg), Pio (5 and 30 mg/kg), or alendronate (ALN) (0.015 mg/kg; 2x/wk, sc). A sham-operated (Sham) group was included. No significant treatment-related differences in body weight or glucose occurred. Veh rats had significantly lower bone mineral density (BMD, mg/cm²) in trabecular regions of the femur than Sham. This BMD loss was prevented by ALN. Sita-treated rats did not differ from Veh in either trabecular or cortical regions. However, Rosi and Pio exacerbated OVX-induced bone loss, and were below both Veh and Sita, particularly in cortical regions (central femur, CFBMD). The mean±SD CFBMD values were as follows: Sham: 229±14; Veh: 220±11, Rosi [5 & 30]: 213±9 and 206±9*; Pio [5 & 30]: 212±8 and 208±7*; Sita [100 & 300]: 214±8 and 222±10; ALN: 225±7; *p<0.05, vs. Veh. The negative effect of the TZDs on BMD was more marked at the central femur, a yellow marrow site, than the distal femur, a red marrow site. In contrast, Sita had no effect on any BMD endpoint. Rats treated with TZDs also gained more fat body mass during the experiment than did Veh or Sita-treated rats. These animal data reveal TZD-associated regional bone mass changes (principally in yellow marrow sites) that appear to parallel the human fracture profile (concentrated in yellow marrow sites, as in cortical foot bones) observed with TZDs in clinical studies and demonstrate potentially important differences between the bone effects of Sita and TZDs.

857

Differential action of pioglitazone and metformin on hepatic fat, substrate metabolism and perfusion in type 2 diabetic patients

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Background and aims: Hepatic steatosis is frequently found in type 2 diabetes (T2DM) and is causally linked to features of the metabolic syndrome, cardiovascular and chronic liver disease. Hepatic steatosis and insulin resistance are closely related phenomena and affect hepatic glucose production, triglyceride output and insulin clearance, but have also been implicated in the impairment of splanchnic glucose and fatty-acid metabolism and hepatic hemodynamics. Consequently, insulin sensitizing and anti-steatotic therapies may be beneficial in correcting hepatic physiology.
Materials and methods: Effects of 24 weeks of treatment with pioglitazone (30mg/day) versus metformin (2000mg/day) and matching placebo in 78 T2DM males (mean age±SE 56.5±0.6 years, BMI 28.7±0.4 kg/m2, HbA1c 7.1±.0.1%) without hepatic or cardiovascular disease on hepatic fat content were determined using proton MR-spectroscopy and positron emission tomography (PET). The following PET measurements were performed: hepatic perfusion, fatty acid and glucose uptake using [15O]H2O, [11C]palmitate under fasting conditions and [18F]-2-fluoro-2-deoxy-glucose under euglycaemic hyperinsulinaemic conditions, respectively.
Results: Seventy-one patients completed the trial and no adverse events were registered. Both pioglitazone and metformin similarly improved glycemic control (P=0.146) and whole-body insulin sensitivity (P=0.501). Pioglitazone versus metformin reduced hepatic fat content (6.9 (2.6-17.4) % to 4.1 (1.9-12.1) % versus 7.7 (3.7-23.9) to 10.7 (5.1-22.0), between-group P<0.001). Pioglitazone, but not metformin, increased insulin-mediated hepatic glucose uptake (P=0.025) and fasting liver perfusion (P=0.044) from baseline. Neither treatment affected fasting hepatic fatty-acid uptake.
Conclusion: In spite of similar effects of pioglitazone and metformin on glycemia and whole body insulin sensitivity, only pioglitazone reduced hepatic fat content in T2DM patients. This was associated with improvements in both insulin mediated hepatic glucose uptake and fasting perfusion.
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858

Erythrocyte metformin concentrations and alteration of renal function

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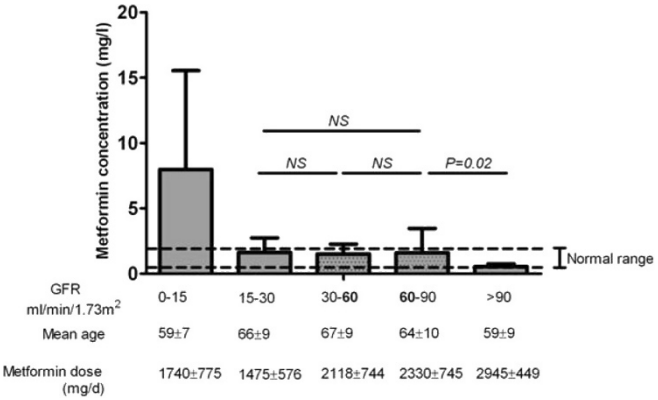
Background and aims: As metformin is eliminated solely by the kidney, renal impairment is a logic contraindication. It is usually recommended not to use metformin when glomerular filtration rate (GFR) is under 60 ml/min/1.73m2. However, since metformin clearance is 4 to 5 times that of creatinine, the degree of renal dysfunction associated with significant metformin accumulation is not known. From transverse data reflecting clinical practice, we therefore studied blood metformin concentrations according to renal function. We chose erythrocyte metformin concentration which is known to be a better marker of chronic accumulation than that in plasma.

Research design and methods: We studied all metformin concentrations measured in 2008. GFR was estimated with abbreviated MDRD equation (Modification of the Diet in Renal Disease). Patients were divided into five groups depending on the GFR corresponding to the stages of chronic kidney disease: GFR 0-15 (stage 5), 15-30 (stage 4), 30-60 (stage 3), 60-90 (stage 2), and >90 (stage 1).
Results: 126 subjects were studied: sex ratio H/F 83/43, mean age of 65±9, mean GFR 47.8±24.1 ml/min/1.73m2, 74.4% having a creatinine clearance < 60 ml/min/1.73m2. The following figure describes the erythrocyte metformin concentrations in the different groups (means ± SD).
Conclusion: Metformin concentrations were somewhat elevated in mild renal failure (stage 2) as compared to stage 1, while remaining within the normal range, but not further in stages 2, 3 and 4. Given that metformin dosage was reduced by half from stage 1 to stage 4, the question arises as to whether metformin administration could be safely continued in mild or moderate renal failure provided the dose is adequately adjusted to renal function.



859

Effect of pioglitazone on proliferation of mature adipocytes
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Background and aims: Adipocyte has been believed be generated from multipotent mesenchymal stem cell via its precursor cell, preadipocyte, whereas mature adipocyte has been considered to be unable to proliferate. On the other hand, thiazolidinedione, anti-diabetic agent, increases subcutaneous fat and decrease visceral fat. However, precise mechanisms have not been fully understood. In this study, we investigate the mechanism that pioglitazone, a thiazolidinedione, increases the number of adipocytes.
Materials and methods: Male Wistar rats were treated with or without 0.005% pioglitazone containing food for 28 day. 5-Bromo-2'-Deoxyuridine (BrdU: 200 mg/kg) was intraperitoneally administered before sacrifice, and then each subcutaneous, epididymal and periintestinal fat was collected. Immunohistochemical staining of BrdU was carried out. Moreover, isolated adipocytes and stromal vascular fraction (SVF) were obtained with collagenase digestion and then DNA was extracted. Equal amount of each DNA was absorbed in nitrocellulose sheet to quantify the BrdU uptake using anti-BrdU antibody. On the other hand, fully differentiated 3T3-L1 adipocytes were incubated with 10μM pioglitazone (Pio), populations in the G0/G1, S and G2/M phases were analyzed with flow cytometry. Morphological analysis and gene expression in these cells were also examined.
Results: Immunohistochemical study using anti-BrdU antibody revealed that BrdU positive cells were identified both in SVF and mature adipocuyes. BrdU uptake into adipocyte DNA was markedly prominent in periintestinal fat compared with subcutaneous fat. Pioglitazone treatment increased BrdU uptake in subcutaneous fats, whereas it decreased in epididymal and periintestinal fats. Cell cycle analysis using flow cytometry revealed that incubation with Pio increased G2/M phase. In contrast, treatment with aphidicolin (Aphi), a DNA polymerase inhibitor, increased the population of G0/G1. 3T3-L1 adipocytes treated with Aphi contained large shape and abundant fat droplets. It also decreased PPARγ and adiponectin mRNA levels and increased MCP-1 and IL-6 mRNA levels.
Conclusion: These results suggest that pioglitazone decreases visceral fat accumulation and increases subcutaneous fat via differentiation of cell cycle, and that finally, mature adipocyte has the ability to proliferate.

PS 71 Insulin sensitisers 2

860

Validation of treatments for metformin monotherapy failure in type 2 diabetic patients. A meta-analysis of randomized clinical trials
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Background and aims: The choice of drugs to add to metformin in type 2 diabetic patients failing to monotherapy should be formulated on the basis of randomized clinical trials (RCTs) performed in this specific population. This meta-analysis compares different agents as add-on treatments for metformin monotherapy failure.
Materials and methods: Placebo-controlled and active comparator RCTs with a duration ≥24 weeks were retrieved from Medline (any date up to November 1st,2008) and included in this meta-analysis. Data for analysis were extracted independently by two observers and potential contrasts were resolved by a senior investigator.
Results: Twelve placebo-controlled and 16 active comparator studies were included. In placebo-controlled studies, thiazolidinediones, alpha-glucosidase inhibitors, DPP-4 inhibitors, and GLP-1 agonists produced a significant reduction of HbA1c at 24 weeks (OR, 95%CI: -0.3[-0.5;-0.2], -0.7[-0.9;-0.5], -0.8[-1.0;-0.6], and -0.7[-0.9;-0.5]%, respectively). In comparisons with thiazolidinediones (N= 6), insulin secretagogues produced a similar reduction of HbA1c (-0.1[-0.2;0.1]%, p=0.33) and increase of BMI (OR: -0.32[-1.18;0.53]Kg/m^2; p=0.46), with a higher hypoglycaemic risk.
Conclusion: Available data are not sufficient to establish the superiority of any class of hypoglycaemic drugs in the treatment of metformin monotherapy failure. Further evidence should be collected in order to formulate a well-validated algorithm.

861

Long-term effects of metformin on endothelial function and inflammation in type 2 diabetes treated with insulin: a randomized, placebo-controlled trial
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Background and aims: Three out of 4 patients with type 2 diabetes will die from cardiovascular disease. Treatment with metformin reduces rates of cardiovascular morbidity and mortality in type 2 diabetes, possibly through improvements of endothelial function and/or reduction of low-grade inflammation. Therefore, we studied the effects of metformin on markers of endothelial function and low-grade inflammation in patients with type 2 diabetes.
Materials and methods: 390 insulin-treated patients with type 2 were randomly allocated to either placebo or metformin. 277 subjects (= 72%) completed the trial. During a follow-up of 4.3 years, plasma samples were taken to measure markers of endothelial function (urinary albumin excretion, plasma levels of vWf, sVCAM-1, sE-selectin, t-PA and PAI-1) and markers of low-grade inflammation (plasma levels of CRP and sICAM-1). For each subject, each variable was expressed as standard deviation of difference from the population mean. Mean standard deviation scores (= z-scores) were calculated as:
• endothelial dysfunction z-score = {urinary albumin excretion + vWf + sVCAM-1 + sE-selectin + t-PA + PAI-1}/6
• inflammation z-score = {CRP + sICAM-1}/2.
Results: Metformin treatment versus placebo was associated with a decrease in vWf of 11% (-16 to -6; p<0.001); a decrease in sVCAM-1 of 5% (-8 to -3; p<0.001); a decrease in t-PA of 15% (-20 to -9; p<0.001); a decrease in PAI-1 of 21% (-31 to -9; p=0.001); and a decrease in the endothelial dysfunction z-score of 7% (-10 to -4; p<0.001). Changes in urinary albumin excretion and sE-selectin were not significant. Metformin treatment versus placebo was associated with a decrease in CRP of 17% (-31 to -1; p=0.036); a decrease in

sICAM-1 of -5% (-8 to -2; p=0.004); and a decrease in the inflammation z-score of -5% (-10 to +1; p=0.074).
Conclusion: This is the first long-term randomized controlled trial showing that metformin treatment is associated with decreases in the plasma concentrations of vWf, sVCAM-1, t-PA, PAI-1, CRP and sICAM-1, reflecting an improvement of endothelial regulation of haemostasis (vWf), of leukocyte adhesion (sVCAM-1), and of fibrinolysis (t-PA and PAI-1), as well as a reduction of low-grade inflammation.
ClinicalTrials.gov Identifier: NCT00375388
Supported by: Altana, Lifescan, E. Merck/Santé, Merck, Sharpe & Dohme, and Novo Nordisk

862

The effects of thiazolidinediones on mitochondrial function are related to their lipophilicity
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Background and aims: We have previously shown that thiazolidinediones (TZDs) inhibit cell respiration by a direct effect on isolated skeletal muscle. This mechanism is independent of peroxisome proliferator-activated receptor-γ (PPAR-γ) and accounts for many actions of TZDs seen *in vitro.* Modulation of cell respiration could also contribute to insulin sensitization and/or adverse effects of TZDs *in vivo.* In the present study, we compared the efficacies of various TZDs as inhibitors of cell respiration and noted an association with their respective lipophilic/hydrophilic properties.
Materials and methods: Isolated strips of soleus muscle from Sprague-Dawley rats were exposed to 10 µmol/l of different TZDs for 24 h. To quantify effects on cell respiration, rates of CO_2 production from palmitate and anaerobic glycolysis (lacate release) were measured during the last hour. ATP and PCr content were determined at the end of the experiment.
Results: 10 µmol/l of almost every tested TZD impaired cell respiration as indicated by reduced CO_2 production and increased lactate release (see table). Only BLX-1002, the most hydrophilic compound, failed to affect respiration at 10 µmol/l, but it also exerted inhibitory action at higher concentrations (% decrease in CO_2 production: 30 µmol/l BLX-1002, -17±12%, ns; 100 µmol/l BLX-1002, -24±8%, p<0.02%; increase in lactate release: 30 µmol/l BLX-1002, +24±11%, p<0.05; 100 µmol/l BLX-1002, +62±14%, p<0.001). Decreased respiration was accompanied by proportionate reductions in the ATP/PCr ratios (% decrease: pioglitazone, -27±6%, p<0.001; rosiglitazone, -16±6%, p<0.05; BLX-1002, -2±6%, ns). The results also revealed that compounds with higher clogP values, indicative of low hydrophilicity and high lipophilicity, were more efficient inhibitors of cell respiration (see table). This conclusion is corroborated by significant correlations between clogP values and relative changes in CO_2 production (r=-0.79; p<0.04) and lactate release (r=0.83; p<0.025).
Conclusion: Direct PPAR-γ-independent impairment of cell respiration is a group effect of TZDs which is more pronounced for more lipophilic compounds. This hints at a possible location of the responsible molecular target in a lipophilic compartment like, e.g., the mitochondrial membrane. TZD-induced impairment of cell respiration can reduce the cellular energy state. Since regular or chronic cellular energy loss seems to be a trigger for insulin sensitization, this mechanism could contribute to the antidiabetic effects of TZDs and explain why some TZDs are effective insulin sensitizers despite carrying relatively weak PPAR-γ-agonistic activities.

clogP values and inhibition of cell respiration by different TZD compounds; *p<0.05

	ClogP	Lactate Release	CO_2 Production
Troglitazone	5.58	+157±14%*	-70±5%*
Ciglitazone	5.07	+82±14%*	-31±3%*
Pioglitazone	3.53	+68±12%*	-64±3%*
Darglitazone	3.29	+71±10%*	-56±7%*
PNU-91325	3.19	+78±8%*	-37±9%*
Rosiglitazone	3.02	+13±6%*	-35±7%*
BLX-1002	0.39	+10±8%	+29±24%

863

Relationship between salt-sensitivity and thiazolidinedione-induced oedema
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Background and aims: Although there is growing recognition that fluid retention and edema are common adverse effects with thiazolidinediones (TZDs), the underlying mechanisms remain unclear. To unravel the relationship between salt-sensitivity and TZD-induced edema, we analyzed sodium excretion before and after administration of TZDs.
Materials and methods: After obtaining the approval of the institutional review board and written informed consent from the patients, we analyzed sodium excretion before and after 8 weeks of administration of pioglitazone to female subjects with type 2 diabetes who did not have heart failure, severe liver or renal dysfunction, or a severe infection. Before administration they were asked to measure daily salt excretion by using a salt monitoring system to measure sodium in overnight urine and to measure their blood pressure daily for 21 days, and when a significant correlation was found between salt excretion and blood pressure (correlation coefficient >0.4, p<0.05), the patient was classified as salt-sensitive.
Results: The data of the 26 patients who satisfactorily completed the follow-up examinations were included in the analysis. Six patients (23.1%) were classified into a salt-sensitive group and 20 patients (76.9%) into a non-salt-sensitive group. There were no differences between the characteristics of the two groups in age, body mass index, HbA1c, blood pressure, or salt excretion before administration of pioglitazone. After 8 weeks of pioglitazone administration, 5 patients (19.2% of the total) had developed edema**,** and, surprisingly, all 5 subjects were salt-insensitive. We therefore divided the subjects into three groups based on the presence of salt-sensitivity and edema, a group that was not salt-sensitive and had edema (group A), a group that was salt-sensitive and did not have edema (group B), and a group that was not salt-sensitive and did not have edema (group C). Salt excretion after administration of pioglitazone was significantly lower than before pioglitazone administration in groups A and B, but not group C (A: -1.16 ± 0.96 gram; p<0.05, B: -0.99 ± 0.72 gram; p<0.05, C: 0.03 ± 0.96 gram), and the hematocrit was significantly lower after administration in group B, but not in group A or C (A: 0.02 ± 0.68 %, B: -1.47 ± 0.55 %; p<0.05, C: -0.21 ± 0.38 %). There were no differences in body weight gain among the three groups.
Conclusion: In subjects who developed edema (group A), TZD caused fluid retention because of sodium reabsorption, and increased fluid in the intravascular space would be mobilized into the extravascular space because of a vascular mechanism, and edema was observed as a result. By contrast, in the subjects classified as salt-sensitive (group B), TZD caused fluid retention, but the increased fluid was retained in the intravascular space, explaining why they did not develop edema. In subjects who were salt-insensitive and did not who develop edema (group C), TZD did not cause fluid retention, therefore hematocrit did not decrease. Thus, TZD-induced edema would be caused not only by fluid retention but also by vascular hyperpermeability. In addition, TZD-induced edema was unrelated to body weight gain. Because administration of a TZD caused fluid retention in the subjects who developed TZD-induced edema as well as in the salt-sensitive subjects, assessment of sodium excretion and salt-sensitivity may be useful in preventing the adverse effects of TZDs.

864

Prospective study on the mechanism of weight gain by pioglitazone in Japanese patients with type 2 diabetes (Manda Study 1). Pioglitazone induced the distribution shift in abdominal adipocytes from visceral fat to subcutaneous fat
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Background and aims: We investigated the mechanism of pioglitazone-induced weight gain by comparing a change in the abdominal distribution between visceral fat and subcutaneous fat.
Materials and methods: Sixty patients with type 2 diabetes without insulin treatment, in addition to the current medication, were taken 15 or 30 mg of pioglitazone throughout the period of this study and their abdominal visceral and subcutaneous fat tissues were compared by computed tomography before

and after 24 weeks of intervention. Several clinical parameters such as glycemic control, congestive and metabolic state were also compared.
Results: Seven patients were withdrawn because of extensive edema (3), muscle pain (1), general fatigue (1), poor compliance (2). The remaining 53 patients were subjected to analysis. HbA1c was improved in 42 patients, indicating that most patients were responder to pioglitazone in terms of glycemic control. Body weight was increased in 40 patients. Whereas a decrease in visceral adipose tissue occurred insignificantly, subcutaneous adipose tissue and V/S ratio were significantly increased. Human atrial natriuretic peptide (ANP) was increased more significantly than brain natriuretic peptide (BNP). The increase in these peptides occurred even in patients without edema, suggesting some mechanism other than pioglitazone-related congestive factor. An increase in adiponectine and the concurrent decrease in high sensitive reactive protein (hCRP) were also seen after pioglitazone treatment. These anti-atherosclerotic beneficial effects were seen even in the patients with weight gain or non-responders with regard to glycemic control.
Conclusion: This study clearly demonstrated the pioglitazone-induced distribution shift in abdominal adipocytes from visceral adipose tissue to subcutaneous adipose tissue. This mechanism may explain pioglitazone-induced beneficial effects both on glucose metabolism in type 2 diabetes and atherosclerotic processes.

changes of parameters after taking pioglitazone

	base-line	6months	p
HbA1c(%)	7.4±1.0	6.5±0.6	<0.0001
BW(kg)	62.4±12.5	64.1±13.1	<0.0001
Viceral fat(V)(cm^2)	103.1±47.7	98.2±51.8	N.S.(0.13)
Subcutaneous fat(S)(cm^2)	148.0±85.2	166.0±99.1	<0.0001
V/S ratio	0.80±0.41	0.68±0.32	0.0001
ANP(pg/ml)	16.2±8.1	22.5±11.8	<0.0001
BNP(pg/ml)	17.5±13.6	19.6±16.9	NS(0.13)
ADP(μg/ml)	10.6±7.9	17.9±13.6	<0.0001
hCRP(mg/dl)	0.091±0.096	0.069±0.096	NS(0.22)

865

The effect of rosiglitazone on fasting plasma retinol binding protein-4(RBP-4) in patients with type 2 diabetes
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Background and aims: Retinol binding protein 4 (RBP4) has recently reported as an adipocyte-secreted molecule that is highly expressed in the adipose tissue of the adipocyte-specific glucose transporter 4 (GLUT4) knockout mice. Transgenic expression or injection of recombinant RBP4 induces insulin resistance, whereas experimentally decreased RBP4 levels ameliorates insulin resistance. In mice lacking GLUT4, rosiglitazone is found to decrease circulating RBP4 levels and to reduce insulin resistance. But the role of RBP4 in human is controversial. In this study, we have investigated whether or not plasma RBP4 levels were different in patients with newly diagnosed type 2 diabetes mellitus and type 2 diabetes mellitus patients with poor glycemic control, and also observed the effects of rosiglitazone treatment on RBP-4 levels in poor glycemic control patients with type 2 diabetes.
Materials and methods: 34 type 2 diabetic patients with poor glycemic control on top of ongoing metformin therapy (11 men, 23 women, age 57±1.5 years, T2DM group) and 30 sex-, age-and BMI-matched patients with newly diagnosed T2DM (12 men, 18 women, age 59±1.2 years, nT2DM group) participated the study. T2DM group patients were treated with rosiglitazone (4mg/d) for 12 weeks. The body composition and metabolic parameters were assesses. Blood samples were drawn after an overnight fast and plasma insulin, FFA, HbA1c, TG, TC, LDL-C, HDL-C were measured. Plasma RBP-4 levels were measured with a radioimmunoassay. The homeostasis model assessment of insulin resistance (HOMAIR) and the homeostasis model assessment of β-cell insulin secretion (HOMAIS) were calculated. The relationship between plasma RBP-4 levels and metabolic parameters was also analyzed.
Results: The plasma RBP-4 levels were found to be markedly increased in type 2 diabetes mellitus patients with poor glycemic control compared with newly diagnosed T2DM patients (P<0.01). The addition of rosiglitazone markedly decreased free fatty acids, HbA1c, fasting plasma glucose, 2h postprandial blood glucose, HOMAIR (all P <0.01) and fasting insulin (P<0.05), but there were no statistical differences in plasma RBP-4 levels between pre- and post-treatment with rosiglitazone (P>0.05). Plasma RBP-4 levels were correlated with sex (r=0.292, P=0.019), triglyceride (r=0.287, P=0.021) and 2h plasma insulin after glucose overload (2hPIns) (r=0.329, P=0.008). Multiple regression analysis showed that only 2hPIns was independent related factor with plasma RBP-4 levels.
Conclusion: The present work indicates that short-term treatment with rosiglitazone can not reduce the fasting plasma RBP-4 levels in patients with type 2 diabetes. Plasma RBP-4 is unlikely to be a useful biomarker of insulin resistance and type 2 diabetes.
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866

Effect of pioglitazone and metformin fixed-dose combination on hs-CRP and adiponectin in patients with type 2 diabetes
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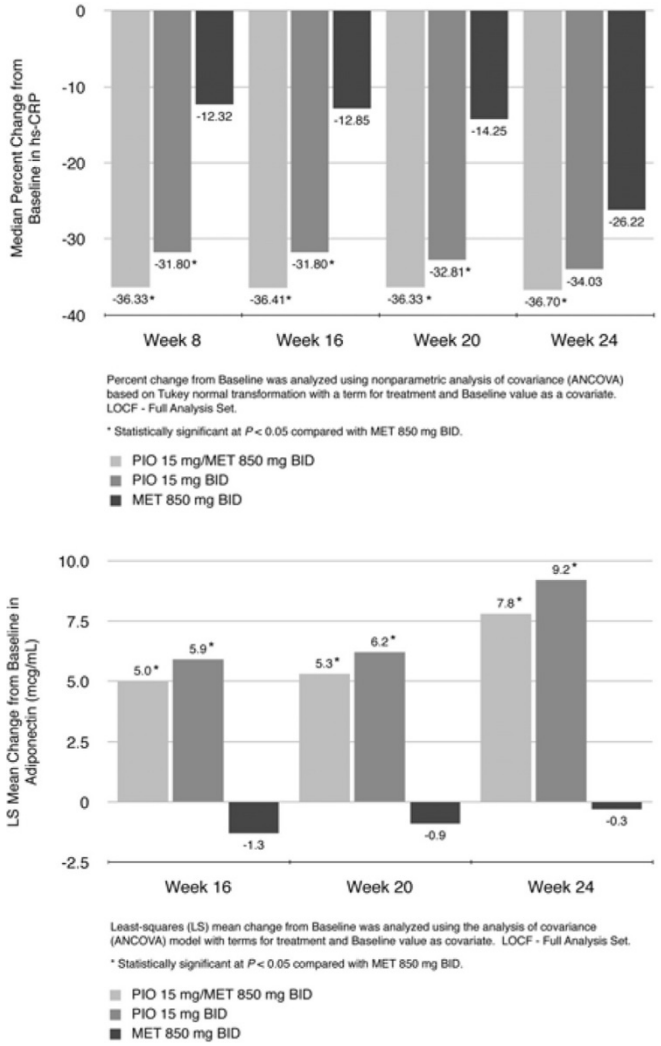
Background and aims: Patients with type 2 diabetes mellitus (T2DM) have evidence of low-grade systemic inflammation, with increased levels of pro-inflammatory markers such as high sensitive C-reactive protein (hs-CRP) and decreased levels of anti-inflammatory molecule adiponectin, which may contribute to their risk for cardiovascular disease. Pioglitazone (PIO) is proven to improve blood sugar control through increased insulin sensitivity.
Materials and methods: This 24-week, double-blind clinical trial compared efficacy and safety of fixed-dose combination (FDC) of pioglitazone/metformin (PIO/MET) (N=201) with PIO (N=189) or MET (N=210) monotherapy in T2DM patients who had stable HbA1c for 3 months and were on no antidiabetes medications. The primary endpoint was change from Baseline in hemoglobin A1c (A1c). Changes in adiponectin and hs-CRP were evaluated as secondary endpoints.
Results: Median Baseline hs-CRP ranged from 2.89 to 3.55 mg/L across groups and decreased by Week 8 through Week 24 in all treatment groups (Figure top). Statistically significant decreases were observed in both PIO/MET FDC and PIO relative to MET at Weeks 8, 16, and 20. A significant increase in adiponectin was observed in both PIO/MET FDC and PIO relative to MET at Weeks 16, 20, and 24 (Figure bottom), with the increase due to PIO alone. Change in levels of adiponectin at the final week in PIO/MET FDC and PIO were 7.8 and 9.2 mcg/mL, in contrast with MET treatment (-0.3 mcg/mL). Analysis of treatment-emergent adverse events showed similar results among all 3 study groups, with 50.7% for PIO/MET FDC and 52.1% and 53.1% for PIO and MET monotherapy, respectively.
Conclusion: PIO/MET FDC improved levels of both hs-CRP and adiponectin, with the major contribution due to PIO.



Supported by: Takeda Global Research & Developoment Center, Inc.

867

Aleglitazar, a balanced dual PPAR α/γ activator, exerts potent insulin-sensitizing and glucose-lowering effects in a nonhuman primate model of metabolic syndrome and type 2 diabetes mellitus

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Background and aims: An ideal peroxisome proliferator activated receptor (PPAR) agonist should provide a therapeutic dose that can achieve good glycemic control and an improved lipid profile while being well tolerated. Aleglitazar is a balanced agonist for PPARα and PPARγ, designed to optimize glycemic and lipid benefits, and minimize PPAR-related adverse effects, weight gain and peripheral edema. Competitive binding assays indicate that aleglitazar is a potent high affinity ligand for both PPARα and PPARγ, which may distinguish it from previously investigated dual PPAR agonists. Under natural and optimal "healthy" diet conditions nonhuman primates frequently develop the metabolic syndrome, and progress to prediabetes and overt type 2 diabetes mellitus (T2DM) with features identical to those of human obesity-associated middle-age onset T2DM. The aim of this study was to evaluate the effect of aleglitazar on insulin sensitivity and glucose homeostasis in obese, prediabetic rhesus monkeys.
Materials and methods: Following pharmacokinetic assessments with a single dose of 0.1 mg/kg aleglitazar and a 4-day washout, 6 insulin-resistant obese monkeys entered a 28-day baseline phase during which they received vehicle (piece of fruit). The monkeys were then treated with aleglitazar (0.03 mg/kg/day) given orally for 42 days. Glucose, HbA1c, insulin, adiponectin (a surrogate marker of insulin sensitivity), triglycerides in circulation, and serum chemistry were measured prior to and at the end of the study.
Results: During euglycemic hyperinsulinemic clamp after insulin-stimulating conditions, glucose infusion rate increased by 60% from a mean of 7.8 mg/fat free mass/min at basal/vehicle conditions to 12.5 mg/fat free mass/min following administration of aleglitazar 0.03 mg/kg/day (p=0.001). This significant improvement in insulin sensitivity was in accordance with an increase in plasma adiponectin levels (from 12.8 to 33.2 μg/mL; p=0.003) and reductions in fasting plasma glucose and fasting insulin. Fasting plasma glucose was reduced by 17% in the whole group from 86.3 to 71.8 mg/dL (p=0.119), and by 30% in a hyperglycemic subgroup (n=3; from 102 to 71.5 mg/dL). Normoglycemia in rhesus monkeys is 65-75 mg/dL following an overnight fast. Fasting insulin was reduced by 61% from 146 to 56.4 μU/mL (p=0.036). HbA1c was also reduced by 13% from 6.7% to 5.8% (p=0.078). In addition, fasting triglyceride levels in circulation decreased by 88% from 392.7 to 48.1 mg/dL (p=0.099). Interestingly, body weight was significantly reduced from 20.5 to 19.5 kg (mean weight loss 4.8%; p=0.040). Biochemistry and hematology profiles showed no concerns about safety, and there were no signs of peripheral edema.
Conclusion: In prediabetic, minimally hyperglycemic monkeys, the balanced dual PPARα/γ agonist aleglitazar mitigated the metabolic imbalance, in particular substantive and significant improvement in insulin sensitivity and reductions in fasting plasma glucose and HbA1c. Given the close accordance between this model and the diabetic state in humans, these data indicate that aleglitazar may have an important role in the reduction of glycemic and cardiovascular risk factors in humans.
Supported by: F. Hoffmann-La Roche AG

PS 72 New therapeutic targets and therapies

868

WISP2, a novel wnt-related adipokine, is a PPARgamma antagonist and promotes inflammation

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Background and aims: Our recent gene expression analysis showed that the expression of the wnt-related molecule, WISP-2, is upregulated in the adipose tissue in obesity characterized by enlarged fat cells. Furthermore, WISP-2 expression correlated with different markers of inflammation and markers of macrophage infiltration. WISP-2 is a secreted protein and, hence, may exert autocrine, paracrine and possibly endocrine actions. Therefore, the purpose of this study was to characterize the possible role of this protein in pathogenesis of adipose tissue inflammation.
Materials and methods: Several cell types including 3T3 L1 preadipocytes, THP-1 cells (a human monocyte cell line), HUVECs (a human umbilical vein endothelial cell line), primary human macrophages and primary human preadipocytes were served as model systems for our investigation. To study the function of WISP-2, we used recombinant WISP-2 protein, and DNA constructs for overexpression or knockdown of the WISP-2 gene. The gene expression was analyzed with real-time RT-PCR (TaqMan). The secretion of cytokines were measured by multiplex electrochemiluminescence using the Meso Scale Discovery technology. Western blot analysis, immunoprecipitations and confocal image analysis are the other methods used in this study.
Results: Interestingly, our *in vitro* studies have shown that WISP-2 is secreted from preadipocytes and has pro-mitogenic, pro-inflammatory and anti-adipogenic properties in these cells consistent with its PPARgamma-inhibitory effect. Remarkably, we have found that conditional shRNA-mediated knockdown of WISP-2 gene in 3T3 L1 preadipocytes induces spontaneous adipocyte differentiation and the induction of several adipogenic markers like aP2, adiponectin and GLUT-4. The pro-inflammatory effect of WISP-2 in preadipocytes also raised the question whether this protein affects macrophage recruitment to the adipose tissue. WISP-2 increased THP-1 cell proliferation as well as their binding to HUVECs. WISP-2 also induced proliferation and secretion of inflammatory cytokines in fully differentiated human primary macrophages. Furthermore, exposure to WISP-2 antagonized the action of rosiglitazone, a PPARγ ligand, and primed human macrophages into the M1 phenotype with marked pro-inflammatory properties.
Conclusion: The wnt-related protein WISP-2 is a novel adipokine which is overexpressed in obesity and has profound effects on preadipocyte differentiation and inflammation. WISP-2 may play an important role for the adipose tissue dysfunction seen in obesity characterized by insulin resistance and inflammation.
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869

DSP-7238, a novel dipeptidyl peptidase (DPP) IV inhibitor, has a superior selectivity and longer duration of action compared with other DPP IV inhibitors

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Background and aims: DSP-7238 is a novel non-cyanopyrrolidine dipeptidyl peptidase (DPP) IV inhibitor currently under clinical development for the treatment of type 2 diabetes. The aims of studies were to investigate DSP-7238 on the enzyme inhibition profiles *in vitro* and to evaluate efficacy on glucose tolerance in Zucker fatty (ZF) rats, pharmacokinetic (PK) and pharmacodynamic (PD) properties in comparison with other DPP IV inhibitors. Moreover, excretion of DSP-7238 was investigated in rats and monkeys.
Materials and methods: The inhibitory potency and property was evaluated using plasma and recombinant enzyme including DPP IV, DPP II, DPP8, DPP9 and fibroblast activation protein (FAP) α with fluorogenic substrates. Substrate selectivity of each DPP IV inhibitor was evaluated by mass spectrometer with the changes in molecular weight of peptide substrates by the release of the N-terminal dipeptides. To examine the PK/PD profile, plasma concentration of compound and plasma DPP IV activity was measured for up to 24 hr after single oral administration of each DPP IV inhibitor. Radio activity was measured in urine and feces after oral administration of [^{14}C] DSP-7238 in rats and monkeys to asses the excretion profile.
Results: DSP-7238 and Sitagliptin (Sita) both competitively inhibited recombinant human DPP IV (rhDPP IV) *in vitro* with Ki of 0.60 and 2.1 nM, respectively. Neither vildagliptin (Vilda, Ki, 2.9 nM) nor saxagliptin (Saxa, 0.59 nM) exhibited competitive inhibition of rhDPP IV. DSP-7238 did not inhibit DPP IV-related enzymes including DPP8, DPP9, DPP II and FAPα (IC50, >50 μM for all enzymes), whereas Vilda and Saxa showed inhibitory potential on DPP8 and DPP9 (respective IC_{50} for DPP8 and DPP9, 1.2 and 0.062 μM for Vilda, 0.16 and 0.053 μM for Saxa). Inhibition of GLP-1 degradation by DSP-7238 was apparently more potent than that of IP-10 or SDF-1α degradation. In contrast, Vilda and Saxa showed comparable inhibition for all substrates tested. Following OGTT in ZF rats, DSP-7238 decreased AUC of blood glucose concentration in dose dependent manner with ED_{50} of 0.089 mg/kg, as well as Sita (0.326 mg/kg) and Vilda (0.172 mg/kg). Inhibition of plasma DPP IV activity was demonstrated with a lower dose of a DSP-7238 single administration at 1 mg/kg, compared with Sita and Vilda (10 and 30 mg/kg, respectively) to produce 80% inhibition over 8 hr. In cynomolgus monkeys, a single oral administration of DSP-7238 exhibited more prolonged plasma DPP IV inhibition and sustained PK profile compared with Sita. Following an oral administration of [^{14}C] DSP-7238, the cumulative urinary excretion of unchanged DSP-7238 up to 48 hr postdose was approximately 5% in rats and monkeys. As a primary elimination pathway is not associated with renal excretions, it is possible that a dose adjustment for the renal insufficiency is not required for DSP-7238, as cautioned for Sita.
Conclusion: These data suggest that DSP-7238 is a selective and long-lasting DPP IV inhibitor without potential concerns on adverse reactions caused by inhibitions of DPP8/9 or chemokine degradation. DSP-7238 can be used as once-daily doses for type 2 diabetes including patients with renal insufficiency.

870

Silibinin fully reverses insulin resistance in high fructose fed-rats through a possible inhibition of glucose-6-phosphatase and pyruvate kinase metabolic pathways

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Background and aims: Fructose-enriched diet causes insulin resistance (IR) and disturbances in glucose metabolism in Wistar rats. The natural antioxidant silibinin (SB) has recently displayed important properties to be used in the treatment of type 2 diabetes. Previous studies *in vitro* of our group have shown that SB inhibits hepatic gluconeogenesis and glycolysis in perifused rat hepatocytes by an inhibition of both glucose-6-phosphatase and pyruvate kinase enzymes. The aim of this study was to explore the capacity *in vivo* of silibinin to reverse the alterations of glucose metabolism related to IR induced in high fructose-fed rats, as well as the underlying mechanisms.
Materials and methods: Male Wistar rats were divided into two batches. One batch received a standard diet, and the other received a fructose-enriched diet. After 4 weeks, each batch of rats was subdivided into two groups. One group received SB (50 mg/kg/day, i.p. injection) for additional two weeks, while the other received SB vehicle. Hepatocytes were isolated from starved rats according to the method of Berry and Friend. They were perifused at 37°C with Krebs-bicarbonate-calcium saturated with O_2/CO_2, then titrated with increasing substrate concentrations of dihydroxyacetone (DHA). We measured glucose, pyruvate and lactate concentrations in the cellular perifusate. Dihydroxyacetone phosphate (DHAP), glucose-6-phosphate (G6P), fructose-6-phosphate (F6P), 3-phosphoglycerate (3PG) and phosphoenolpyruvate (PEP) concentrations were measured fluorimetrically in the cellular fraction.
Results: In perifused hepatocytes, fructose diet increased both gluconeogenesis ($J_{Glucose}$) and glycolysis (J_{L+P}) as compared with control, by 25% and 22% respectively (DHA 2.4 mM, $p<0.001$). Administration of SB fully reversed these metabolic alterations in fructose fed rats to normal values (DHA 2.4 mM, $p<0.001$). Also, fructose-enriched diet changed the relationship between $J_{Glucose}$ and DHAP, G6P or F6P as compared with control. Dietary treatment

increased DHAP, F6P and G6P concentrations at each steady state, giving a double relationship between $J_{Glucose}$ and DHAP, G6P and F6P. SB reversed this effect and normalized the relationship between $J_{Glucose}$ and DHAP, G6P or F6P. On the other hand, fructose-treated rats showed an increase of PEP concentration, leading to a double relationship between J_{L+P} and DHAP or PEP compared with control rats. SB also prevented this alteration.
Conclusion: SB reverses metabolic alterations proper to insulin resistance in rats by a possible inhibition of glucose-6-phosphatase and pyruvate kinase enzymes. We suggest that SB could be beneficial as complementary therapy for the treatment of type 2 diabetes.

871

Impact of INT131, a selective PPARγ modulator (SPPARM), on glycaemic control in patients with type 2 diabetes in a 24 week phase 2b Study (INT131-007)

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Background and aims: Insulin resistance is a key etiologic factor in Type 2 Diabetes Mellitus (T2DM). INT131 is a novel, potent, non-TZD selective PPARγ modulator (SPARRM) designed to improve insulin sensitivity and glucose metabolism while minimizing the typical side effects of the full PPARγ agonists: excessive weight gain, fluid retention, and CHF. In rodent models of T2DM, INT131 demonstrated similar insulin sensitizing and glucose lowering activity as full agonist TZDs with little or no side effects. Further, INT131 demonstrated no significant weight gain, edema, cardiac hypertrophy, or adipocyte replacement of bone marrow in 6 month safety studies in rodent and monkey. A 4 week phase 2a study of INT131 in patients with T2DM on no medications for their diabetes has previously demonstrated potent insulin sensitizing activity and glucose lowering with 1 and 10 mg INT131 QD. The magnitude of glucose lowering of 1 mg INT131 in that study was consistent with modeled glucose lowering of 45 mg QD pioglitazone, but without significant TZD side effects. Based on these data, a 24 week head-to-head dose-finding clinical study of INT131 compared to 45 mg pioglitazone and placebo was carried out.
Materials and methods: INT131-007 is a 24 week double blind, placebo and active controlled phase 2b study with either INT131 (0.5. 1, 2, or 3 mg QD), pioglitazone 45 mg QD, or placebo in patients with inadequately controlled T2DM (HbA1c 7.5-10.0) on a stable dose of sulfonylurea with or without metformin. Enrollment across the US and Mexico of 372 patients was completed on March 10, 2009. Patients underwent 2 screening visits separated by 1 week, followed by a 1 week lead-in period with home glucose monitoring. A glucose rescue policy focusing on FPG and HbA1c was monitored throughout the 24 week study.
Results: Key baseline characteristics (± SD) for the INT131-007 study patients include: age 55.9 ± 9.5 yrs, duration of diabetes 8.4 ± 6.1 yrs, 47% women, BMI 32.0 ± 5.5, FPG 9.59 ± 2.30 mmol/l (172.6 ± 41.4 mg/dl), and HbA1c 8.32 ± 0.72%. The objective of the study is to assess change from baseline in glycemic control (FPG and HbA1c) after 24 weeks of INT131 (0.5. 1, 2, and 3 mg QD), or full dose (45mg QD) pioglitazone or placebo.
Conclusion: Available data support the potential of INT131 to function as a SPPARM to provide potent insulin sensitization and glucose reduction in patients with poorly controlled T2DM separate from the undue effects of full-agonist TZDs such as weight gain and fluid retention. The design of the trial, baseline demographics, adverse events, and key efficacy assessments will be presented.

872

ARRY-403, a glucokinase activator with potent glucose-dependent anti-hyperglycaemic activity in animal models of type 2 diabetes mellitus

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Background and aims: Glucokinase (GK) is the rate-limiting, glucose-sensing enzyme in several key tissues involved in glucose homeostasis. GK stimulates insulin release in pancreas in a glucose-dependent manner, regulates glucose utilization and production in hepatocytes. We sought to utilize structure-based drug design to identify a potent, efficacious and safe GKA, and to further characterize that molecule.
Materials and methods: ARRY-403 was tested in a variety of *in vitro* assays (EC_{50}, $S_{0.5}$, predicted metabolism, permeability, physiochemical determinations, broad selectivity panels for kinases, receptors, ion channels) and orally in normal and diabetic animal models (C57Bl/6J, *ob/ob*, ZDF and NONcNZO10/LtJ) as monotherapy and in combination with standard of care therapies. Nonfasted glucose, and $AUC_{glucose}$ in OGTT studies, were monitored.
Results: Through iterative optimization, we identified ARRY-403 for advanced preclinical evaluation. ARRY-403 potently activates human GK *in vitro* (EC_{50} = 79 nM at 5 mM glucose), with an $S_{0.5}$ = 0.93 mM glucose (ARRY-403 at 5 μM) and V_{max} = 134% compared to the no activator control. It possesses good *in vitro* properties (aqueous solubility, cell permeability, low potential for drug-drug interactions, low predicted hepatic clearance), and selectivity against broad panels of receptors and enzymes. PK analyses (1 mg/kg IV and 10 mg/kg PO) showed low IV clearance and high oral exposure in a number of species. ARRY-403 elicited glucose-dependent glucose-lowering activity in an acute OGTT study in C57Bl/6J mice (dosed 3, 10, and 30 mg/kg, with AUC reductions of 15, 32, and 37% respectively, vs. 10% for sitagliptin dosed 30 mg/kg PO), with no safety issues. In a 28-day study in *ob/ob* mice, dose-dependent glucose lowering in an OGTT was demonstrated (reductions of 30, 49, and 62% compared to vehicle at 3, 10 and 30 mg/kg, respectively, on day 28). ARRY-403 treatment resulted in fasted and nonfasted blood glucose levels comparable to those of normal mice. A 14 day study in NONcNZO10/LtJ mice dosed at 10 mg/kg resulted in a 56% AUC decrease from control and was as efficacious as metformin (100 mg/kg) or sitagliptin (30 mg/kg). Combination therapy of ARRY-403 with metformin, sitagliptin or pioglitazone in diabetic high fat-fed ZDF female rats resulted in greater efficacy than the comparator compounds alone (fasting, non-fasting, and postprandial blood glucose endpoints).
Conclusion: Based on these data, ARRY-403 shows significant promise as a therapeutic agent for Type 2 diabetes, and has advanced into Phase 1 clinical testing.

873

BI 10773, a novel and selective SGLT2 inhibitor, lowers blood glucose and improves glycaemic control in diabetic rodent models

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Background and aims: BI 10773 is a novel potent and selective inhibitor of sodium-dependent glucose cotransporter 2 (SGLT2). SGLT2 inhibitors are a new and promising class of oral antidiabetic agents, which increase urinary glucose excretion. BI 10773 is currently under clinical development for the treatment of type 2 diabetes. Here, we show the *in vitro* potency and selectivity of BI 10773 together with the *in vivo* efficacy in comparison to remogliflozin etabonate, another SGLT2 inhibitor from a different structural class being in clinical development by GSK.
Materials and methods: described under results.
Results: BI 10773 has high *in vitro* potency for inhibiting human SGLT2 (IC_{50} 3.1±0.7 nM). The selectivity of BI 10773 for SGLT2 over SGLT1, SGLT4 and SGLT5 is >2500-fold, >3500-fold and >250-fold, respectively, whereas remogliflozin is less potent (IC_{50} 12±4 nM) and less selective (SGLT1 (>650-fold), SGLT4 (>125-fold) and SGLT5 (>40-fold)). Both compounds induced urinary glucose excretion (UGE) and reduced blood glucose in *db/db* mice and ZDF rats. While a single oral dose of 1 mg/kg of each compound led to a similar increase of UGE within the first three hours post application in both animal models, the UGE achieved with BI 10773 over 24 hours was significantly higher than that achieved with remogliflozin etabonate (*db/db* mice 24 h UGE: BI 10773: 17.7 mmol glucose/ kg body weight vs. remogliflozin etabonate: 9.2 mmol glucose/ kg body weight), due to a longer duration of action of BI 10773. Furthermore, blood glucose was lowered in *db/db* mice and ZDF rats after single oral dosing of BI 10773 for more than 7 h. In ZDF rats, blood glucose 7 h post dose was still significantly lowered by 36% with 3 mg/kg BI 10773, while remogliflozin etabonate at the same dose was ineffective 7 h after administration. In an OGTT in *db/db* mice, performed with 1 mg/kg of both compounds, remogliflozin etabonate did not reduce glucose excursion. In contrast, BI 10773 reduced glucose AUC by 35%. Multiple once daily oral dosing of ZDF rats with BI 10773 achieved a reduction of blood

glucose under fed and fasting conditions. After 5 weeks of treatment, HbA1c was also decreased vs. vehicle treated animals by 1.5% and 2.2% with 1 mg/kg and 3 mg/kg BI 10773, respectively. Thus, the glucose lowering achieved with the SGLT2 inhibitor BI 10773 after single dosing is reflected in an improved glycemic control after multiple dosing.
Conclusion: In conclusion, our data show that our SGLT2 inhibitor BI 10773 has a favourable potency and selectivity profile. In comparison to remogliflozin etabonate, BI 10773 has a longer duration of action in rodents *in vivo*, which most likely reflects a superior pharmacokinetic profile. Our preclinical data show that BI 10773 is a promising drug candidate for the treatment of type 2 diabetes.

874

The fructose-1,6-bisphosphatase inhibitor CS-917 generates an N-acetylated metabolite that alters cellular lactate homeostasis
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Background and aims: CS-917 is the 1st member of a new class of antidiabetic agents that reduces the overproduction of glucose by gluconeogenesis, an abnormality which is commonly observed in patients with T2DM. Following its absorption, CS-917 undergoes conversion to MB05032, a potent inhibitor of fructose-1,6-bisphosphatase (FBPase; IC_{50} 16 nM). In humans, MB05032 is extensively metabolized by N-acetylase-1 to MB05099, a long-lived metabolite ($t_{1/2}$ ~30 h) that is not an inhibitor of FBPase. MB05099 achieves high, variable plasma levels (~3-20 uM) that exceed those of MB05032 at steady state. To better understand the clinical profile of patients treated with CS-917, the metabolic effects of MB05032 and MB05099 were studied in cells. Metformin, a mild mitochondrial respiratory chain inhibitor that is known to affect cellular lactate homeostasis, was included in the assays.
Materials and methods: Cultures of human hepatocytes, rat hepatocytes, and HepG2 cells were exposed to MB05032 or MB05099 (3-100 μM), a prodrug of MB05099 (10-100 μM), and/or metformin (30-3000 μM) for up to 24 h. LAC, pyruvate (PYR), and LAC dehydrogenase (LDH) were measured by enzyme-coupled spectrophotometric assays. Redox potential and ATP content of cells were analyzed by a dye (MTT) reduction assay and a luciferase-based assay, respectively. Respiration was measured with a Clark-type O_2 electrode. Drug uptake was assessed by HPLC.
Results: Exposure of rat hepatocytes to MB05099 or metformin, but not MB05032, increased the intracellular LAC/PYR ratio in a concentration-dependent manner by up to 2-fold, indicating a reduction in oxidative capacity. Further, overnight exposure of human hepatocytes to MB05099 resulted in a concentration-dependent increase in the LAC/PYR ratio (up to 3-fold) as well as an increase in extracellular LAC levels (from 0.5 up to 1.0 mM). Treatment with MB05032 also increased lactate release by human hepatocytes, but had no effect on the LAC/PYR ratio. MB05099, which is an anion at physiological pH, did not penetrate HepG2 cells and was pharmacologically inactive in this cultured cell line. However, 6- to 24-h exposure to the prodrug of MB05099 (30 μM) yielded high intracellular levels of MB05099 and resulted in reduced ATP content (55%), lowered redox potential (74%), and increased LAC levels in the growth medium (by ~100 μM). At 100 μM, the prodrug increased LDH release and reduced LAC release by HepG2 cells, which likely reflects loss of function. Consistent with the effects on cellular redox state, MB05099 reduced glucose-driven respiration by rat hepatocytes and succinate-driven respiration by rat liver mitochondria with ~12% and ~15% inhibition, respectively, at 30 μM.
Conclusion: The current studies suggest CS-917 treatment may not only reduce lactate clearance by inhibition of gluconeogenesis (via generation of the active metabolite, MB05032), but also by reducing mitochondrial oxidation (via generation of the N-acetylated metabolite, MB05099). The mitochondrial effects may predispose patients to disturbances of acid-base balance as has been observed with other drugs that affect mitochondrial function. The current findings at clinically relevant concentrations of MB05099 may have safety implications for 2nd generation FBPase inhibitor MB07803, which generates an N-acetylated metabolite with similar properties to MB05099 but at >100-fold reduced levels in humans relative to CS-917.

875

Pronounced and rapid glucose lowering with the fructose-1,6-bisphosphatase (FBPase) inhibitor MB07803 in poorly controlled type 2 diabetes mellitus (T2DM) subjects
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Background and aims: MB07803, an oral prodrug of a potent, selective FBPase inhibitor, was studied in T2DM subjects to determine safety, tolerability, pharmacokinetics, and glucose lowering response.
Methods: A randomized, double-blind, placebo (PBO)-controlled, ascending dose (50, 200, 400 mg Q12h), 14-day domiciled study was conducted in 42 noninsulin-requiring T2DM subjects (mean baseline FPG=221 mg/dL, HbA1c=8.8%; T2DM duration=7.l years) after a 2-week wash off of antidiabetic therapy. Change from baseline in glycemic endpoints was analyzed.
Results: Plasma MB07803 active metabolite exposure, assessed by AUC(0-tau)ss, increased proportionally from 50 to 200 mg Q12h dose, but not from 200 to 400 mg Q12h dose. Dose-related glucose lowering was observed.

Change from Baseline - Glucose Parameter

MB07803 Treatment Group (Q12h)	PBO-Adjusted LSM	PBO-Adjusted 95% CI	P-value vs. Placebo
FPG AUC 0-6 hrs (mg•hr/dL) Day 13/14			
50 mg	-143	-351.7, 65.7	0.1726
200 mg	-349.4	-557.1, -141.8	0.0017
400 mg	-439.4	-656, -222.8	0.0002
FPG (mg/dL) Day 15			
50 mg	-16.4	-63, 30.1	0.4791
200 mg	-58.2	-104, -12.4	0.0142
400 mg	-55.1	-104.6, -5.6	0.0302
24h Glucose AUC (mg•hr/dL) Day 13/14			
50 mg	-763.5	-1503.5, -23.5	0.0435
200 mg	-1185.7	-1926.8, -444.6	0.0026
400 mg	-1507.9	-2278.5, -737.3	0.0004
24h Weighted Mean Glucose (mg/dL) Day 13/14			
50 mg	-30.4	-61.3, 0.5	0.0537
200 mg	-49.8	-80.7, -19	0.0024
400 mg	-62.2	-94.3, -30.1	0.0004

In the 400 mg group, 5 of 10 subjects manifested vomiting. Of these 5 subjects, 4 were down-dosed to 200 mg Q12h and completed the trial. In the 200 mg group, 4 subjects experienced at least 1 episode of mild nausea but all completed the study. In the 50 mg group, no patient experienced nausea or vomiting and the group's overall adverse event profile was similar to PBO. Neither lactic acidosis nor consecutive lactate > 4.5 mM occurred. Nonconsecutive, asymptomatic lactate > 4.5 mM occurred in 1 subject in association with glucose <60 mg/dL and symptoms of hypoglycemia during the last 2h of an 18h fast. 4 other subjects had asymptomatic glucose <60 mg/dL while fasting at least 12h.
Conclusion: MB07803 was safe at all doses and well-tolerated to a dose of 200 mg Q12h. All doses demonstrated statistically and clinically significant effects on 24h glycemic measures. The 200 and 400 mg doses also showed statistically and clinically significant reductions on FPG AUC 0-6 hrs and FPG. These results suggest that FBPase inhibitors are capable of pronounced and rapid reduction of fasting and postprandial glycemia in patients with well-established and poorly-controlled T2DM.

876

The glucokinase (GK) activator mk-0599 lowers plasma glucose concentrations in healthy non-diabetic subjects

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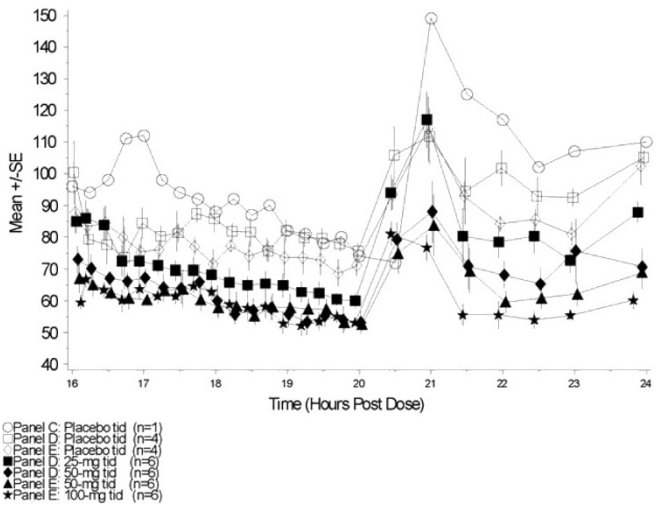
Background and aims: Glucokinase (GK) activators are expected to be associated with a dual mechanism for lowering blood glucose concentrations - enhancement of glucose uptake, metabolism and glycogen synthesis in the liver and potentiation of glucose-dependent insulin secretion from pancreatic β-cells. MK-0599 is an orally active, potent and specific allosteric activator of GK. A double-blind, randomized, placebo-controlled, sequential panel study was conducted to assess the safety, tolerability, pharmacokinetics and glucose-lowering effects of MK-0599 in healthy non-diabetic adult male subjects.
Materials and methods: Separate study panels consisting of 8 subjects each were randomized to receive MK-0599 or matching placebo in a 3:1 ratio, respectively, in up to 5 treatment periods. Study drug was administered after an overnight fast and blood samples for determination of plasma glucose concentrations (glucometer measurements) were taken as frequently as every 5 to 15 minutes for up to 24 hours postdose. Single 0.4- to 160-mg doses and 13- to 100-mg doses given 3 times daily (TID) were administered to study participants.
Results: The pharmacokinetics of MK-0599 were less than dose-proportional after administration of single doses up to 160 mg, whereas the AUC was increased by approximately 3-fold with TID administration compared with a similar dosage given as a single administration. After administration of 50 mg TID or more, clinically significant (compared with placebo) glucose-lowering effects were observed after administration of the last dose (16 to 24 hours after administration of Dose #1; figure).
Conclusion: Hence, GK activators lower plasma glucose concentrations in healthy non-diabetic (normoglycemic) subjects and are considered to be a potentially novel treatment for patients with type 2 diabetes.



877

Colesevelam improves insulin resistance in diet-induced obesity (F-DIO) rat models by increasing release of GLP-1

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Background and aims: Bile acid sequestrants have been shown to improve glycemic control in humans. This study investigated how colesevelam improves glucose levels.
Materials and methods: Studies were carried out in an insulin-resistant rat model: the high energy chow-fed F-DIO rat. There were four treatment groups: F-DIO rats fed regular chow (control); high energy chow-fed F-DIO alone (HE); high energy chow containing 2% colesevelam (WC); and high energy chow containing 0.02% SC-435 (SC). Colesevelam HCl is a bile acid sequestrant and SC-435 is an ASBT inhibitor which also reduces intestinal bile acid absorption. After 4 and 8 wks of treatment, oral glucose tolerance test (OGTT) was performed and GLP-1 levels were assessed.
Results: In both the HE and SC groups, plasma glucose and insulin levels remained significantly higher ($p<0.001$) than the baseline throughout the OGTT. In contrast, insulin in the WC group reached peak level in 15-30 min, and then returned to baseline within 30-60 min. This pattern was similar to that in the control group. In the WC group, plasma glucose returned to baseline during OGTT and was lower ($p<0.001$) than that in the HE (-23%) or SC (-18%) at the 120 min timepoint. After 4 wks treatment, fasting total GLP-1 values in the WC group were 60% and 65% higher than in the HE and SC groups, respectively. After 8 wks of treatment, the fasting GLP-1 levels were 100% higher ($p<0.05$) with the WC compared to the HE group. During OGTT, the plasma GLP-1 levels in WC treated rats was 100% ($p<0.05$), 61% ($p<0.05$), and 97% higher ($p<0.001$) than the HE group at 15, 30 and 60 min, respectively. Concentrations of the potent FXR ligands (chenodeoxycholic, deoxycholic and cholic acids) in the portal blood did not decrease in the HE group but were significantly reduced in both WC (71%, $p<0.001$) and SC (77%, $p<0.001$) groups.
Conclusion: The results from this study suggest that colesevelam reduces plasma glucose levels by improving both insulin-resistance and first phase insulin release in an insulin-resistant rat model. This effect is not due to reduced bile acid flux returned to the liver but may be related to increased fasting and post-prandial GLP-1 secretion. Further studies are warranted to explore how colesevelam results in increased GLP-1 release.
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PS 73 Diabetes in childhood

878

Postprandial glucagon levels associates with glycaemic control in Danish children and adolescents with new onset type 1 diabetes

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Background and aims: Glucagon release is often perturbed in patients with type 1 and type 2 diabetes leading to a state of hyperglucagonaemia. The role of hyperglucagonemia on metabolic control is not fully understood. The aim of this study is to investigate the role of glucagon on the glycaemic control as assessed by stimulated blood glucose, HbA1c and insulindose adjusted HbA1c (IDDA1c) during the first 12 months after diagnosis.
Materials and methods: 130 children and adolescents aged < 17 years from 4 centres in Denmark with newly diagnosed type 1 diabetes were followed for 12 months. A 90-minutes Boost-test (mixed meal) was carried out in each patient at 1, 3, 6, and 12 months after diagnosis to characterise the residual beta cell function (stimulated C-peptide, and rise in post-prandial blood glucose) and stimulated glucagon. Postprandial glucagon, HbA1c and insulin dose adjusted HbA1c were analysed using linear regression in a compound symmetric model over 1, 3, 6 and 12 months. Postprandial blood glucose, gender, age and stimulated C-peptide were included as explanatory factors. The measure for IDAA1c was developed by a multiple regression analysis, where a negative association between stimulated C-peptide (dependent variable) and HbA1c (-0.21, regression coefficient, p<0.001) and insulin dose (U/kg bodyweight) (-0.94, regression coefficient, p<0.001) (independent variables) and was calculated as: IDAA1c = actual HbA1c (%) + [4 x insulin dose (U/Kg/24h)]
Results: The postprandial levels of glucagon were highly associated with the rise in postprandial glucose (p=0,0001). We also observed a significant positive association between stimulated glucagon levels and both HbA1c (est.: 0.18, p=0.02) and IDAA1c (est.: 0.24 and p=0.03). In a separate model we found no significant relationship of glucagon and postprandial blood glucose at 1 month when residual beta cell function is present. However, by 3, 6 and 12 months after diagnosis the rise in postprandial glucose was associated with increased glucagon levels (est.: 2.55, 2.78, 3.08 with corresponding p-values: <0.0001, 0.0191, 0.0016). In the same model postprandial glucagon levels associated significantly with HbA1c at 3 and 6 months (p=0.05 and <0.0001) after diagnosis and with IDDA1c only at 6 months (0.0052).
Conclusion: The rise in blood glucose associates with postprandial glucagon. Our data also suggest that postprandial glucagon associates with worsening of the glycaemic control. Therapies aimed at blocking the effects of glucagon might improve glycaemic control.

879

Diabetic ketoacidosis at diagnosis in Austrian children - a population based analysis 1989-2008

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Background and aims: Diabetic ketoacidosis (DKA) is a life-threatening complication of type 1 diabetes present in 15- 67 % of children at the time of diagnosis. Studies on the prevalence vary widely but most of them are hospital based. The aim of our study was to analyze the prevalence of onset ketoacidosis during a period of 20 years (1989-2008) population based in the whole of Austria.
Materials and methods: In a prospective population based incidence study all newly diagnosed patients with diabetes ≤15 years of age were registered by the Austrian Diabetes Incidence Study Group. The completeness of the case-ascertainment was >93 % with a uniform completeness of ascertainment over time. The registered data set comprises blood glucose, pH, ketonuria and clinical symptoms of DKA at manifestation. DKA was defined as pH < 7.3 and severe DKA as pH < 7.1. Time trends were estimated by linear regression models
Results: During the study period 3331 children < 15 years of age (1797 boys and 1534 girls) were registered with type 1 diabetes in the incidence data base. 1238 (37.2%) presented with DKA, 855 (25.7%) had a mild DKA (pH 7.1) and 383 (11.5%) a severe form (pH<7.1), one patient died at onset. The frequency of DKA was negatively associated with age at onset. In children <2 years the frequency of DKA was 60 % (p < 0.0001). In this age group a trend to more DKA episodes in girls was observed (70% vs. 54%, p <0.05). Mean blood glucose at diagnosis was significantly higher in children presenting with DKA (563.8 ±226.4 mg/dl vs. 443.4 ± 183.3 mg/dl, p < 0.0001). Despite a significant increase in diabetes incidence in Austria during the observation period from 8.4/100 000 to 18.5/100 000 (p < 0.0001) no significant change in the prevalence of DKA at manifestation could be observed within the same time period.
Conclusion: The overall frequency of DKA in children with newly diagnosed type 1 diabetes in Austria is still high and did not change in the last 20 years despite a clear increase in the manifestation rate. Especially young children < 2 years of age have a high risk of DKA at onset. Efforts to reduce the occurrence of onset DKA must be focused upon earlier diagnosis and intervention in newly diagnosed patients.

880

Ketoacidosis at diabetes onset is still frequent in children and adolescents: a multicentre analysis of 14,664 patients from 106 institutions

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Background and aims: We aimed at analyzing the frequency, clinical characteristics, risk factors and trends associated with the occurrence of ketoacidosis at the onset of type 1 diabetes mellitus, on the basis of long-term follow-up data.
Materials and methods: A total of 106 paediatric diabetes centres in Germany and Austria participated in this study. Data was collected between 1995 and 2007 by means of a diabetes software programme for prospective documentation (DPV). The data of 14,664 patients with type 1 diabetes were suitable for evaluation. Ketoacidosis (DKA) was defined and classified according to the ISPAD consensus guidelines: mild DKA, 7.2 ≤ pH < 7.3; moderate DKA, 7.1 ≤ pH < 7.2; severe DKA: pH < 7.1).
Results: DKA (pH values < 7.3) was observed in 21.1% of patients. The frequency of DKA, including the severe form, remained unchanged throughout the 13 years' observation period. The frequency of DKA was particularly striking among children < 5 years of age (26.5%) and in patients with a migration background (27.3%). DKA occurred more frequently among girls (21.8%) than in boys (19.4%).
Conclusion: Ketoacidosis occurring at diabetes onset continues to be a difficult problem. Our data show no significant change in the frequency and magnitude of DKA over the last 13 years.
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881

YKL-40 is elevated in type 1 diabetic children which show components of metabolic syndrome compared to those without, as well as to healthy control subjects

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Background and aims: Despite therapeutical advances in glycemic control in type 1 diabetes mellitus (T1DM) in children and adolescents, the risk for cardiovascular morbidity and mortality before the age of 40, is still increased up to 30-fold compared to non-diabetic controls. The mechanisms behind are still not clear. Recent studies showed, that the protein YKL-40, which is in-

volved in endothelial dysfunction and vascular inflammation, is co-responsible for plaque rupture in cardiovascular disease (CVD). Thus we investigated YKL-40 in T1DM children and adolescents.
Materials and methods: In the current study YKL-40 was measured in 153 children and adolescents with T1DM. As control group (CO) 24 healthy gender-matched subjects from 7 to 19 years were defined. The presence of risk factors for CVD, metabolic syndrome and vascular inflammation was studied. YKL-40 levels were measured using a commercially available ELISA (Quidel Corporation, San Diego, CA). Inter-assay coefficient of variation (CV) was 12.4%, and intra-assay CV was 6.9%. Statistical analyses were done with students' unpaired t-test, ANOVA, correlation analysis and multivariate regression modeling as appropriate.
Results: T1DM children and adolescents showed significantly elevated YKL-40 levels: T1DM: 44.08± 46.04 ng/ml vs. CO: 34.04±11.83 ng/ml; p=0.025. Analysing the T1DM patients responsible for the difference, we discovered a sub-group of patients with high YKL-40 levels. We defined a cut-off limit of mean of the total study population plus one standard deviation; approximately 80 ng/ml. Thus we obtained 138 patients below this limit and 15 above. The T1DM sub-group with YKL-40 > 80 ng/ml showed significant differences (T1DM, YKL-40 80 ng/ml) for systolic blood pressure (BP): 114±15 vs. 122±13 mmHg; p=0.034; for body mass index (BMI): 21.4±4.0 vs. 24.0±3.9 kg/m2; p=0.021; for triglycerides (TG): 108±80 vs. 213±172 mg/dl; p<0.001; for VLDL: 19±11 vs. 28±10 mg/dl; p=0.009; fasting glucose: 155±84 vs. 155±85 mg/dl; p=0.971; for gamma-glutamyl-transferase (GGT): 16±5 vs. 24±12 U/l; p=0.027; C-reactive protein (CRP): 0.3±0.4 vs. 0.5±0.5 mg/dl; p=0.046.
In a next step, univariat regression analysis in the T1DM patients was performed. Univariat significant predictors for YKL-40 (age, diastolic BP, BMI, TG, GGT, CRP) were further analyzed by multivariate regression. GGT (beta=0.379, p<0.001) and TG (beta=0.318, p<0.001) were revealed to be significantly associated with YKL-40. The single most independent predictor was GGT (beta=0.538, p<0.001).
Conclusion: This is the first study which investigated YKL-40 in non-diabetic as well as in T1DM children and adolescents. YKL-40 was significantly higher in T1DM and especially high in those with concomitant cardiovascular risk factors like hypertension, hyperlipidemia, elevated weight, and systemic inflammation, all parameters being involved in an insulin resistance/metabolic syndrome. Recently it was postulated, that the increased CVD risk in T1DM might be associated with an underlying "metabolic" like syndrome. Our finding of elevated YKL-40, which is involved in plaque rupture, might explain how this "metabolic" like syndrome evolves to cardiovascular events.

882

Predictors of recurrent diabetic ketoacidosis in children and adolescents with type 1 diabetes. Experience from a large multicenter data base
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Background and aims: Diabetic ketoacidosis (DKA) remains the leading cause of hospitalisation and death in children and adolescents with established type 1 diabetes despite DKA preventing strategies in diabetes education. Thus the aim of this study was to determine the risk factors for recurrent DKA in a large cohort of children and adolescents with previously diagnosed type 1 diabetes.
Materials and methods: This study is an observational investigation using the DPV (Diabetes-Patienten-Verlaufsbeobachtung) -Wiss data base containing clinical data on 28 770 type 1 diabetic patients < 20 years of age at follow up from Germany and Austria. DKA was defined as pH< 7.3 and/or hospital-admission due to DKA. Data are presented as mean ± SEM of the latest year of therapy if not otherwise stated. DKA at manifestation was not included in the analysis. DKA rate is given as incidence of DKA per 100 patient years. Statistical analyses were performed using Wilcoxon rank sum test and multiple Poisson regression analyses.
Results: Mean age of the study cohort was 13.96 ± 3.97 years (mean ±SD) (47.9% females). 94.12% had no episode of DKA, 4.85% of patients presented with 1 episode and 1.03% of patients with recurrent DKA (≥ 2 episodes). When comparing patients without, with one or recurrent DKA, age at manifestation (p<0.01), HbA1c (p<0.001) and insulin dose (p<0.001) were significantly higher in patients with recurrent DKA. According to multiple Poisson regression incidence of DKA was found to be significantly higher in female patients (females: 7.3 ±0.46 males: 5.81±0.23; p=0.03) and in patients with positive migration background (migration background: 7.81±0.56 vs. no migration background: 6.33±0.27; p=0.02). Children of the age group 10-15 years were at significantly higher risk (<5y.: 3.23 ±0.66, 5-10y.: 3.34 ±0.30, 10-15y.: 8.04 ±0.34; 15-20y.: 6.68 ±0.44; p<0.01) as well as children with longer duration of diabetes (≤ 2 y: 5.69 ±0.49, 2-5 y.: 6.68 ±0.30, 5-10 y.: 6.19 ±0.27; > 10 y.: 7.60±1.20; p<0.05). No significant association was found with the type of treatment (CT: 5.44±0.42; ICT: 7.01±0.35, CSII 5.71±0.33) or centre size (<100 patients: 6.49 ±0.26 vs.> 100 patients: 7.09±0.72).
Conclusion: In a large cohort of European paediatric patients with type 1 diabetes the rate of DKA was found to be significantly higher in females, in children with a positive migration background in early teenage years. Further risk factors were age at onset and duration of diabetes.

883

Rapid GlucoseSpray: an innovative tool to control hypoglycaemia and improve HbA_{1c} in children with type 1 diabetes
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Background and aims: Hypoglycaemia continues to be a composite situation to tackle especially in children up to 5 years of age because of difficulties in administering the correct dose of glucose as well as child compliance. Aim of the this study was to evaluate the effect of administering small amounts of glucose through the Glucose RapidSpray (GRS) during first signs of hypoglycaemia in children affected by type 1 diabetes (T1D) to estimate whether such treatment was able to improve the metabolic control over a period of 9 months follow up. This device consists of a 19 ml bottle containing 10 g glucose solution with the addition of artificial flavours and excipients to facilitate buccal absorption delivering by spray puffing glucose in quantities as small as 0.5 grams.
Materials and methods: We designed an open randomized trial in 27 T1D children up to 5 years of age. They were randomly allocated into two groups: group A) GRS (10 to 20 puffs of GRS, 0.5 g to 1 g glucose) depending on hypoglycaemic symptoms; group B) traditional treatment of hypoglycaemia episodes (sugar, fruit juice, candies, etc). The following factors were evaluated at entry into the trial, at 3 and 6 months follow-up, and at the end of the study (9 months): HbA1c, number and characteristics of hypoglycaemic episodes, compliance to treatment and quality of life of children and their parents.
Results: HbA1c at at entry was 9.4% ± 1.2 in group A and 9.1% ± 0.6 in group B (p NS). After 3 months both groups showed a significant improvement in HbA1c with no differences between groups (group A: 8.7% ± 1, group B: 8.6% ±0.7) which further improved at 6 months (group A: 8.4% ± 0.9, group B: 8.4% ±0.6) and at the end of the study (group A: 7.8% ± 0.5, group B: 8.2% ± 0.3). There was a statistically significant difference in the improvement of the HbA1c in the GRS treated group vs. control group over the period of observation (1.63% vs. 0.86% difference, respectively, p=0.02, paired T test). Moreover, children in group A showed a tendency for less severe hypoglycaemic episodes during the day compared to group B during the whole observational period.
Conclusion: A more acceptable and practical tool to control and manage hypoglycaemia especially in toddlers and young children represents a very relevant therapeutic issue. GRS is able not only to reduce the risk of hypoglycaemia but to improve the overall metabolic control, as shown by a reduction of HbA1c.

884

Phenotypes of prediabetes and non alcoholic fatty liver disease in obese children and adolescents

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Background and aims: Obesity is becoming epidemic especially in the youth. It associates not only with disturbed carbohydrate metabolism but also with non alcoholic fatty liver disease.
Aim of this study was to assess the association between fatty liver, diagnosed by ultrasound, and disordered metabolic status.
Materials and methods: We have studied 541 children (age 8-18 years) with various degrees of obesity (BMI 24.4-62 kg/m2). In all subjects we evaluated metabolic profile, glucose tolerance by OGTT (using a dose of 1.75 g glucose/kg body weight to a maximum of 75 g), presence of fatty liver (FL) by ultrasonography, insulin resistance by HOMA and Matsuda index, beta cell function by the ratio of AUC of insulin to glucose measured every 30min during OGTT.
Results: Children with FL had higher levels of fasting glucose, insulin, triglycerides, ALT, AST, GGT, mean OGTT glucose. Moreover, FL subjects had impaired glucose tolerance (IFG/IGT/DM was present in 5/55/3 and 0/24/2 of children with and without FL, p<0.001), increased insulin resistance (HOMA and Matsuda-index) and slight decreased, although not significant, beta cell function. In a multivariate model, fatty liver was the strongest predictor of 2h and mean OGTT glucose and of reduced insulin sensitivity (Matsuda-index), after correcting for gender, BMI, age and liver enzymes. No significant association was found with altered beta cell function.
Conclusion: Screening for presence of fatty liver by ultrasound can identify young subjects with altered glucose metabolism and be a target for improving metabolic dysfunction.

885

Effect of overweight/obesity on cardiometabolic risk profile in children with type 1 diabetes

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Background and aims: Although cardiometabolic risk factors are abundantly present in overweight children, only few studies have assessed the prevalence of these factors in paediatric cohorts with type 1 diabetes (T1DM). The aim of this study was to determine and compare the prevalence of traditional cardiometabolic risk factors and to assess the effect of overweight/obesity on these risk factors in a cohort of normal and overweight/obese children with T1DM.
Materials and methods: This cross-sectional study included 283 consecutive patients (aged 3-18 years) attending a dedicated out-patient clinic for T1DM care. After stratification for weight status, the prevalence of cardiometabolic risk factors, the metabolic syndrome (according to a modified paediatric definition), and high alanine aminotransferease (ALT; a marker of liver steatosis), were assessed.
Results: Out of 283 children (median age 12.8 years, interquartile range [IQR] 9.9-16.0, median diabetes duration 5.3 years, IQR 2.9-8.6), 67.5% was aged ≥11 years, and 38.5% were overweight/obese (Z-BMI ≥1.1). Median HbA1C levels were 8.2%, IQR 7.4-9.8, and HbA1C ≥7.5% was present in 73.9%. Microalbuminuria was found in 17.7%, high triglycerides in 17.3%, high LDL-cholesterol in 28.6%, low HDL-cholesterol in 21.2%, and high blood pressure in 13.1% of the children. The vast majority had a positive family history for either type 2 diabetes or cardiovascular disease (63.3%), while smoking was only reported by 3.9%. In total, 95.1% of children had one or more of the nine forementioned cardiometabolic risk factors, 31.4% of children showed exactly two risk factors, and the percentage of children with more than three risk factors was still 20.8%. A higher prevalence of the metabolic syndrome (7.3% vs. 1.7%), ALT >30 IU/L (15.6% vs. 4.5%) and high blood pressure (22.9% vs. 5.7%) were found in overweight/obese as compared to normal-weight children (all $P<0.05$).
Conclusion: Cardiometabolic risk factors were frequently present a paediatric cohort of children with T1DM, however, only hypertension, the metabolic syndrome and ALT >30 IU/L were more abundant in overweight/obese compared to normal weight children.

886

Calcitriol administration in the youth reduces bone turnover and may prevent bone loss in type 1 diabetes

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Background and aims: In patients affected with type 1 diabetes (T1D), some factors such as a lack of anabolic effects of insulin and amylin on bone mass cause both a bone loss in adults and a lower peak of skeletal mass in younger subjects. According to several studies, vitamin D may reduce bone turnover and prevent bone loss in non diabetic patients. However, considering also the effect of vitamin D in reducing insulin requirement in type 1 diabetes patients, this compound might be considered as a novel therapeutic adjuvant in this disease. The objective of this study was to determine the effect of 1 year treatment with calcitriol on bone turnover in subjects with T1D by analyzing Osteocalcin (a bone formation marker) and β-CrossLaps (a bone resorption marker).
Materials and methods: In a double blind study designed to evaluate the effect of calcitriol to preserve beta cell function in recent onset T1D, we have investigated the effect of this treatment on bone turnover markers. A group of 25 subjects with recent-onset T1D and baseline C-peptide > 0.25 nM, were randomized to calcitriol at 0.25 μg daily dose or placebo (1:1) and followed-up for 1 year. Osteocalcin and β-CrossLaps, were evaluated by ECLIA method (modular E170, Roche Diagnostics, Mannheim, Germany) at diagnosis and at 1 year follow-up.
Results: At 1 year follow-up osteocalcin and β-CrossLaps dropped by 38.6% and 47.3%, respectively in the calcitriol treated group but their levels were not significantly different compared to diagnosis due to high variability. No significant differences were also found at 1 year comparing calcitriol vs. the placebo group for both osteocalcin (25.1±3.6 (sem) ng/mL vs 46.1±14.2 (sem) ng/mL, respectively; P=0.157) and β-CrossLaps (0.29±0.6 (sem) ng/mL vs 0.48±0.1 (sem) ng/mL, respectively; P=0.151). However, by stratifying patients according to age, we found that at 1 year follow-up as compared to diagnosis, calcitriol treated patients <18 years of age (mean age 16 years ± 1.46) showed statistically significant 61% drop of osteocalcin (68.8±17.6 (sem) ng/mL vs 26.8±11.5 (sem) ng/mL, respectively, p=0.04) and a 67% reduction in β-CrossLaps (0.92±0.77 (sem) ng/mL vs 0.31±0.08 (sem) ng/mL, respectively, p=0.09). In this age range, patients on calcitriol therapy vs. placebo showed at 1 year follow-up a trend for lower osteocalcin (74.2±23.7 (sem) ng/mL vs 26.8±4.8 (sem) ng/mL, respectively, p=0.08) and significantly lower β-CrossLaps (0.76±0.15 (sem) ng/mL vs 0.31±0.1 (sem) ng/mL respectively, p=0.03). Differences were not statistically significant in patients older than 18 years of age.
Conclusion: Our preliminary data suggest that in young subjects with T1D calcitriol therapy for 1 year at dose of 0.25 μg daily decreases bone remodelling and may contribute to preserve bone mass in T1D related osteopenia.

PS 74 Insulin analogues

887

Changes of glycaemic control, anthropometric parameters and fasting lipid profiles in patients with type 2 diabetes mellitus starting insulin therapy with premixed insulin analogues BID

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Background and aims: Weight gain associated with initiation of insulin treatment in type 2 diabetes mellitus (T2DM) and its metabolic consequences remain an important concern in clinical practice. It is not well understood how initiation of conventional insulin regimen with insulin analogues affect distribution of adipose tissue and body weight changes. We report here anthropometric and lipid endpoints from a study of premixed insulin analogues (PIA; 75% insulin lispro protamine suspension, 25% insulin lispro or 70% insulin aspart protamine suspension, 30% insulin aspart).
Materials and methods: Body weight (BW), BMI and distribution of adipose tissue (waist circumference [WC], hip circumference [HC], WHR) and fasting lipid profiles were recorded along with HbA_{1c} in T2DM pts starting PIA BID after failure of oral antihyperglycemic medication (OAM) in a 1-year, prospective, observational study conducted in Europe, Middle East and Asia. Data were summarized using means (SD), 95% CIs, percentages. *P*-values for changes from baseline were calculated using *t*-tests.
Results: Out of 1139 enrolled, 991 (87%) pts completed the study (595 [52.2%] males, age 57.9 (10.1) years, diabetes duration of 9.3 (5.9) years, body weight 81.5 (15.9) kg, BMI 29.2 (4.7) kg/m^2, baseline HbA_{1c} 9.9 (1.8) %). Initial daily dose of PIA 35.9 IU (13.7) increased to 41.4 IU (19.4). 951(83.5%) patients received insulin in combination with one or more OAMs (656 [69%] pts with 1, 267 [28.1%] with 2, 27 [2.8%] with 3, and 1 [0.1%] patient with 4 OAMs). 546 patients (48.6% of enrolled pts) received at least one hypolipaemic medication (504 [92.3%] pts 1, 41 [7.5%] 2, 3 [0.2%] pts 3) during the observation period. In patients who completed 12 months of follow-up, following mean changes were observed: HbA_{1c} decreased by 2.5% (CI 2.4-2.7); BW and BMI increased by 1.5kg and 0.6kg/m^2 (Table); WC and HC increased by 0.7cm (CI 0.5-1.0; p<0.001) and by 0.5cm (CI 0.3-0.8; p<0.001). There was no observed change in WHR. Total and LDL cholesterol and triglycerides decreased (p<0.001) whereas HDL cholesterol increased from baseline (< 0.001) (Table).
Conclusion: Following introduction of premixed insulin analogues in a group of patients with type 2 DM observed for 12 months glycemic control and fasting lipid profiles improved. No changes in mean WHR were observed despite increases in mean body weight, BMI, waist and hip circumferences.

Change of HbA1c, weight and fasting blood lipids (N=1139; n=patients with observed measures)

Characteristic	Baseline mean (SD), n	After 12 months mean (SD), n	Change from Baseline mean (95% CI), n	*P*-value
HbA_{1c} (%)	9.9 (1.8), 1098	7.3 (1.1), 951	- 2.5 (2.4-2.7), 938	< 0.001
Weight (kg)	81.5 (15.9), 1139	83.6 (14.9), 978	+ 1.5 (1.3-1.8), 978	< 0.001
BMI (kg/m^2)	29.2 (4.7), 1138	29.9 (4.4), 978	+ 0.6 (0.5-0.7), 978	< 0.001
Waist-to-hip ratio	1.0 (0.1), 1013	1.0 (0.1), 880	0.0 (0-0), 870	0.472
Total cholesterol (mg/dL)	207.4 (46.9), 1057	191.5 (34.8), 806	- 17.2 (14.3-20.0), 778	< 0.001
HDL (mg/dL)	44.8 (13.1), 988	46.7 (15.8), 791	+ 2.1 (0.9-3.2), 749	< 0.001
LDL (mg/dL)	125.8 (38.1), 982	115.0 (30.8), 791	- 10.9 (8.5-13.3), 737	< 0.001
Triglycerides (mg/dL)	200.3 (133.9), 1050	167.5 (83.7), 813	- 37.0 (29-45.1), 786	< 0.001

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888

Improved glycaemic control in over 11,000 elderly patients from the IMPROVE™ Study of biphasic insulin aspart 30/70 (BIAsp 30) in eight countries

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Background and aims: IMPROVE™ is an international, open-label, non-randomised, non-interventional, 26-week observational study of biphasic insulin aspart 30/70 (BIAsp 30) in patients with type 2 diabetes in routine clinical practice. The analysis presented here examines whether elderly patients, given their generally poorer disease status and longer diabetes duration, can benefit from BIAsp 30 therapy.
Materials and methods: Enrolled patients aged ≥65 years (N=11,988) were initiated on, or switched to, BIAsp 30 during routine care. Safety and effectiveness data were obtained at baseline and 26 weeks. Where available, analysis was also performed according to pre-study treatment (no pharmaceutical therapy, oral antidiabetic drugs [OADs] only, and insulin±OADs).
Results: Patients had a mean (standard deviation, SD) age of 71.3 (5.2) years, weight of 69.8 (14.5) kg, body mass index (BMI) of 26.3 (4.9) kg/m^2, diabetes duration of 10.4 (7.4) years and HbA_{1c} of 9.18% (1.88%). Mean HbA_{1c} decreased by 2.1% by study end (p<0.0001; Table 1). Significant reductions in HbA_{1c} were also observed when analysed according to pre-study therapy: no therapy -3.2%, OADs only -2.1%, insulin±OADs -1.7% (all p<0.0001). Mean fasting blood glucose (FBG) and postprandial plasma glucose (PPBG) after all main meals also improved significantly. The rate of major and minor hypoglycaemic events decreased significantly during the study (both p<0.0001). There was a small increase in body weight. BIAsp 30 dose increased by 0.06 IU/kg from baseline to final visit. The HbA_{1c} target of <7% with no hypoglycaemia was achieved by 33.1% of patients (n=3972, mean HbA_{1c} [SD] 6.3% [0.5%]).
Conclusion: The IMPROVE™ study demonstrates that BIAsp 30 exerts beneficial effects in terms of blood glucose control in patients aged ≥65 years with long diabetes duration. These positive effects appear independent of prior diabetes therapy, and are accompanied by significant reductions in rates of hypoglycaemia. BIAsp 30 therapy may be of particular advantage to older patients, as starting and intensifying therapy is simplified when only one type of insulin is used.

Table 1

Variable N=11,988	.	Mean (SD)	n
HbA_{1c}, %Hb	Baseline	9.2 (1.9)	7184
	Final visit	7.1 (1.1)	
	Change	-2.1 (1.9)*	
FBG, mmol/L	Baseline	10.5 (3.3)	8128
	Final visit	6.7 (1.4)	
	Change	-3.9 (3.4)*	
PPBG (breakfast), mmol/L	Baseline	14.5 (4.6)	5901
	Final visit	8.5 (1.9)	
	Change	-6.0 (4.6)*	
PPBG (lunch), mmol/L	Baseline	13.3 (4.1)	2911
	Final visit	8.5 (1.8)	
	Change	-4.8 (4.1)*	
PPBG (dinner), mmol/L	Baseline	12.3 (3.6)	2093
	Final visit	8.0 (1.5)	
	Change	-4.3 (3.8)*	
Major hypoglycaemic events per patient year	Baseline	0.15	9954
	Final visit	0.01	
	Change	-0.14*	
Minor hypoglycaemic events per patient year	Baseline	3.84	9959
	Final visit	3.06	
	Change	-0.78*	
Weight, kg	Baseline	69.1	9866
	Final visit	69.3	
	Change	0.2*	
BIAsp 30 dose, IU/kg	Baseline	0.38 (0.20)	9834
	Final visit	0.44 (0.20)	
	Change	0.06 (0.17)	

*p<0.0001 for comparison of baseline vs. final visit. SD, standard deviation; FBG, fasting blood glucose; PPBG, postprandial plasma glucose; BIAsp 30, biphasic insulin aspart 30/70.

Supported by: Novo Nordisk

889

Lower treatment costs with insulin glargine compart to insulin detemir as part of a basal-bolus regime in type 2 diabetes: results from the LIVE-COM study in Germany

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Background and aims: The basal insulin analogues glargine (GLA) and detemir (DET) are well-established in the treatment of diabetes. In addition to clinical aspects the economic impact of basal insulin analogues on health care expenditures is of importance. The aim of this study was to compare resource utilization and costs incurred by a basal-bolus insulin regimen in type 2 diabetes (T2D) patients when using either GLA or DET as basal insulin from the perspective of the Statutory Health Insurance (SHI) in Germany under real-life conditions.
Materials and methods: LIVE-COM was a non-interventional, cross-sectional, retrospective study performed between April and September 2008 in randomly selected primary care practices in Germany. T2D patients with SHI status were eligible for documentation if aged ≥18 years and either using GLA or DET as part of a basal-bolus regimen for ≥6 months prior to documentation. The primary objective was to compare the mean direct diabetes-specific treatment costs (DTC) between the GLA and DET groups over 6 months. DTC comprised antidiabetic medication (insulins, oral antidiabetics), needles, blood glucose self-monitoring (test strips, lancets), and hypokits to treat severe hypoglycaemia. Mean costs were adjusted for relevant influencing factors by analysis of covariance (ANCOVA). Differences in means were regarded significant with a 2-sided p<0.05.
Results: LIVE-COM enrolled a total of 1731 T2D patients from 138 primary care practices (GLA, 1150; DET, 581). Patient characteristics for the GLA (53.3% male) and DET (48.7% male) groups were (mean±SD): age (66.0±10.7 vs. 64.7±10.1 years, p=0.01), BMI (31.3±5.8 vs. 32.7±6.2 kg/m^2, p<0.01), HbA1c (7.5±1.2 vs. 7.7±1.2 %, p<0.01), and fasting blood glucose (140±46 vs 148±48 mg/dl, p<0.01). Diabetes duration (>10 years: 60.0% vs. 59.0%, n.s.,onset of first insulin therapy (>5 years: 61.6% vs. 64.4%, n.s, and number of diabetic complications and risk factors (mean±SD: 3.0±1.7 vs. 2.9±1.6, n.s.) were similarly distributed in both groups. Adjusted mean DTC per patient and 6 months were lower in the GLA compared to the DET group (€932 vs. €1060, p<0.01). The adjusted average DTC savings were €128 (95% CI: €90; €167; p<0.01) per patient. Adjusted mean single costs in the GLA and DET groups were: basal insulin €223 vs. €246 (p<0.01), bolus insulin €241 vs. €289 (p<0.01), needles €67 vs. €80 (p<0.01), test strips €347 vs. €393 (p<0.01), lancets €14 vs. €16 (n.s.), oral antidiabetics €37 vs. €36 (n.s.), and hypokits €2 vs. €1 (n.s.). Mean daily total insulin dose of 27.7/40.3 U (basal/bolus) was found to be significantly lower in the GLA compared to 32.1/47.1 U in the DET group (p<0.01). GLA patients had fewer basal insulin injections per day (1.1 vs. 1.3, p<0.01) and required also less test strips per day for blood glucose self-monitoring (3.2 vs. 3.6, p<0.01) compared to DET patients. Reported hypoglycaemic events, hospitalization rates and frequency of physician contacts did not differ between the GLA and DET groups.
Conclusion: Under real-life conditions, a glargine vs. detemir based basal-bolus regimen was associated with considerable lower costs of diabetes care from the SHI perspective in a 6-month head-to-head comparison in T2D patients.
Supported by: LIVE-COM

890

Insulin glulisine has a faster onset of action than insulin aspart

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Background and aims: Insulin glulisine (GLU) might have a faster onset of action than do other short-acting analogs owing to its zinc-free formulation. We compared the pharmacokinetics and pharmacodynamics (PK/PD) of GLU with those of insulin aspart (ASP) in a euglycaemic clamp study performed in healthy volunteers.
Materials and methods: Twelve healthy subjects (six male; mean±SD age, 42±12 years; BMI, 24.6±2.2 kg/m^2; normal HbA1c and fasting plasma glucose) participated in this randomised, double-blind, crossover, euglycaemic glucose-clamp trial. After an overnight fast, subjects were connected to a Biostator and, after establishing baseline for a period of 90 minutes, received 0.2 U/kg of either GLU or ASP sc in the abdomen. Glucose infusion rates (GIRs) were automatically adjusted every minute by the Biostator to maintain blood glucose levels at 5% below the individual fasting value for 10 hours post-dosing.
Results: Shortly after injection, insulin concentrations and early metabolic activity were significantly greater with GLU than with ASP, as indicated by a higher AUC[INS 0-30min]/AUC[INS total] ratio (p=0.0001) and a nearly twofold higher area under the GIR curve (AUC[GIR 0-30 min]; p=0.0421) (Table). Shorter times to 10 and 20% of INS[max] (p=0.0005 each) and a 50% shorter time to 10% of GIR[max] (p=0.0146) showed a significantly faster insulin absorption and onset of action for GLU than for ASP, whereas INS t[max] and total metabolic activity (AUC[GIR total]) were comparable.
Conclusion: Similar to previous comparisons with insulin lispro, GLU also shows an earlier onset of action than does ASP, probably because of its zinc-free formulation.

Insulin glulisine versus aspart: PK/PD results

	GLU	ASP	p-value
AUC[INS 0-30 min]/AUC[INS total]	0.07 (0.04)	0.04 (0.03)	0.0001
INS t[10%] (min)	3 (0-15)	12 (1-15)	0.0005
INS t[20%] (min)	8 (1-22)	19 (5-22)	0.0005
INS t[max] (min)	90 (40-120)	90 (50-150)	0.6406
AUC[INS t10%] (min)	38 (22-55)	45 (26-58)	0.0164
AUC[GIR0-30 min] (mg/kg)	30 (26)	16 (18)	0.0421
AUC[GIR total] (mg/kg)	2167 (715)	2256 (588)	0.6066
GIR t[10%] (min)	9 (2-23)	17 (3-20)	0.0146
GIR t[20%] (min)	14 (7-34)	22 (9-30)	0.0435

Data are mean (SD) or median (range)

Supported by: sanofi-aventis

891

Use of rapid-acting insulin aspart reduces costs and cardiovascular complications in type 2 diabetes when compared with human insulin

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Background and aims: The NICE study was a five-year, open-label, randomized controlled trial which compared cardiovascular outcomes in Japanese type 2 diabetes patients intensively treated with human insulin (HI) or insulin aspart (IAsp), a rapid-acting insulin analog. The within-trial cost-effectiveness of IAsp versus HI was evaluated from the perspective of a third-party healthcare payer.
Materials and methods: A cost-effectiveness model was developed in Microsoft Excel® based on mortality data, cardiovascular events and resource use information from 5 years of observation in the NICE study. Cardiovascular events/interventions captured in the model included: myocardial infarction, angina pectoris, percutaneous coronary intervention, coronary artery bypass grafting, transient ischemic attack and cerebral infarction. Mortality and cardiovascular event probabilities were derived from the annual rates observed during the trial period. Health-related quality of life (HRQOL) was estimated in the model by applying HRQOL scores in the year of the event and, in the case of myocardial and cerebral infarction, in subsequent years as a health state utility. Event costs were expressed in 2008 Japanese Yen (JPY) and were calculated from hospital receipt data supplied by the Japanese Medical Data Center. Insulin costs were obtained from the trial. Other pharmacy costs (e.g. oral antidiabetic agents and concomitant medications) were assumed to be the same in both treatment arms and were not captured in the analysis. Life expectancy, quality-adjusted life expectancy, cardiovascular event rates and costs were evaluated over a 5-year time horizon (in line with the NICE study). All future costs and clinical benefits were discounted at 3% annually. Sensitivity analyses were performed.
Results: Over five years of treatment, IAsp was dominant (life- and cost-saving) versus HI. Compared with HI, IAsp was associated with a small improvement in discounted life expectancy of 0.005 years (4.688 versus 4.683 years) and an improvement in quality-adjusted life expectancy of 0.034 quality-adjusted life years (QALYs) (3.787 versus 3.753 QALYs). IAsp also incurred lower costs (JPY 470,753 versus 579,974, difference -109,221) when compared with HI. The reduction in cost resulted from the decreased inci-

dence of cardiovascular events with IAsp (0.013 events per patient year versus 0.030 on HI). Breakdown of costs indicated that drug costs were higher with IAsp (JPY 337,066 versus 267,781), however these costs were more than offset by the reduced costs associated with cardiovascular complications over five years of treatment.
Conclusion: In a Japanese type 2 diabetes population, prescribing rapid-acting insulin aspart significantly reduced cardiovascular complications, resulting in increased quality of life and decreased costs when compared with human insulin.

892

Insulin detemir and insulin glargine have similar effects on hepatic glucose metabolism

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Background and aims: Insulin detemir (Det) is an insulin analog that binds reversibly to albumin. These studies were designed to compare direct effects of Det and insulin glargine (Glg), another basal insulin analog, on hepatic glucose production. The studies also compared the indirect effects of the two analogs, elicited via delivery of insulin into the peripheral circulation and the brain.
Materials and methods: Studies were carried out in chronically catheterized overnight fasted conscious dogs using arteriovenous difference and tracer techniques. Pilot studies were done to establish Det and Glg infusion rates that would suppress net hepatic glucose output (NHGO) ≈50% during hepatic portal vein insulin infusion (Low Insulin; 0.1 and 0.3 mU·kg^{-1}·min^{-1}, respectively). For the primary (non-pilot) studies, a primed continuous infusion of [3-^{3}H]glucose began at 0 min, basal sampling occurred from 90-120 min, and a euglycemic pancreatic clamp was carried out from 120-540 min. During the clamp, somatostatin was infused i.v. and glucagon was replaced intraportally at a basal rate. Dogs received Det or Glg into the hepatic portal vein (Po), a peripheral vein (IV), or the head (H; bilateral carotid and vertebral arteries) (6 groups, *n*=5-6/group). Low Insulin was infused from 120-420 min and High Insulin (4× Low Insulin) was infused from 420-540 min. Biopsies from the liver and hypothalamus were taken at the end of study.
Results: Arterial plasma glucose was 114±1, 110±1, and 108±1 mg/dl (Basal, Low Insulin, and High Insulin, respectively), and arterial plasma glucagon was 38±2, 38±2, and 34±1 ng/l. Neither parameter differed among groups. Basal NHGO (1.5±0.2 mg·kg^{-1}·min^{-1}); glucose R_a (2.2±0.3 mg·kg^{-1}·min^{-1}); and arterial plasma NEFA (916±45 μM) did not differ among groups. Data from the last h of Low and High Insulin are shown in the Table.
In Det Po, IV, and H, respectively (*n*=3/group), liver p-Akt (μg/mg protein) was 100±17, 115±10, and 139±46; in hypothalamus, it was 26±0.8, 36±14, and 28±10. In Glg Po, IV, and H, respectively (*n*=3/group), liver p-Akt was 71±19, 113±21, and 132±48; hypothalamus p-Akt was 21±2, 26±9, and 28±5 (no significant differences between groups or among routes).
Conclusion: Det and Glg have similar effects on the regulation of hepatic glucose metabolism in vivo. Portal and IV Det delivery reduces circulating NEFA to a greater extent than Glg, suggesting a greater indirect effect on hepatic glucose production.

Clamp data (mean±SE)

	NHGO (mg·kg^{-1}·min^{-1})		Glucose R_a (mg·kg^{-1}·min^{-1})		GIR (mg·kg^{-1}·min^{-1})		NEFA (μM)	
	Low Insulin	High Insulin	Low Insulin	High Insulin	Low Insulin	High Insulin	Low Insulin	High Insulin
Det Po	0.8±0.2	0.1±0.3	1.1±0.2	0.6±0.4	3.2±0.9	8.3±1.9	143±24	86±21
Glg Po	0.6±0.2	0.1±0.1	0.9±0.1	0.4±0.3	2.4±0.6	6.8±1.3	306±76*	123±41
Det IV	0.5±0.5	-0.3±0.3	2.3±0.5	1.6±0.5	4.3±1.3	12.5±3.6	125±39	90±38
Glg IV	0.1±0.3	-0.7±0.2	1.6±0.3	1.2±0.2	5.2±2.2	13.4±2.8	243±28*	62±22
Det H	0.7±0.4	-0.6±0.4	1.7±0.2	0.8±0.6	2.0±0.7	7.8±0.9	249±38	138±31
Glg H	0.4±0.3	-0.9±0.3	1.7±0.3	0.9±0.3	2.8±0.7	11.6±1.8	194±40	113±46

Negative values=net uptake. GIR, glucose infusion rate. *P<0.05 vs corresponding Det group

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893

Baseline HbA_{1c} predicts the likelihood of reaching the 7.0% HbA_{1c} target with structured titration of add-on insulin glargine: patient-level analysis of 12 studies in type 2 diabetes mellitus

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Background and aims: Addition of insulin glargine with systematic dosage titration can improve glycemic control to 7.0% HbA_{1c} or better in many patients with type 2 diabetes mellitus (T2DM) not well-controlled with oral agents. Baseline HbA_{1c} may be a useful predictor of reaching this target.
Materials and methods: We report here an analysis of patient-level data pooled from 12 randomized controlled studies with similar design, including structured basal insulin titration seeking fasting plasma glucose ≤100 mg/dL (≤5.5 mmol/L), to determine the likelihood of reaching 7.0% HbA_{1c} and the frequency of hypoglycemia when starting from various baseline HbA_{1c} levels.
Results: Data are from 2312 T2DM patients (mean [±SD] age 58.4±10.1 y; BMI, 31.0±5.45 kg/m^2; baseline HbA_{1c} 8.78±1.06%) after 24 weeks of treatment. Among 12 studies , at study end (mean 24weeks), HbA_{1c} was 7.07% (range 6.73%-7.45%) and 55.0% of patients (range 41%-78%) reached ≤7.0%. Mean HbA_{1c} reduction from baseline was 1.71% and the value at 24 weeks correlated strongly with baseline HbA_{1c}; continuous data for both variables was used (Pearson correlation coefficient=0.39, P<0.0001). Higher baseline HbA_{1c} correlated with greater reduction from baseline (Pearson correlation coefficient=-0.62, P<0.001). The table shows HbA_{1c} reductions from baseline, percentages of patients achieving HbA_{1c} ≤7.0%, and rates of symptomatic or severe hypoglycemia for ranges of baseline HbA_{1c}. Among patients with baseline HbA_{1c} <8.0%, 75.4% achieved the target HbA_{1c} ≤7.0%.
Conclusion: Despite greater reductions from baseline HbA_{1c}, those patients in higher baseline HbA_{1c} ranges were less likely to reach ≤7.0%. The risk of hypoglycemia was not significantly related to baseline HbA_{1c}, using this structured titration of basal insulin glargine.

Range of Baseline HbA_{1c}, %	N	Mean Baseline HbA_{1c}, % (SD)	Mean Change in HbA_{1c}, 24-wk, % (SD)	Mean HbA_{1c}, 24-wk, % (SD)	Patients at HbA_{1c} ≤7.0% by 24 wk, %	Mean Symptomatic Hypoglycemia, [a] Events-per Patient-Year (SD)	Mean Severe Hypoglycemia, [b] Events-per Patient-Year (SD)
<8.0	581	7.56 (0.324)	−0.90 (0.693)	6.66 (0.679)	75.4	5.56 (9.950)	0.065 (0.623)
8.0-8.4	436	8.19 (0.141)	−1.37 (0.771)	6.82 (0.763)	62.8	4.62 (8.177)	0.024 (0.220)
8.5-8.9	360	8.69 (0.141)	−1.64 (0.848)	7.05 (0.842)	55.8	4.57 (10.19)	0.068 (0.578)
9.0-9.4	327	9.20 (0.149)	−2.03 (0.841)	7.17 (0.836)	46.5	5.38 (11.48)	0.030 (0.269)
≥9.5	608	10.19 (0.635)	−2.60 (1.210)	7.59 (1.125)	33.9	3.89 (8.960)	0.080 (1.292)
P[c]			<0.0001	<0.0001	<0.0001	0.0713	0.7528

[a]Any symptomatic hypoglycemia event with blood glucose <70 mg/dL. [b]Any symptomatic hypoglycemia event that required assistance and had either a recorded blood glucose value <36 mg/dL or had prompt recovery after oral carbohydrate, i. v. glucose, or glucagon administration. [c]For continuous variables, analysis of variance models including study and baseline as factors; for categorical variables, Cochran-Mantel-Haenszel statistics, between baseline HbA1c and corresponding column variable controlling for studies.

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894

Glycaemic outcomes 1 year after initiation of insulin glargine or detemir in type 2 diabetes in the US

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Background and aims: To date, there are limited data on glycemic control comparing initiation of insulin glargine (GLAR) or detemir (DET) in the clinical setting.
Materials and methods: A database of 47 US managed care plans was analyzed to determine the real-world experience with the 2 therapies in patients enrolled between March 2005 and 2008 who were treated with oral antidiabetic agents before initiating GLAR (*n*=11,861) or DET (*n*=2285).

Results: Patient characteristics from the 6 month (baseline) prior to initiation of GLAR vs DET included mean age of 54 years in both groups. Fewer patients initiating GLAR (12%) vs DET (25%) were seen by endocrinologists. Baseline A1C was similar in both groups (9.4% [GLAR] vs 9.6% [DET], p=0.49). The percentage of patients with any medical claim for hypoglycemia during the 6-month baseline period was 4.8% in GLAR vs 3.9% in DET (p=0.073). 23% of GLAR vs 10% of DET patients had been hospitalized (p<0.001) during the baseline period with an average of 8.2 vs 5.4 hospital days, respectively. 23% in GLAR vs 16% in DET used emergency services (p<0.001). Hospital insulin initiation occurred in 9.4% of GLAR vs 3.7% of DET patients (p<0.001). Unadjusted 1 year A1C change from baseline was -1.3% with GLAR (n=1303) vs -1.0% with DET (n=289) (p=0.037). The unadjusted percentage of patients who achieved A1C < 7% was 26.7% in GLAR group vs 22.6% in DET group (p<0.069) one year after initiation. Adjustment for age, sex, baseline A1C, hypoglycemia, diabetes medication, access to an endocrinologist, hospitalization, Charlson comorbidity index score, concomitant medication, and total daily insulin dose resulted in an adjusted mean A1C at 1 year of 7.8% for GLAR vs 8.2% for DET (Δ=0.4%, p=0.04) and 8.1% vs 8.6% (Δ=0.5%, p=0.01) among patients who had initiated insulin in an ambulatory clinic (excluding patients who initiated insulin in a hospital). Excluding patients with hospitalizations during the baseline period, adjusted mean A1C at 1 year was 8.0% for GLAR vs 8.6% for DET (Δ=0.6%, p=0.005). Average daily insulin dose was modestly higher in DET vs GLAR (36 vs 31 U, p<0.001). The 2 groups did not differ in the rate of claims for hypoglycemia (7.3% for GLAR vs 6.6% for DET, p=0.275).
Conclusion: Patients initiating GLAR vs DET as basal insulin in real-world clinical settings demonstrated greater A1C improvement with no increase in medically coded hypoglycemia.
Editorial support provided through the sanofi-aventis US Group

895

Effects of insulin glargine vs thiazolidinediones on glycaemic and lipid variables in type 2 diabetes mellitus: the impact of obesity
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Background and aims: Obesity is a common risk factor for diabetes and may affect the therapeutic response to antidiabetic medications. We compared the effect of baseline BMI on glycemic and lipid variables in patients with uncontrolled type 2 diabetes mellitus (T2DM) (glycated hemoglobin A1C [A1C] >7.0%).
Materials and methods: This post hoc analysis of pooled data from 2 similarly designed randomized controlled studies compared insulin glargine (GLAR) vs thiazolidinediones (TZDs) added to oral therapy: rosiglitazone (ROSI) added to metformin (MET) + sulfonylurea (SU) combination therapy or pioglitazone (PIO) added to SU or MET monotherapy. This analysis was stratified by obesity class: BMI<30 kg/m^2 (n=183); BMI ≥30-34 kg/m^2 (n=170); BMI ≥35-39 kg/m^2 (n=103); and BMI ≥40 kg/m^2 (n=95).

Mean Change From Baseline at 24 Weeks										
	Overall		BMI <30 kg/m²		BMI ≥30-34 kg/m²		BMI ≥35-39 kg/m²		BMI ≥40 kg/m²	
	Baseline Value		Baseline Value		Baseline Value		Baseline Value		Baseline Value	
	Mean Change		Mean Change		Mean Change		Mean Change		Mean Change	
	GLAR	TZD	GLAR	TZD	GLAR	TZD	GLAR	TZD	GLAR	TZD
A1C, %	9.14	9.10	9.24	9.11	9.01	9.05	9.05	9.19	9.28	8.99
	-2.04[a]	-1.68	-2.28[a]	-1.48	-2.06[b]	-1.68	-1.82	-1.92	-1.97	-1.85
FPG, mg/dL	209.06	209.08	205.93	201.39	205.28	209.24	210.89	211	222.95	217.51
	-81.33[a]	-57.91	-84.08[a]	-48.98	-73.00[c]	-60.28	-94.04[b]	-60.43	-81.02	-67.08
Weight, kg	96.78	97.85	80.42	79.33	93	95.59	107.2	108.38	125.22	124.87
	1.83[d]	2.98	1.81	2.42	1.43[c]	2.96	2.57	2.75	1.91[c]	4.43
	Baseline Value Mean Change, %		Baseline Value Mean Change, %		Baseline Value Mean Change, %		Baseline Value Mean Change, %		Baseline Value Mean Change, %	
	GLAR	TZD	GLAR	TZD	GLAR	TZD	GLAR	TZD	GLAR	TZD
TG	194.27	198.79	170.05	185.56	203.37	199.35	204.21	215.03	218.23	202.34
	-18.47[a]	-12.37	-17.59	-12.42	-20.9	-13.38	-18.92	-16.55	-22.51	-18.09
LDL-C	109.21	106.67	114.18	108.56	105.84	104.53	107.4	100.16	109.3	113.46
	-1.85[a]	6.62	-6.08	2.99	-2.01[c]	9.00	-5.25[c]	9.83	4.03	4.6
HDL-C	42.29	41.46	44.44	42.84	41.40	40.79	41.42	40.64	41.57	41.15
	1.08[a]	9.35	1.41[d]	12.69	1.95[a]	10.10	-0.71[d]	8.98	4.36	9.16
TC	195.58	194.24	197.70	195.22	193.42	191.58	194.08	192.69	198.70	197.97
	-4.17[a]	3.97	-5.40[d]	4.37	-4.81[d]	3.90	-6.56[d]	4.76	-1.89	0.12
NEFA	52.92	51.15	46.8	46.63	56.43	47.87	55.04	57.47	57.2	58.76
	-25.77[f]	-19.95	-26.77[c]	-14.24	-31.44	-18.57	-26.09	-25.59	-24.37	-26.09

GLAR vs TZD: [a]P<0.0005; [b]P<0.01; [c]P<0.05; [d]P<0.005; [e]P=0.504; [f]P=0.0528. From ANCOVA with terms for study and baseline value.
Analysis population for glycemic control: GLAR (N=264) and TZD (N=288).
Analysis population for lipid variables: GLAR (N=258) and TZD (N=278).

Results: Overall, reductions in A1C, fasting plasma glucose (FPG), low-density lipoprotein cholesterol (LDL-C), and total cholesterol (TC) were greater in the GLAR arm (N=264) than in the TZD arm (N=288) (P<0.0005). In patients with BMI <30 and BMI ≥30-34, GLAR significantly reduced A1C and FPG compared with TZDs (P<0.05); this difference was attenuated with increasing BMI. GLAR was associated with more favorable changes in triglycerides (TG), LDL-C, and TC in all BMI groups, and in non-esterified fatty acids (NEFA) at all BMIs except ≥40. Differences were statistically significant for the following: TC in all BMI groups except ≥40 (P<0.005); LDL-C when BMI was ≥30-34 and ≥35-39 (P<0.05); and NEFA when BMI was <30 (P<0.05). High-density lipoprotein cholesterol (HDL-C) increased significantly (P<0.005) with TZDs in all BMI groups. There was no consistent pattern in TG, NEFA, and TC changes by BMI. Effects on lipid variables varied with the TZD used in each study, with more favorable changes associated with PIO vs ROSI. Hypoglycemia was more frequent with GLAR. Weight gain was greater with TZDs (P=0.0024) and there was a progressive increase in weight gain across BMI groups with TZDs, but not with GLAR.
Conclusion: Despite more frequent episodes of hypoglycemia, GLAR more effectively improved glycemic control than did TZDs, particularly at lower BMI, and was associated with less weight gain and more favorable changes in TG, LDL-C, TC, and NEFA in less obese patients.
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PS 75 Clinical aspects of oral medication

896

Treatment patterns and persistence among elderly patients with type 2 diabetes: data from a US managed care claims database

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Background and aims: The ADA/EASD consensus statement for the management of type 2 diabetes mellitus calls for lifestyle intervention plus metformin, followed by the addition of basal insulin or sulfonylurea. In the elderly population, diabetes remains suboptimally managed owing to greater frailty, impaired cognition, and comorbidities, with <50% of patients following current guidelines. While a general treatment consensus needs to be better defined for this population segment, achieving glycemic control for most elderly patients should be prudent and individualized as functional disabilities and/or comorbidities often increase the complexity of diabetes management.
Materials and methods: This longitudinal retrospective cohort study assessed treatment patterns among patients (≥65 years; n=182,458) with at least 1 diabetes medication prescription claim from 2004 to 2007 using a national managed care claims database of 46 health plans, and tracked medication changes from their initial treatment record in the data: 1 oral antidiabetic drug (OAD) 39.75%, 2 OADs (28.06%), ≥3 OADs (9.80%), OAD + insulin (11.46%), and insulin only (10.93%) therapies.
Results: From 2004 to 2007 the number of patients staying on therapy decreased as the number of OADs increased. Treatment persistence remained highest in patients using insulin who were more likely to stay on therapy: patients staying on 1 OAD were 87.58% in 2004 vs 86.39% in 2007; 2 OADs, 82.23% vs 78.25%; ≥3 OADs, 79.36% vs 69.55%; OAD + insulin, 83.95% vs 80.74%; and insulin only, 93.79% vs 93.94%. Compared with a younger cohort (18-64 y), a smaller proportion of elderly patients used metformin (43.90% vs 63.20%), but not sulfonylurea (41.96% vs 21.31%). Transition of therapy occurred in a greater proportion of patients from metformin or sulfonylurea monotherapy, as well as 2 OADs, to multiple combinations including the use of insulin glargine over the same period (Table). Average prescription cost was also less for patients on insulin and insulin + OAD therapies compared with ≥3 OADs or other combination therapies with exenatide.
Conclusion: Although metformin is the preferred initial oral agent, it may not be appropriate for older patients with comorbid conditions, such as renal or cardiac disease. As more elderly patients with diabetes are switching to multi-agent regimens, greater adherence to recommended treatment algorithms should be emphasized and individualized to improve outcome in diabetes-related quality measures, such as those covered under the Physician Quality Reporting Initiative, and avoid unnecessary costs.
Editorial support provided through the sanofi-aventis US Group

Table. Diabetes Treatments and Associated Costs in Patients Aged ≥65 Years

Therapy Type	Patients on Therapy (%) (2004)	(2007)	Mean Rx Cost for Patients on Therapy (US$)
Met	15.44	11.68	53.72
Met + basal insulin	–	1.54	–
Met + SU	16.89	17.8	70.88
Met + TZDs	4.73	5.00	240.92
Met + GLP-1	–	0.43	–
Met + TZDs + SU	8.48	8.32	240.21
Met + basal insulin	–	1.54	–
SU	17.57	11.68	27.82
SU + TZD	5.23	4.06	220.71
TZD	5.82	3.01	254.11
Glargine + 1 OAD	1.25	2.67	184.53
Glargine + 2 OADs	1.35	2.58	217.82
Glargine	0.91	2.51	167.50
Lispro	1.80	2.19	225.20
NPH	2.57	1.99	143.08
Met + SU + Sit	–	1.54	239.18
NPH + RHI	2.26	1.42	–
Met + TZD + Sit	–	1.41	292.14
RHI	1.77	1.29	120.90
Exenatide + 2 OADs	–	1.23	301.5
Aspart	–	1.05	268.96
Glargine + 3 OADs	–	0.96	256.91
NPH + 1 OAD	1.46	0.94	–
Met + Sit	–	0.91	242.71

GLP-1 = glucagon-like peptide 1; Met = metformin; OAD = oral antidiabetic drug; RHI = regular human insulin; Sit = sitagliptin; SU = sulfonylurea; TZD = thiazolidinedione.

897

Medical costs in a US managed care cohort with oral antidiabetics maintenance therapy in type 2 diabetes patients who achieved HbA_{1c} <7.0

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Background and aims: One-year medical costs were evaluated for patients with type 2 diabetes mellitus (T2DM) treated with sulfonylurea (SU), pioglitazone (PIO), or metformin (MET) as maintenance therapy after achieving a target HbA1c < 7.0%.
Materials and methods: T2DM patients treated with SU, PIO, or MET monotherapy were identified in the Ingenix Impact database (01/01/00-03/31/07), a large managed care claims database including medical, pharmacy and enrollment records. Inclusion criteria were: 1) continuous insurance enrollment 6 months before and 12 months after the date of glycemic goal achievement of HbA1c < 7.0% (the index date); 2) a baseline HbA1c value > or = 7.0%; 3) exclusive use of the study medication during the 6-month baseline period; 4) at least one drug refill after the index date; and 5) age 18 years or greater at the index date. Medical, pharmacy, and total costs were compared over the 1-year post-goal achievement period using a non-parametric Wilcoxon test. Subgroup analysis was performed among patients with 24 months of post-index continuous insurance enrollment. A generalized linear model with log-link function and gamma distribution was used to compare costs when adjusting for baseline characteristics such as demographics, prior comorbidities, medications, and resource utilization.
Results: Final cohorts included 1,869 SU, 427 PIO, and 1,163 MET patients. Over the first year of follow-up, descriptive results showed numerically higher medical costs for SU patients (8,362 US dollars) than PIO patients (6,881 US dollars) and MET patients (8,198 US dollars), $P<0.05$. Specific components of the medical costs that were lower for the PIO cohort compared to the SU cohort included diabetes-related costs, emergency-room costs, office visits, and inpatient costs, although these were not all statistically significant. Using multivariate regression, PIO patients had risk-adjusted medical costs of 7,849 US dollars, which was 1,298 US dollars ($P=0.0211$) less than medical costs for SU patients (9,147 US dollars). MET patients had risk-adjusted medical costs of 8,174 US dollars, which was 973 US ($P=0.0155$) lower than medical costs for SU patients. Risk-adjusted total healthcare costs (including prescription and medical costs) were 12,083 US dollars for SU, 12,146 US dollars for PIO, and 11,543 US dollars for MET. Neither of these comparisons were statistically significant. Among the subgroup of patients with 24 months of post-index continuous insurance enrollment, PIO showed risk-adjusted medical costs of 16,251 US dollars while SU showed medical costs of 20,435 US dollars, a 2-year cost savings for PIO of 4,184 US dollars ($P=0.0110$). MET also showed lower 2-year medical costs (18,170 US dollars) compared to SU.
Conclusion: Patients receiving PIO as maintenance therapy incurred significantly lower risk-adjusted 1- and 2-year medical costs compared with patients receiving SU as maintenance therapy after glycemic goal achievement in the US managed care setting.
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898

The high prevalence of malnutrition and unnecessary drug therapy in very old diabetic patients

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Background and aims: Type 2 diabetes usually occurs in the context of obesity and associated insulin resistance. Current treatment recommendations are based on lifestyle modifications and incremental drug therapy to achieve optimal glycemic control. However, this approach may lead to inappropriate priorities upon aging, when diabetes may be compounded by malnutrition.

Materials and methods: We undertook a systematic evaluation of glycemic and nutritional parameters in a cohort of very old diabetic patients admitted to our geriatric service. We also implemented nutritional support therapy and a scheme of drug therapy adjustment. Oral hypoglycemic agents (OHA) withdrawal was attempted in case of good glycemic control (HbA1c <7.5% or fasting blood sugar (FBS) <7.5 mmol/l) or when contra-indications to OHA were present.
Results: 80 diabetic patients (mean age 82.8+7.4 years, 22% men, 78% women) have been included in this ongoing survey. Mean BMI was 29.6+6.6 kg/m2, and mean HbA1c was 6.8+1.2%. 75% patients had an HbA1c 85% of which on metformin), 33% on insulin and 5% on combined insulin/OHA therapy. Malnutrition, as evidenced by a MNA (Mini nutritional Assessment) score <23.5, indicating "Malnutrition" or "risk of malnutrition", was observed in 71% patients. Severe hypoalbuminemia (<30 g/l), usually without proteinuria, was observed in 49% patients. We also observed a high prevalence of vitamin B12 (< 200 pmol/l) and 25-hydroxy vitamin D (<30 nmol/l) deficiency (34 and 32% respectively). Among patients treated by OHA, complete drug withdrawal was attempted in 72% patients (mean HbA1c 6.2+1.0%, mean FBS 6.9+1.8 mmol/l), much more often than new drugs were added (p=0.002 by McNemar's test). In spite of in-hospital nutritional therapy, FBS remained <7.5mmol/l (mean value 6.6+1.0 mmol/l) at hospital discharge without new drug therapy in 78% patients.
Conclusion: The prevalence of malnutrition is high in very old diabetic geriatric patients. Malnutrition should be screened for in these patients, and when present should prompt a revision in diet and drug therapy. In particular, the possibility of reducing unnecessary drug therapy should be considered. Malnutrition and consequently reduced insulin resistance (occurring even in overweight/obese patients) is most likely a key factor for successful OHA withdrawal.

899

Effect of glitazones on cancer mortality in type 2 diabetes
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Background and aims: Numerous studies have observed an association between type 2 diabetes (T2DM) and cancer, with recent reports suggesting a modulating role of antidiabetic therapy. There is little epidemiologic information, however, exploring the effect of the glitazones and cancer mortality in T2DM. We hypothesized a decreased risk of cancer mortality associated with glitazones use, compared to sulfonylurea monotherapy use.
Materials and methods: This was a population-based retrospective cohort study using administrative data from Saskatchewan Health. We identified new users of metformin or sulfonylureas from January 1, 2000 to December 31, 2005, with follow-up until death, departure from the province, or December 31, 2006. We compared cancer mortality between glitazone users (plus metformin), metformin and sulfonylurea combination users, metformin monotherapy users, and sulfonylurea monotherapy users (reference group).A time-varying Cox analysis was used to estimate the hazard ratio (HR) of cancer mortality, accounting for oral antidiabetic therapy, insulin therapy, age, sex, and chronic disease score (CDS). Exposure to oral antidiabetic therapies was defined by use of each agent (i.e., yes/no) within 1-year time windows. We examined the dose-response gradient for insulin exposure. Firstly, we created a count of insulin dispensations for each 1-year time window. Cumulative insulin exposure/year was then calculated by summing insulin counts at the end of each year and dividing by the person years of insulin use since insulin index. We stratified the cumulative insulin exposure level into high, low, and no exposure to insulin.
Results: We identified 20,448 new users of metformin or sulfonylureas during the index period, with an average (SD) follow-up of 3.5 (1.8) years. The mean age for the cohort was 61.3 (14.3) years and 53.8% were men. Unadjusted cancer mortality was 9.8% (154/1,577) for sulfonylurea monotherapy users and 1.3% (43/3,365) for glitazone (plus metformin) users (p < 0.0001). After adjusting for age, sex, CDS, and insulin use, glitazone (plus metformin) users had a significantly lower risk of cancer mortality compared with the sulfonylurea monotherapy cohort (HR: 0.42, 95% CI: 0.24 - 0.72; p=0.002). The adjusted HRs (95% CI) for insulin use were 1.33 (0.83 - 2.13) and 7.08 (5.27 - 9.51) for <12 and ≥12 cumulative insulin dispensations/year, respectively, compared to those using no insulin.
Conclusion: These finding add further support that antidiabetic therapies may play a role in the relationship between type 2 diabetes and cancer outcomes. Our results suggest that patients with type 2 diabetes exposed to glitazones had a significantly decreased risk of cancer mortality compared to patients exposed to sulfonylurea monotherapy. It is uncertain whether this decreased risk is related to a protective effect of glitazones or a toxic effect of sulfonylureas.
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900

Severe hypoglycaemia: are patients correctly selected for sulphonylurea therapy?
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Background: Hypoglycaemia is a well recognised side effect of some treatment modalities for diabetes mellitus, particularly in patients with tight glycaemic control. Recent NICE guidelines recommend hypoglycaemia advice for those with Type 2 Diabetes Mellitus (T2DM) before starting insulin or sulphonyurea therapy (SU).
Aims: To further define the different forms of therapy associated with hypoglycaemia and patient groups at greater risk.
Materials and methods: We performed a retrospective analysis of patients who developed severe hypoglycaemia in our Trust over a 4 month period. We included episodes of severe hypoglycaemia referred to the Diabetes service from Dec 2008 to March 2009. We defined severe hypoglycaemia as any hypoglycaemic event that required emergency services intervention. Data was also obtained from regional ambulance service. Trust approval was obtained prior to the commencement of the analysis
Results: A total of 76 patients suffered severe hypoglycaemia over the study period. The median age was 63 years (IQR 43 to 78 years) and 40 patients were male and 36 female. The median HbA1c was 8.1%. (31 T1DM, 33 T2DM, 11 not recorded, 1 non diabetic). Of the 76 patients, 61 (80%) patients were taking insulin (56 on insulin only and 5 in combination with Metformin), 10 (13%) patients were taking SU (5 with Metformin and 2 with thiazolidinediones(TZD)) and 1 patient on both insulin and SU. Therapy in 3 patients was not recorded and 1 patient developed hypoglycaemia due to severe liver failure. The results of the analysis is summaried in Table 1 and Table 2. When the SU treated and non SU treated group was compared, patients on SU were significantly older and had significantly lower HbA1c. Five patients in the SU group had HbA1c levels < 6.0%.
Conclusion: Our analysis shows that almost 15% of patients who suffered from severe hypoglycaemia were on SU therapy. Patients from this group were more elderly and had lower levels of HbA1c's. This study is inherently limited by its retrospective component and relatively small numbers. However, we feel that whilst the use of SUs are effective in the treatment of T2DM, caution is advised when considering this therapy in the more elderly population in particular those with tight glycaemic control. Access to self capillary glucose monitoring and crucially the ability to recognise and treat this condition must be considered when commencing treatment with SU in each individual patient. Whilst national HbA1c targets are useful for clinicians to ascertain glycaemic targets, this should not apply in all cases.

Table 1 * Mann Whitney U

	Type 2 Patients On Insulin (T2I)	Type I Patients	Type 2 Patients on Sulphonylurea (T2S)	p value p1: T2I vs T2S p2 = T1 vs T2S
n	23	31	10	
Median hbA1c	8.5%	8.2%	6.6%	p1: <0.001 p2:<0.0001
Median Age	70.5 (IQR 61-78	46 (IQR 36-56)	79 (IQR 76-83 years)	p1: 0.01 p2<0.0001

901

Older patients with newly diagnosed type 2 diabetes are less likely to be treated with oral antihyperglycaemic agents compared to younger patients
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Background and aims: This study compared older (≥ 65 yrs) and younger patients with newly diagnosed type 2 diabetes (T2DM), and evaluated factors associated with oral antihyperglycemic agent (OAHA) initiation.

Materials and methods: This is a retrospective cohort study using the General Electric (GE) Centricity electronic medical record database in the US. Patients aged ≥30 yrs with newly-diagnosed T2DM (Jan 2003 to Dec 2005) were included. Newly-diagnosed was defined as no diabetes diagnosis or treatment within 2 years prior to the new T2DM diagnosis. Their medical records 1 year prior to (baseline) and 2 years after (follow-up) diagnosis were extracted. OAHA initiation was estimated based on the first OAHA prescription during follow-up. Multivariable Cox proportional hazards regression model was fitted. Untreated patients were censored at the end of 2 years follow-up.
Results: Among 10,760 newly-diagnosed T2DM patients, 55% were female, 43% Caucasian. The mean age at T2DM diagnosis was 61.3(±12.5), with 4,617 (43%) ≥65 yrs. At baseline, older patients relative to younger patients had lower HbA_{1c} (6.7% vs. 7.2%, with only 38% vs. 32% attaining HbA_{1c} <7% goal), fasting blood glucose (117.9 vs. 132.8 mg/dL) and BMI (30.6 vs. 35.2), higher rates of acute myocardial infraction (MI) (2.1% vs. 0.9%), heart failure (5.1% vs. 1.8%), stroke (3.1% vs. 0.8%), and renal disease (17.5% vs. 6.2%), and more co-medications (all p-values <0.05). Within the 2 years after T2DM diagnosis, 56% older and 40.5% younger patients (P<0.001) had not received OAHA. Older patients were 16% less likely to be treated than younger patients (adjusted hazard ratio 0.84, p<0.001). Additionally, patients with MI or renal disease at baseline were less likely to be treated (p<0.05). Higher BMI or HbA_{1c} at baseline, and heart failure, initiating antihypertensive or lipid lowering drugs after T2DM diagnosis were factors associated with increased likelihood of OAHA treatment (p<0.05).
Conclusion: This study found that older patients with newly diagnosed T2DM had milder hyperglycemia but more comorbid conditions, and experienced more delayed OAHA therapy than younger patients.
Supported by: Merck & Co., Inc.

902

The effect of nateglinide combined with metformin on the repairment of abnormal glucose tolerance in new-onset type 2 diabetic patients and its mechanisms
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Background and aims: To analyze the effects of nateglinide combined with metformin on the repairment of abnormal glucose tolerance as well as insulin resistance and islet β cells' early phrase insulin secretion in patients with new-onset type 2 diabetes mellitus.
Materials and methods: 60 new-onset type 2 diabetic patients were divided into nateglinide combined with metformin group(nateglinide group, 32 patients)and acarbose combined with metformin group(acarbose group, 28 patients)randomly. Oral glucose tolerance test and insulin releasing test were performed before and after 4 months' treatment. We compared oral glucose tolerance before and after treatment and observed the changes in insulin resistance, islet β cells' insulin secretion and glucose disposition index. The insulin resistance was evaluated by the ratio of area under insulin curve/area under glucose curve (AUC_I / AUC_G) and HOMA-IR. HOMA-β,MBCI ,early phrase insulin secretion (represented by ΔI30/ΔG30) and the second-phase insulin secretion (represented by AUC_I)were calculated to estimate islet β cells secretary function.
Results: After 4-month treatment, 1 case restored normal glucose tolerance and 7 cases changed from diabetes to impaired glucose tolerance in the nateglinide group, the AUC_G decreased from 30.21±7.47 to 27.45±5.91(P<0.05); 5 cases changed from diabetes to impaired glucose tolerance in the acarbose group, the AUC_G decreased from 7.45±6.15to24.86±4.96(P<0.05). Before treatment, no statistical difference existed in blood glucose level during OGTT between the two groups. After 4-month treatment, the peak blood glucose value was observed at 60-minute point during OGTT in the nateglinide group and at 120-minute point in acarbose group. Compared with pretreatment, HOMA-IR was obviously improved in the two groups, it changed from 8.29±1.64 to 7.09±1.27(P<0.01)in the nateglinide group and 8.41±1.82 to 6.89±1.31(P<0.05)in the acarbose group; The longer the course of disease, the lower MBCI appeared in the two groups(P>0.05). After treatment, the HOMA-βdecreased slightly in the two groups(P>0.05); and the $\Delta I_{30}/\Delta G_{30}$ was recovered from 5.03±0.85 to 6.72±0.79(P<0.05)in the nateglinide group, compared with the acarbose group, the $\Delta I_{30}/\Delta G_{30}$ was also extremely improved after treatment(P<0.05). The AUC_I in the two groups was decreased after treatment(P<0.05). After 4-month treatment, the peak serum insulin level was observed at 60-minute point during OGTT in the nateglinide group and at 120-minute point in acarbose group, and there is statistical difference at 60min,120min and 180min points during OGTT between this two groups. The DI increased from 0.711±0.161 to 0.964±0.206(P<0.01)in the nateglinide group.
Conclusion: The treatment of nateglinide combined with metformin ameliorated impaired glucose tolerance in patients with new-onset type 2 diabetes mellitus via improving the insulin resistance and promoting the recovery of islet β cell's first phrase insulin secretion.

903

The efficacy of lowering HbA_{1c} with a gliclazide modified release-based intensive glucose lowering regimen in the ADVANCE trial
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Background: Intensive glucose control with a gliclazide modified release (MR)-based regimen in people with type 2 diabetes in the ADVANCE trial (Action in Diabetes and Vascular disease: Preterax and Diamicron Modified Release Controlled Evaluation), reduced the risk of combined microvascular and macrovascular events, predominantly through reductions in the development of new or worsening nephropathy. In the present analyses we assess the efficacy of this strategy in reducing glycated haemoglobin (HbA1c) to the target level of 6.5%.
Methods: All 11,140 patients randomised to receive either intensive (n=5,571) or standard glucose control (n=5,569) were included in analyses of treatment efficacy as either the absolute HbA1c reduction, the HbA1c level achieved or the percentage of patients reaching various HbA1c targets (<=7.0%, <=6.5% and <6.0%) at the end of follow-up (median 5 years). Simple linear and multiple regression models were also used to assess treatment efficacy in those assigned to intensive glucose control according to subgroups defined by age, duration of diabetes, body mass index (BMI), HbA1c level and glucose lowering treatment (diet alone, monotherapy or combination therapy with 2 or more oral hypoglycaemic drugs) at study entry.
Results: At the last study visit, HbA1c was reduced to a mean of 6.5% with 81, 65 and 21% achieving HbA1c levels of <=7.0%, <=6.5% and <6.0% respectively in those assigned to intensive glucose control, amongst whom, 70% were receiving the maximum daily dose of gliclazide MR of 120mg. In those assigned to standard control, HbA1c was reduced to7.3% with only 50, 29 and 8% achieving comparable HbA1c levels. With intensive glucose treatment, substantial reductions in HbA1c were observed across all subgroups defined by baseline age, duration of diabetes, BMI, HbA1c or glucose lowering treatment at study entry (all p<0.0001). By the end of the study HbA1c was reduced to 6.1% in those receiving gliclazide MR alone, 6.4% in those on other oral regimens including gliclazide MR and 6.8% in those also requiring insulin. In a model including all these baseline variables, HbA1c and BMI emerged as the only independent predictors of reduction in HbA1c (p<0.001). While severe hypoglycaemia was more frequent in the intensive control group, it was uncommon overall (0.7 vs. 0.4 episodes per 100 patient years in the intensive versus standard care groups). Furthermore the reductions in HbA1c were achieved without weight gain.
Conclusion: Intensive glucose control with a gliclazide MR-based regimen was effective in lowering HbA1c, irrespective of age, duration of diabetes, BMI or HbA1c at study entry, and irrespective of initial glucose lowering treatment. The decrease in HbA1c was more marked in those with higher HbA1c levels and lower BMI at baseline. This regimen was well tolerated with acceptable rates of severe hypoglycaemia and without weight gain.
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904

Cost-related medication underuse and perceptions about medication in patients with type 2 diabetes
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Background and aims: Medication adherence may be linked to improved outcomes in people with type 2 diabetes. Previous research has identified

multiple factors influencing cost-related medication underuse (that is, taking less medication than prescribed due to costs) such as patient-provider communication, health literacy, income, and prescription drug coverage. Patients' perceptions about their diabetes medication, such as perceived side effects and convenience, may also be factors in affecting cost-related medication underuse. The objective of this study was to determine the association between perceptions about diabetes medication and cost-related medication underuse in patients with type 2 diabetes.
Materials and methods: A United States-based online survey was administered to participants with type 2 diabetes. Survey measures included the validated Perceptions about Medication for Diabetes-25 (PAMD-25) instrument containing four scales: Perceived Effectiveness, Convenience and Timing, Emotional Side Effects, and Physical Side Effects. Higher scores indicate more positive perceptions about medication for all scales except for the Physical Side Effects scale where higher scores indicate more negative perceptions about medication. Two items exploring the participants' perceptions of the amount paid for diabetes medication as being reasonable/unreasonable and financially draining/not draining were also included. Covariates included socio-demographic information, patient characteristics such as general health status, physician-patient prescribing relationship, polypharmacy, and financial factors such as out of pocket costs for diabetes medication. Analyses were based on binary logistic regression models to test univariate effects of the perceptions about medication and covariates on cost-related medication underuse, the dependent variable. Significant factors were entered in a forward stepwise logistic regression analysis to determine the effect of diabetes medication perceptions on cost-related medication underuse while controlling for covariates. Significance was determined at $p < 0.05$ for all analyses.
Results: The analytic sample included 604 participants (male = 53.0%; Caucasian = 85.6%; mean age [SD] = 58.6 [11.5] years). Overall, 61 subjects (10.1%) reported cost-related medication underuse. All four scales of the PAMD-25 were significant variables in the univariate regression analyses: Perceived Effectiveness (OR = 0.58; 95% CI, 0.41 - 0.83, $p < 0.01$), Convenience and Timing (OR = 0.27; 95% CI, 0.18 - 0.42; $p < 0.001$), Emotional Side Effects (OR = 0.32; 95% CI, 0.22 - 0.45; $p < 0.001$), and Physical Side Effects (OR = 2.07; 95% CI, 1.61 - 2.66; $p < 0.001$). Also significant were the statements that the amount paid for diabetes medication was reasonable as compared to unreasonable (OR = 0.15; 95% CI, 0.09 - 0.26; $p < 0.001$) and that the amount paid was not financially draining (OR = 0.08; 95% CI, 0.05 - 0.14; $p < 0.001$). Controlling for covariates, Emotional Side Effects (OR = 0.45; 95% CI, 0.28 - 0.72; $p < 0.01$) and stating that the amount paid for diabetes medication was not financially draining (OR = 0.19; 95% CI, 0.09 -0.40; $p < 0.001$) retained their significance in the final model (Nagelkerke $R^2 = 0.42$).
Conclusion: Patients' perceptions about diabetes medication, particularly emotional side effects and perceived financial burden, may moderate the effects of other factors such as income and insurance coverage on patients' response to medication cost pressures.

PS 76 Clinical aspects of insulin therapy

905

Predictors of responders to insulin therapy at one year among adult patients with type 2 diabetes: a retrospective study
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Background and aims: Among patients with type 2 diabetes (T2D), the decision to initiate insulin often becomes necessary when lifestyle intervention and oral pharmacotherapy have failed to achieve glycaemic targets. The effectiveness of insulin therapy to lower blood glucose levels however may depend on a variety of factors. This study aims to determine baseline parameters that might predict responders to insulin therapy.
Materials and methods: This was a retrospective UK population based study derived from 358 general practices electronic dataset. We used standardised computerised routines to extract codes data on demographic, medical, biochemical and drug prescription history. T2D was defined as non-insulin requiring within 12 months of diagnosis. We included all patients with T2D, diagnosed at the age of >18 years old and who were initiated on insulin from January 2000 to December 2006. Insulin responders were defined as HbA1c of <7.5% and/or HbA1c reduction by >1% at 12 months post insulin initiation.
Results: Mean (SD) 6032 patients were identified. Baseline age was 63.7(11.7). 61% of patients (3696) responded to insulin therapy. At 1 year post insulin initiation, HbA1c was significantly reduced (9.8 v 8.4%, P<0.001) and BMI increased (30.3 v 31.1, P<0.001). Using a logistic regression model, older age (P<0.001), lower BMI (P=0.046), higher Hba1c (P<0.001) and basal bolus insulin therapy and premixed insulin, compared to basal insulin alone at baseline were independent predictors of responders to insulin. Gender and Townsend quartile were not significant predictors of insulin responders. A BMI of <35.3 was derived as a cut-point for response to insulin (P=0.038)
Conclusion: Overall, insulin therapy confers significant HbA1c reduction and weight increase in patients with T2D. In this cohort, responders to insulin at 1 year was seen in 61% of patients.The responsiveness to insulin therapy appears to depend on a variety of baseline factors which includes age, BMI, HbA1c and insulin regime. Clinicians should to take these factors into account when making a decision to initiate insulin therapy in patients with T2D.
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906

Treatment satisfaction was impaired in patients with diabetes type 2 using ICT with human insulin and who were obliged to keep a regular injection to meal interval. A randomised cross-over study
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Aims: In the pre-analogue-insulin era diabetic patients were usually instructed to keep an injection meal interval (IMI) of 20-30 minutes to compensate for the delayed onset of the action of regular insulin. All studies examining IMI were performed exclusively in patients with diabetes mellitus type 1. We studied the influence of IMI on HbA1c and hypoglycaemia in patients with diabetes mellitus type 2 (DM2).
Patients/methods: In a randomized cross-over-study (ClinicalTrials.gov NCT00529165) 100 patients with DM2 (age 66.9 years; diabetes duration 12.7 years; HbA1c 7.1%; 93% keeping an IMI before study-onset) on prepandial insulin therapy with human insulin (minimum 1 year) and stable glycaemic control, were randomised in two groups (Group A: IMI 20min then no IMI, group B: no IMI then IMI 20min). All patients were obligated to attend 8 visits during the study, 4 visits in each period. Visit 4 (V4) took place after the first period, before the cross-over was initiated, visit 8 (V8) at study end. Treatment satisfaction was assessed with the DTSQ (C. Bradley, max. score 33) and diabetes dependent quality of life with the ADDQoL (C. Bradley). The study was performed in a general practice. The dropout rate was 4%.
Results: At baseline HbA1c in group A was 6.96 vs. group B 6.85% (p=0.437) Treatment satisfaction (max. score 36) was in group A: 32.5 vs. group B 33.3;

(p=0.165). In period 1 treatment satisfaction increased more in the group without IMI than with IMI (9,4 vs. 4,2 points; p=0.001). After cross-over treatment satisfaction decreased in the group without IMI (period 1 IMI 20min) by 1.9 points and increased substantially in the group without IMI (period 1 IMI 20min) by 9.0 points (p=0.001). Diabetes dependent quality of life did not show differences at any time during the study, regardless of doing an IMI or not, neither did HbA1c nor hypoglycaemia. At the end of the study 86.5% of the patients decided against a meal injection interval,, irrespective of having or not having applied an IMI during the last study period.
Conclusion: Treatment satisfaction in patients with diabetes mellitus type 2 and good glycaemic control on preprandial therapy with human regular insulin is lower with a meal injection (IMI) interval of 20 minutes compared to insulin injection immediately before the meal and did not improve HbA1c. Diabetes dependent quality of life is not influenced by an IMI. Hence, a meal-injection-interval can not be regularly recommended for patients with type 2 diabetes on? preprandial therapy with human regular insulin.

907

Randomized 2-phases-crossover-study to examine the necessity of an injection-to-meal-interval in patients with diabetes mellitus type 2 and flexible insulin therapy with human insulin

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Background and aims: In the pre-analogue-insulin era diabetic patients were usually instructed to use an interval of 20-30 minutes between injection and meal to overcome the slow onset of regular insulin preparations. All studies deal with injection-meal-intervals (IMI) in patients with diabetes mellitus type 1. We studied influence of IMI on HbA1c and hypoglycaemia in patients with diabetes mellitus type 2 (DM2).
Materials and methods: In a randomized Cross-over-Study (ClinicalTrials.gov NCT00529165)with two phases of 3 months 100 patients with diabetes type 2 (age 66.9 years; diabetes duration 12.7 years; HbA1c 7.1%; 93% keeping IMI) on prepandial insulin therapy with human insulin (minimum 1 year) have been randomized to group A (Phase 1: IMI 20min, Phase 2: no IMI) or group B (Phase 1: no IMI, Phase 2: IMI 20min). All patients received 8 visits, visit 4 (V4) was after phase 1 and visit 8 (V8) was after phase 2. The study was held in a general practice. The drop out was 4%. The study was powered on the expectation that 100 patients would be evaluated. This had 80% power to detect no difference of HbA1c (α=0.15).
Results: HbA1c was not different at baseline (V1: A: 6.96 vs. B 6.85%; p=0.437) neither at the end of phase 1 (V4: 6.70 vs. 6.76%; p=0.669) nor at the end of phase 2 (V8: 6.73 vs. 6.83%; p=0.434). In group A HbA1c decreased in phase 1 (IMI 20min) 0.32% (p=0.001) and increased in phase 2 (no IMI) 0.13% (p=0.005). In group B HbA1c decreased in phase 1 (no IMI) 0.09% (p=0.097) and did not change in phase 2 (IMI 20min). The incidence of mild hypoglycaemia did not differ between group with IMI or no IMI at any time of the study (V1: 0.69 vs. 1.04; p=0.367; V4: 0.81 vs. 0.67; p=0.578; V8: 0.79 vs. 0.73; p=0.836). In group A the incidence of hypoglycaemia increased in phase 1 (IMI 20min) by 0.12 episodes per month (p=0.604) and decreased in phase 2 (no IMI) by 0.08 (p=0.647) and decreased in group B phase 1 (no IMI) by 0.37 (p=0.276) and increased in phase 2 (IMI 20min) by 0.125 (p=0.612). There was no case of severe hypoglycaemia in the study.
Conclusion: We did not find differences in HbA1c or mild hypoglycaemia if patients obey an injection-to-meal-interval or not. Marginal, clinical not relevant changes in HbA1c and mild hypoglycaemia were observed within the groups. An injection-to-meal-interval is not necessary for patients with type 2 diabetes and preprandial therapy with human regular insulin.

908

Real-life prescription patterns of insulin for patients with type 2 diabetes in The Netherlands

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Background and aims: Studies have suggested that in type 2 diabetes (T2D) insulin analogues may improve the balance between glycaemic control and hypoglycaemia compared with human insulins; for example, basal analogues provide similar glycaemic control with less hypoglycaemia. The aim of this study was to assess the real-life prescription patterns of insulin use in patients with T2D in The Netherlands, in particular with respect to the prescribing of human insulins or analogues.
Materials and methods: The PHARMO database contains community pharmacy drug dispensing records from primary and outpatient secondary care for approximately 2.5 million patients in The Netherlands. We selected patients with T2D starting a new insulin treatment (human or analogue basal/premix/basal-bolus) in the period 2004-6. The patients were categorised into insulin-naïve users (no insulin in previous year) and prior users. We determined the insulin used at start for naïve and prior users. For prior users we also determined the insulin type used before start, to identify treatment changes.
Results: The study included 10,739 patients with T2D of whom 7116 (66.3%) were insulin naïve and 3623 (33.7%) were prior insulin users. Among the 7116 naïve patients 52.0% started on a basal regimen, 32.7% started on premixes and 15.3% started with a basal-bolus regimen (Table 1). Within each regimen type the majority of patients received analogues rather than human insulin (range 54.9-61.0%; overall proportion receiving analogues 58.7%). Among the 3623 prior insulin users 17.1% started on a basal regimen, 33.3% started on premixes and 49.6% started with a basal-bolus regimen. As with the insulin-naïve users the majority of patients received analogues rather than human insulin (range 67.3-87.4% among regimen types; overall proportion receiving analogues 74.1%). Of the 541 patients starting on basal analogues, 60.4% were switched from neutral protamine Hagedorn (NPH) insulin and 10.2% from human premix. Of 812 patients starting on premix analogues, 33.0% were switched from human premix, 26.7% from NPH insulin and 26.1% from basal analogue. Of 1331 patients starting on basal-bolus analogues, 48.0% were switched from human basal-bolus and 13.2% from human premix.
Conclusion: Among patients with T2D starting a new insulin treatment the majority were prescribed an insulin analogue, which indicates that both general practitioners and internists in The Netherlands prefer long-acting insulin analogues or analogue premixes over human insulin.
Table 1. Proportions of patients starting on different insulin regimens and using human or analogue insulins within each regimen.

Table 1

	Insulin naïve N=7116		Prior users N=3623	
New regimen	**n**	**%**	**n**	**%**
Basal[a]	**3702**	**52.0**	**619**	**17.1**
Neutral protamine Hagedorn[b]	1444	39.0	78	12.6
Basal analogue[b]	2258	61.0	541	87.4
Premix[a]	**2324**	**32.7**	**1206**	**33.3**
Human[b]	1002	43.1	394	32.7
Analogue[b]	1322	56.9	812	67.3
Basal-bolus[a]	**1090**	**15.3**	**1798**	**49.6**
Human[b]	492	45.1	467	26.0
Analogue[b]	598	54.9	1331	74.0

[a]For each regimen type, % of overall naïve or prior-use patients is shown.
[b]Within each regimen type, % using human or analogue insulins in that type is shown.

Supported by: Novo Nordisk

909

Sex differences in insulin dose and postprandial glucose (PPG) as BMI increases in patients with type 2 diabetes (T2D)

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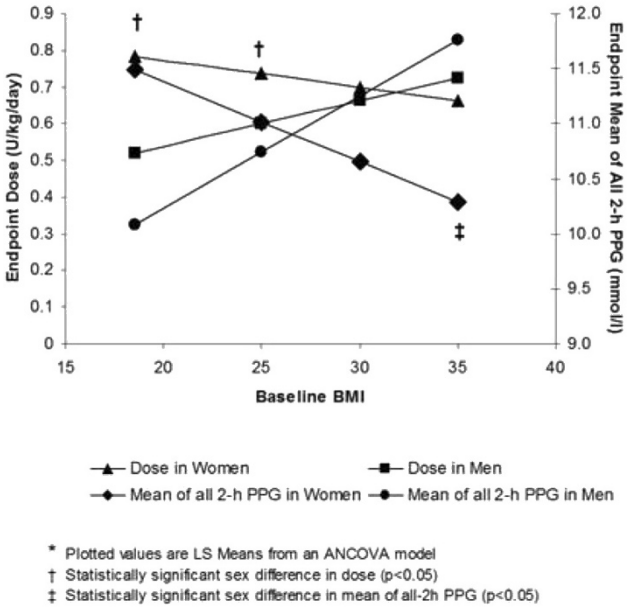
Background and aims: To make sex-specific comparisons of efficacy and safety, which are rarely included in the development and reporting of clinical trials (CTs) in T2D.
Materials and methods: This post-hoc analysis examined sex differences in efficacy and safety parameters within groups of patients on lispro mixtures twice daily (BID) (LMBID, n=319) or three times daily (TID) (LMTID, n=344), basal bolus therapy (BBT=glargine [G] with lispro TID, n=184), NPH (n=355),

or G (n=203). An integrated CT database was evaluated for CTs which were at least 12 weeks in duration and included only insulin-using T2D patients (±oral agents). Six such trials were identified and used for this analysis.
Results: At endpoint, with increasing baseline BMI, 2-h PPG (LMBID) and insulin dose (LMBID and NPH) were significantly different in women vs men (figure-LMBID), although change in HbA1c was not significantly different. For NPH-treated patients, weight gain was also greater in women than men (all LS mean±SE) (1.45±0.23 vs 0.21±0.29 kg; p=0.005). With LMTID, women had lower endpoint 2-h PPG after lunch and dinner than men (8.08±0.19 vs 8.66±0.18 mmol/l, p=0.028; and 8.55±0.21 vs 9.14±0.21 mmol/l, p=0.046, respectively), while women on LMBID and G had less hypoglycaemia (LMBID: 0.56±0.10 vs 0.87±0.11 episodes/patient/30 days, p=0.035; G: 1.06±0.19 vs 1.64±0.21 episodes/patient/30 days, p=0.042). BBT had no significant differences between sexes in any variables.
Conclusion: In conclusion, sex differences in response to insulin therapy in T2D appear to exist, and may have implications for the safety, efficacy, and optimal utilisation of insulin therapy. Thus, sex differences should be explored and considered in future CT design and analyses in diabetes.

Figure. Sex Difference: Predicted Endpoint Insulin Dose and PPG at Different Baseline BMI for LMBID Patients*



* Plotted values are LS Means from an ANCOVA model
† Statistically significant sex difference in dose (p<0.05)
‡ Statistically significant sex difference in mean of all-2h PPG (p<0.05)

Supported by: Lilly USA, LLC

910

Escalating insulin dose with intensified insulin therapy regimens may achieve greater glycaemic efficacy than basal-only therapy, particularly in obese type 2 diabetes patients

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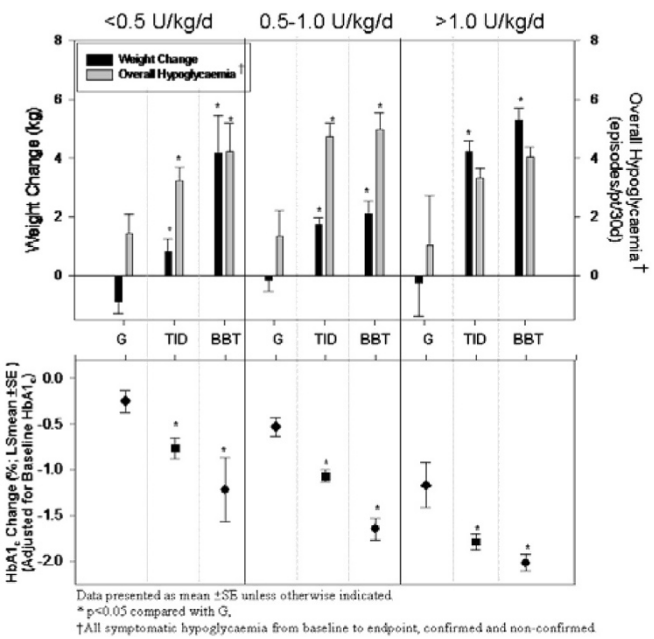
Background and aims: To investigate the relationship of endpoint insulin dose to baseline parameters, efficacy, and safety for different insulin regimens in type 2 diabetes (T2D).
Materials and methods: This pooled post-hoc analysis compared the effect of insulin dose and baseline BMI on change in $HbA1_c$, weight, and symptomatic hypoglycaemia between groups of T2D patients (pts) on glargine (G, n=159), lispro mixtures TID (LMTID, n=345), or basal bolus therapy (BBT=G with lispro TID, n=187). Two trials in an integrated CT database - one comparing G vs LMTID (n=317, baseline $HbA1_c$ 6.5-11.0%), and the other LMTID vs BBT (n=374, baseline $HbA1_c$ 7.5-12.0%)-met criteria of being 24 weeks in duration and including insulin-using T2D pts (±oral agents).
Results: For 3 insulin dose categories (≤0.5 U/kg/day [d], 0.5-1.0 U/kg/d, and >1.0 U/kg/d), the effects of G, LMTID, and BBT on change in $HbA1_c$ (adjusted for baseline $HbA1_c$), weight, and hypoglycaemia are shown in Figure 1. For reaching target $HbA1_c$<7.0%, therapies were similar when insulin dose was low (<0.5 U/kg/d), but for 0.5-1.0 U/kg/d, more pts on BBT reached $HbA1_c$<7.0% vs G (65% vs. 36%, p<0.05), and for >1.0 U/kg/d, more pts reached A1C<7.0% in both LMTID and BBT vs G (53% and 63% vs 21%, p<0.05). When pts were grouped by baseline BMI categories, insulin dose was larger with LMTID and BBT vs G (BMI<30 kg/m^2: 0.86±0.05 and 1.58±0.09 vs 0.54±0.07 U/kg/d; BMI ≥30 kg/m^2: 0.97±0.03 and 1.34±0.04 vs 0.63±0.05 U/kg/d; all LMTID and BBT p<0.05 vs G). $HbA1_c$ change mirrored insulin dose in LMTID and BBT vs G (BMI <30 kg/m^2: -1.0±0.1% and -1.7±0.2% vs -0.8±0.1%; BMI ≥30 kg/m^2: -1.4±0.06% and -1.6±0.08% vs -0.6±0.1%; all LMTID and BBT p<0.05 vs G). For BMI <30 kg/m^2, only BBT had more pts reach $HbA1_c$<7.0% vs G (74% vs 46%, p<0.05), while more pts reached $HbA1_c$<7.0% with both LMTID and BBT vs G at BMI ≥30 kg/m^2 (54% and 59% vs 32%, both p<0.05). LMTID and BBT also had significantly more weight gain and hypoglycaemia in both BMI categories (p<0.05). Severe hypoglycaemia did not differ between treatments at any insulin dose or baseline BMI category. A study limitation is that G and LMTID may have been less aggressively titrated in the first study than in the LMTID vs BBT study, which may understate the efficacy of G and LMTID and overstate the efficacy of BBT.
Conclusion: This post-hoc analysis suggests escalating insulin dose with intensified regimens may achieve greater $HbA1_c$ reduction and attainment of targets, especially in obese pts and those requiring ≥0.5 U/kg/d of insulin. Intensified insulin therapy may be associated with more weight gain and hypoglycaemia compared to basal-only therapy.



Data presented as mean ±SE unless otherwise indicated.
* p<0.05 compared with G.
†All symptomatic hypoglycaemia from baseline to endpoint, confirmed and non-confirmed

Supported by: Lilly USA, LLC

911

Basal insulin treatment of type 2 diabetes mellitus is associated with greater increases in satisfaction and HRQoL than treatment alternatives: an empirical review

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Background and aims: We examined current treatments for Type 2 diabetes mellitus (DM) to determine whether differences in patient-reported treatment satisfaction and HRQoL exist between three medication groupings (basal insulins, rapid acting insulin and oral agents).
Materials and methods: Data were obtained from an empirically-based systematic literature review of 280 randomized controlled trials (RCTs) of

treatments for Type 2 DM conducted since 1998. After restricting the articles to adult samples and removing duplicates, we identified 230 articles for consideration. Seventeen of these studies included measures of treatment satisfaction and/or health-related quality of life (HRQoL) in ways that allowed for the quantitative assessment of change-from-baseline treatment effects. Separate fixed-effects meta-analyses compared the standardized effect sizes of changes in patient outcomes within the RCT treatment arms of basal insulin (+/-OAD) vs. rapid acting insulin (+/- OAD) vs. oral agents.

Results: The effect sizes associated with change from baseline of treatment satisfaction outcomes were significantly greater among patients on basal dosing of intermediate and long acting insulin regimens (Std Diff in Means = .44 (95% C.I. .39 - .48) than the rapid acting insulin administered two or more times per day (Std Diff in Means = .15 (95% C.I. .091 - .20) or among patients on only oral medications (Std Diff in Means = .10 (95% C.I. .075 - .13). Within the injectable medication groups, treatment effects assessed across treatment satisfaction measures were larger than those associated with HRQoL measures (see Table).

Conclusion: Treatment satisfaction and HRQoL changes were highest among patients treated with basal insulin. We conjecture that Basal insulin may be associated with improved health status due to greater medication effectiveness; while greater patient satisfaction is due to less frequent dosing and lower self-monitoring requirements than regimens involving shorter acting insulin. Differences in the patient samples (e.g., by disease progression) could be an influential variable. Across all treatment types patients' responses to treatment satisfaction versus HRQoL scales may be differentially effected by response bias, since patients are often not blind to the efficacy of their medication, and by improvements in recently developed treatment satisfaction measures.

Patient Reported Dimension	Basal Insulin Std Diff in Mean (95% C.I.)	Rapid Insulin Std Diff in Mean (95% C.I.)	Oral Medications Std Diff in Mean (95% C.I.)
Treatment Satisfaction	.55 (95% C.I. .49 - .61) (8 studies, 1062 patients)	.29 (95% C.I. .18 - .38) (4 studies, 391 patients)	.05 (95% C.I. .008 - .098) (6 studies, 1871 patients)
Health Related QoL	.23 (95% C.I. .15 - .31) (4 studies, 327 patients)	.078 (95% C.I. .017 - .16) (4 studies, 231 patients)	.14 (95% C.I. .10 - .17) (4 studies, 698 patients)

Supported by: sanofi-aventis, US

912

Comparison of mealtime pramlintide, insulin, or a combination of both, when added to basal insulin treatment in patients with type 2 diabetes

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Background and aims: Addition of mealtime injections of insulin to basal insulin therapy can increase glycaemic control for many patients with type 2 diabetes (T2DM); however, this approach increases the risks of weight gain and hypoglycaemia. Pramlintide can improve glycaemic control and reduce weight without increasing insulin-induced hypoglycaemia; therefore the addition of prandial pramlintide instead of or in conjunction with prandial insulin may offer additional therapeutic options for T2DM patients using basal insulin. This study examined the safety and efficacy of intensification of basal insulin therapy with prandial pramlintide (PRAM, 120 µg TID), rapid-acting insulin (RAI), or both agents.

Materials and methods: This was a randomised, open-label, multicenter, 36-week study in patients with T2DM (N=112, age 54 ± 11 y, HbA_{1c} 8.2 ± 0.8%, FPG 8.9 ± 2.5 mg/dL, BMI 36 ± 6 kg/m^2, mean ± SD) and it consisted of 2 phases. Phase 1 (24 weeks) compared PRAM with RAI when added to basal insulin. Phase 2 (12 weeks) explored additional prandial therapy for patients failing to achieve HbA_{1c} ≤6.5% at W24 (PRAM patients added RAI at W26, n = 31; RAI patients added PRAM at W26, n = 36). Patients with HbA_{1c} ≤6.5% continued their Phase 1 treatment (PRAM, n = 17; RAI, n = 14).

Results: In Phase 1, PRAM and RAI resulted in similar HbA_{1c} reductions (-0.9 ± 0.2% vs -1.1 ± 0.2%; LS mean ± SE) and FPG (6.7 ± 0.4 vs 6.8 ± 0.3 mmol/L) at Week 24 (W24), however significant weight gain was seen with RAI treatment (RAI: 4.2 ± 0.6 kg, p<0.001; PRAM: -0.3 ± 0.7 kg, p = 0.978 vs baseline). Throughout Phase 2, HbA_{1c} levels were stable for PRAM (6.3 ± 0.1% at W24 vs 6.4 ± 0.1% at W36) and RAI subjects (6.1 ± 0.1% vs 6.4 ± 0.1%). Despite the addition of another prandial agent, HbA_{1c} levels also remained relatively stable for combinations (PRAM+RAI: 7.7 ± 0.2% at W24 vs 7.4 ± 0.2% at W36; RAI+PRAM: 7.3 ± 0.2% at W24 vs 7.4 ± 0.2% at W36). No significant weight change occurred in Phase 2. Addition of PRAM in patients using RAI allowed a reduced RAI dose (38.6 ± 3.8 U/d at W24 vs 19.4 ± 3.2 U/d at W36). The incidence of hypoglycaemia was lower with PRAM compared to RAI (55% vs 82% in Phase 1; 41% vs 71% in Phase 2) with no severe hypoglycaemia. Patients on combination prandial regimens experienced comparable hypoglycaemic rates (PRAM+RAI, 58%; RAI+PRAM, 53%). Nausea was observed in some patients initiating PRAM (21% in Phase 1; 11% in Phase 2).

Conclusion: Compared with RAI, PRAM added to basal insulin therapy resulted in similar improvements in glycaemic control, a lower risk of hypoglycaemia, and no weight gain. Addition of the alternate prandial agent in Phase 2 resulted in maintenance of HbA_{1c} and weight. Addition of PRAM in Phase 2 for subjects on RAI in Phase 1 allowed a decrease in daily RAI dosage.

913

Day-to-day variation of insulin requirements in type 2 diabetic patients with end-stage renal disease treated by maintenance haemodialysis

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Background and aims: We aimed to evaluate day-to-day variations of insulin needs in Type 2 diabetic patients with end-stage renal failure on maintenance haemodialysis (HD), as significant glycaemic excursions are frequent in this population.

Materials and methods: We developed a 24-hour euglycaemic clamp applicable to free living conditions in patients receiving 2200 calories with 55% carbohydrate in a standardized 3-meal and 2-snack regimen per day. Insulin was infused intravenously at variable rates, adjusted every 30 minutes to clamp the glycaemia at 5.55±1.11mmol/l over 24 hours pre-HD, during HD session, and 24 hours post HD. We studied 10 volunteers (6 men and 4 women, age: 55.7±8.7 years, duration of DM: 11.9±4.5 years, BMI: 22.7±4.5kg/m2, dry weight: 61.6±10.7 kg, duration of HD: 2.3±2.3 years).

Results: The mean capillary glycaemia was 5.5±0.3mmol/l pre-HD and 5.3±0.2mmol/l post-HD (p=0.39). This was achieved with the infusion of 23.6±7.7 IU/24h pre-HD vs. 19.9±4.9 IU/24h post-HD, indicating an overall 15.3% decrease (p=0.09). The basal insulin needs per hour were decreased significantly from 0.4±0.1/hour pre-HD to 0.3±0.1/hour post-HD (p=0.01). The total boluses were decreased by 2.2±3.1 IU (-18.6%, p=0.15). The change in blood urea induced by HD did not correlate with the change in daily insulin needs (r=0.1, p=0.79).

Conclusion: In conclusion, the 15% reduction in insulin needs to achieve normoglycaemia after versus before HD observed in these type 2 diabetic patients , being equivalent to almost 4 IU per day is likely to be clinically significant despite marginal statistical significance. Further investigations with intermittent subcutaneous protocol based on the present results are warranted to further guide clinical practice recommendations.

Supported by: North East Diabetes Trust, UK

PS 77 Safety of insulin therapy

914

Lower rate of hypoglycaemia but comparable glycaemic control with insulin detemir compared to NPH insulin in patients with type 1 diabetes

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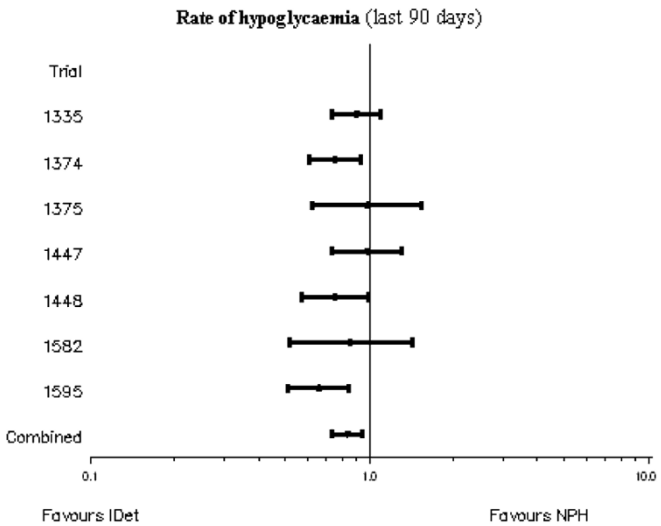
Background and aims: In clinical trials, focus is often on efficacy, and these trials are rarely powered for analysis of difference in hypoglycaemia between treatment arms. Here we wish to present a meta-analysis to explore the relationship between glycaemic control as measured by last recorded HbA_{1c} on treatment and hypoglycaemia (plasma glucose level < 3.1 mmol/L) in subjects with type 1 diabetes treated with either insulin detemir (IDet) or NPH insulin (NPH) once or twice daily as the basal component of a basal-bolus therapy.
Materials and methods: Data were collected from seven randomised clinical trials more than 16 weeks (112 days) in duration reported up to March 2008 and with individual patient data (IPD) available. The trials included 1801 subjects on IDet and 1080 on NPH; treatment was from 16 to 104 weeks. A negative binomial regression model was used to analyses the number of hypoglycaemic episodes (last 90 days of treatment), with trial and treatment as factors, HbA_{1c} as covariate and log-transformed exposure time as offset. No heterogeneity was found (p=0.33 for test of trial and treatment interaction).
Results: As shown in the Forest plot a significantly lower rate of hypoglycaemia was estimated with IDet than with NPH (p<0.0001) which amounted to a lower estimated rate of hypoglycaemia of 19%. A model of HbA_{1c} versus hypoglycaemia indicates that the lower the HbA_{1c}, the larger the difference between treatments for risk of hypoglycaemia.
Conclusion: IDet was associated with a lower rate of hypoglycaemia than NPH at all levels of glycaemic control (HbA_{1c}).

Figure 1



915

Estimating number-needed-to-treat with insulin glargine compared with NPH insulin to avoid a hypoglycaemic episode in people with type 2 diabetes mellitus: a meta-analysis

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Background and aims: Studies have shown that for similar blood glucose control, insulin glargine (glargine) is associated with a decreased risk of hypoglycaemia compared with NPH insulin (NPH) in type 2 diabetes mellitus (T2DM). We have evaluated the number of people needed to be treated (number-needed-to-treat [NNT]) with glargine vs NPH to save one person from experiencing hypoglycaemia based on a meta-analysis of trials comparing glargine with NPH.
Materials and methods: The formula $NNT=1/(p_N-p_G)$ was used, where p_N and p_G represent the risk of ≥1 hypoglycaemia episode in an NPH- or glargine-treated person, as derived from meta-regression adjusted for change in HbA_{1c}. Where NNT is large (>100), glargine is favoured over NPH, but the clinical advantage is small; where NNT is smaller (e.g. <20) the treatment difference is clinically important. Study selection criteria were: randomized, parallel-group design; basal glargine vs basal NPH (both with oral agents); comparable dosing regimens; ≥24-week duration. Hypoglycaemia rates (severe [study definition] or symptomatic with self-monitored levels of <2.0 or <3.9 mmol/l) were extracted and adjusted for HbA_{1c} change from baseline. Five comparative studies using evening injections (n=2641) were found and analyzed. A random effects model was used if there was significant heterogeneity (p≤0.100) between studies; otherwise a fixed effects model was used.
Results: Evening glargine vs NPH treatment resulted in a significantly lower risk of nocturnal hypoglycaemia for all categories (symptomatic <3.9 mmol/l, NNT=8, p<0.001 [OR=0.52, p=0.009]; symptomatic <2.0 mmol/l, NNT=107, p=0.008 [OR=0.44, p=0.003]; severe: NNT=112, p=0.047 [OR=0.52, p=0.0498]). Daytime symptomatic hypoglycaemia was to be numerically lower with glargine vs NPH (NNT=184, p=0.085 [OR=0.64, p=0.073] and NNT=35, p=0.136 [OR=0.88, p=0.136] with self-monitored plasma glucose <2.0 and <3.9 mmol/l, respectively), but these differences were not statistically significant.
Conclusion: Overall, these results confirm that glargine has the benefit of a risk reduction of nocturnal hypoglycaemia vs NPH, with one person in every eight treated avoiding a confirmed (self-monitored plasma glucose <3.9 mmol/l) nocturnal event, and additional avoidance of other events at lower frequency.
Supported by: sanofi-aventis

916

Examining the glycaemic and hypoglycaemic benefits with rapid-acting insulin analogues: a meta-analysis of insulin aspart versus regular human insulin in randomised controlled trials

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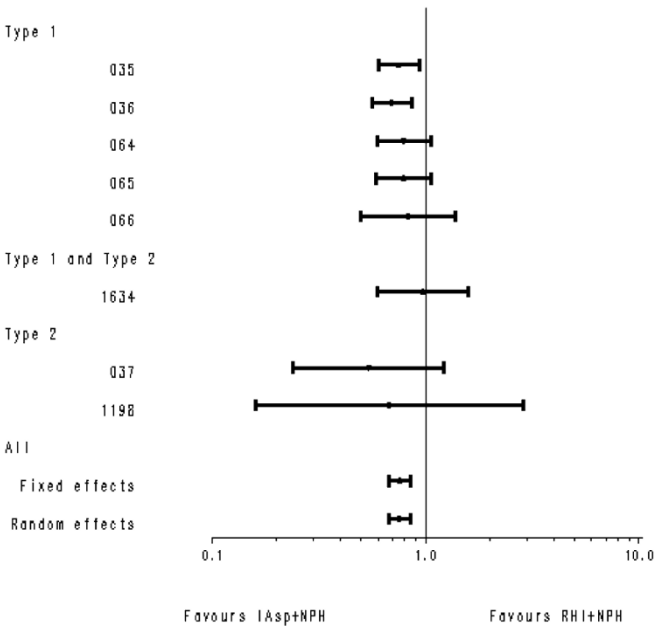
Background and aims: A meta-analysis of the efficacy and safety of insulin aspart (IAsp) vs regular human insulin (RHI) in a basal-bolus regimen with NPH insulin in subjects with type 1 or type 2 diabetes was undertaken with focus on a comparison of rate of hypoglycaemia.
Materials and methods: Data were collected from 10 randomised, controlled clinical trials (≥ 12 weeks) including 3727 subjects (53.9% male), with mean age 41.4 year, mean body mass index 25.5 kg/m² and mean HbA_{1c} 8.2% at baseline. Change in HbA_{1c} was analysed in a linear mixed model using repeated measurements and a spline for time, with a knot at 12 weeks controlling for trial. Hypoglycaemic episodes were modelled using negative binomial regression.
Results: The model found a significant difference in HbA_{1c} in favour of IAsp+NPH after 12 weeks of treatment, with a fixed estimate = -0.10 CI [-0.15; -0.04], a difference that was maintained over the next 12 weeks. A statistical test showed that heterogeneity was not significant (see figure). After 12 weeks the average prandial blood glucose increment was significantly lower

with IAsp+NPH, overall estimate = -0.61, CI [-0.88; -0.34] whereas no group difference was found for change in fasting plasma glucose. The incidence of hypoglycaemic episodes (overall and daytime) was similar in both groups, but the risk of nocturnal episodes was significantly lower with IAsp+NPH; overall estimate = 0.76, CI [0.67; 0.85].
Conclusion: After 12 weeks of treatment and onwards, glycaemic control was modestly but significantly improved with IAsp+NPH compared to RHI+NPH. The risk of overall and daytime hypoglycaemia was similar with the two regimens, but with IAsp+NPH the risk of nocturnal hypoglycaemia was significantly lower by about 25%, a finding not reported in previous systematic reviews or meta-analyses



917

Basal/bolus with prandial inhaled Technosphere insulin plus insulin glargine vs biaspart 70/30 insulin in type 2 diabetes inadequately controlled on insulin with/without oral agents

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Background and aims: Technosphere Insulin (TI) is a fast-acting inhaled insulin with a pharmacokinetic profile well suited for earlier control of postprandial plasma glucose (PPG). This randomized, active-control, parallel-group study compared the efficacy and safety of basal/bolus prandial TI plus bedtime glargine insulin (G) vs. premixed biaspart 70/30 insulin BID (BPA 70/30) in type 2 diabetes mellitus inadequately controlled (HbA_{1c} >7.0% and ≤11.0%) despite insulin with or without oral antihyperglycemic therapy.
Materials and methods: Subjects were randomized to a 52-week course of TI+G (n=334) or BPA 70/30 (n=343) with insulin adjustments according to investigator discretion to achieve predefined glycemic goals but without enforcing a structured titration regimen. Primary outcome was change in HbA_{1c}. Secondary objectives were proportion of subjects reaching specific HbA_{1c} levels, PPG, and fasting plasma glucose (FPG).
Results: Mean baseline characteristics were similar for TI+G and BPA 70/30 (age 55.9, 55.9 years; disease duration 13.1, 13.6 years; baseline HbA_{1c} 8.7%, 8.7%; BMI 31.55, 31.07 kg/m^2). HbA_{1c} was reduced by 0.58% and 0.70% in the TI+G and BPA 70/30 groups (intent-to-treat last observation carried forward), respectively, and the proportion of subjects achieving HbA_{1c} <7.0% were comparable between treatments (22% vs. 27%). Mean FPG at week 52 was 7.8 mmol/l for the TI+G group and 8.7 mmol/l for the BPA 70/30 group, and the FPG change from baseline was 2.0 vs. 1.0 mmol/l (p=0.0029). The absolute 1-h PPG (9.5 vs. 11.6 mmol/l; p<0.0001) was significantly lower with TI+G. TI+G produced significantly less weight gain (0.9 vs. 2.5 kg; p=0.0002) and significantly less mild/moderate and severe hypoglycemia (Table). The final insulin doses were TI, 198 U (approximately equivalent to 53 IU of rapid-acting insulin); G, 47 IU; and BPA 70/30, 88 IU. Mean changes from baseline to week 52 in forced expiratory volume, forced expiratory vital capacity, and carbon monoxide diffusing capacity were similar in the two groups.
Conclusion: TI+G vs. BPA 70/30 resulted in comparable HbA_{1c} reductions but lower 1-h PPG with less weight gain and less hypoglycemia.

Hypoglycemia Incidence, Event Rate, and Odds Ratio

Hypoglycemia	Incidence (%)		Event Rate		Odds Ratio	Odds Ratio *p* Value
	TI+G	BPA 70/30	TI+G	BPA 70/30		
Mild/moderate	47.99	68.88	0.40	0.59	0.417	<0.001
Severe	4.33	9.97	0.01	0.02	0.409	<0.007
Total	47.99	68.88	0.41	0.61	0.417	<0.001

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918

Reduced incidence and frequency of hypoglycaemia in an integrated analysis of pooled data from clinical trials of subjects with type 2 diabetes using prandial inhaled Technosphere insulin

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Background and aims: Technosphere Insulin (TI) is a rapid-acting insulin with a pharmacokinetic profile well suited for earlier control of postprandial plasma glucose (PPG). This integrated analysis includes the pooled data from six phase II/III clinical trials in subjects with type 2 diabetes mellitus inadequately controlled (HbA_{1c} ≥6.6% and ≤12.0%) despite insulin with or without oral antihyperglycemic therapy.
Materials and methods: Subjects were randomized to treatment regimens to achieve predefined glycemic goals: TI (n=1,795) or s.c. insulin (n=942), which included BPA 70/30, or insulin aspart and "usual care," with insulin adjustments according to investigator discretion in five trials and forced titration in one trial. A structured titration regimen was not enforced. When experiencing hypoglycemic-like symptoms, subjects were instructed to confirm the event with a blood glucose reading. Subjects experiencing a severe hypoglycemic episode were required to report the details of third-party assistance (if needed), the presence of neurologic symptoms, and the specifics of treatment.
Results: Mean baseline characteristics were similar for TI and s.c. insulin (age 56.2, 55.6 years; disease time since diagnosis 10.8, 12.4 years; baseline HbA_{1c} 8.82%, 8.84%; BMI 31.07, 31.07 kg/m^2). Subjects treated with TI experienced statistically significantly fewer hypoglycemic episodes in regard to both incidence and frequency compared with subjects treated with s.c. insulins. For incidence, significantly fewer subjects reported hypoglycemia with TI: 31.8% vs. 49.6% for total hypoglycemia; 31.6% vs. 49.4% for mild/moderate hypoglycemia; and 2.8% vs. 7.5% for severe hypoglycemia, with the three comparison p values <0.0001. For frequency, TI also had significantly fewer events, evaluated by event rate (number of events per 100 subject-months): 23.87 vs. 38.78 for total hypoglycemia (p<0.0001); 23.16 vs. 37.32 for mild/moderate hypoglycemia (p<0.0001); and 0.66 vs. 1.37 for severe hypoglycemia (p<0.0184).
Conclusion: TI, often in combination with a basal insulin, consistently reduced the incidence and frequency of both mild/moderate and severe hypoglycemic events under conditions of comparable glycemic control. The reduction in the risk of hypoglycemia in patients with type 2 diabetes under inadequate control may facilitate the introduction of insulin therapy.

Hypoglycemia	Incidence(%)		Odds Ratio	Odds Ratio *p* Value	Event Rate (per 100 subject-months)		Event Rate *p* Value
	TI	s.c. insulin			TI	s.c. insulin	
Mild/moderate	31.6	49.4	0.466	<0.0001	23.16	37.32	<0.0001
Severe	2.8	7.5	0.359	<0.0001	0.66	1.37	0.0184
Total	31.8	49.6	0.466	<0.0001	23.87	38.78	<0.0001

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919

Pulmonary function tests remain similar in patients who received Technosphere insulin and in patients currently receiving standard antidiabetic therapy

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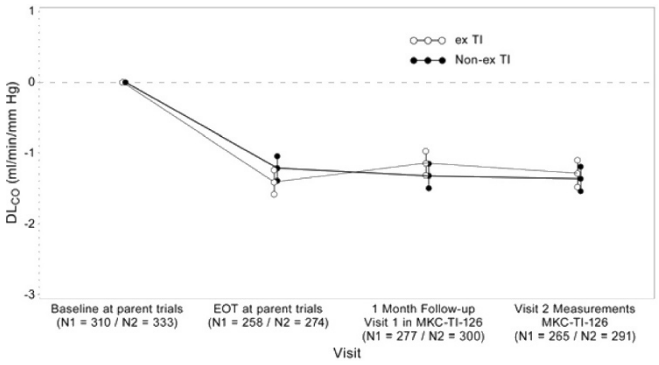
MannKind Corporation, Valencia, United States.

Background and aims: Previous controlled clinical studies have demonstrated that regimens of basal insulin plus Technosphere Insulin (TI) were as effective as basal insulin plus rapid-acting s.c. insulin in patients with diabetes. In previously reported studies, we have been unable to detect a consistent change in pulmonary function tests (PFTs). Small but clinically non-significant differences have been observed. This clinical trial was designed to assess the changes in pulmonary function after cessation of TI therapy and resumption of standard antidiabetic treatment in patients with type 1 or type 2 diabetes.
Materials and methods: Adults with diabetes who participated in any of four controlled clinical trials of TI were invited to participate in this follow-up trial to evaluate changes in PFTs after completing the study and being switched to usual antidiabetic therapy without TI. Patients were followed for a total of 3 months after cessation of study therapy. PFTs were assessed at the end of the parent trial and 1 and 3 months after subjects completed the parent trial.
Results: Of 649 patients in this study, 315 subjects (121 with type 1 diabetes, 194 with type 2 diabetes) received TI and 334 subjects (129 with type 1 diabetes, 205 with type 2 diabetes) received the antidiabetic regimen without prandial TI during the parent trials. Small, non-progressive treatment group differences in mean changes from baseline in forced expiratory volume in 1 second (FEV_1) and carbon monoxide diffusing capacity (DL_{CO}) observed during the comparative phase of the controlled trials disappeared when comparing the two groups at 3 months after cessation of TI therapy and resumption of standard antidiabetic therapy (FEV_1: -0.08 l in the TI group, -0.11 l in the non-TI group [p=0.1388]; DL_{CO}: -1.29 ml/min/mm Hg in the TI group, -1.37 ml/min/mm Hg in the non-TI group [p=0.9360]). In addition, there was no statistical difference in FEV_1 between the two groups when examining subjects with type 1 and type 2 diabetes (p=0.6158 and p=0.1795, respectively).
Conclusion: These data suggest that the pattern and magnitude of PFT changes associated with the use of TI in subjects with type 1 and type 2 diabetes are not likely due to any structural alterations in the lungs and are not clinically meaningful.



920

Pulmonary functions (over 2 years) in diabetic subjects treated with Technosphere insulin or usual antidiabetic treatment

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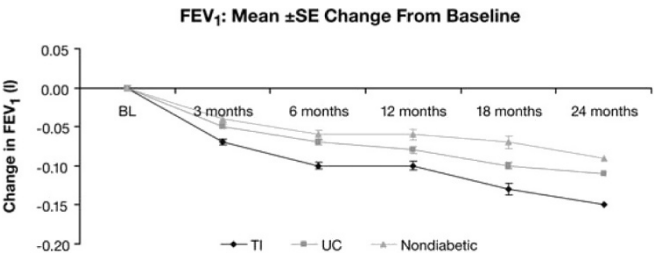
Background and aims: Technosphere Insulin (TI) is a rapid-acting inhaled insulin with an action profile that mimics early meal-related insulin release. The goal of this multicenter study was to evaluate and compare changes in lung function in diabetic subjects treated with TI or with usual antidiabetic treatment (UC).
Materials and methods: Pulmonary function tests (PFTs), including forced expiratory volume in 1 second (FEV_1), forced vital capacity, total lung capacity, and carbon monoxide diffusing capacity (DL_{CO}), were prospectively followed over a 2-year period in subjects with type 1 and type 2 diabetes mellitus receiving TI (n=730) or UC (n=824), along with a cohort of nondiabetic subjects who did not receive any specific therapy (n=145).
Results: The treatment groups were similar for age (TI group: 50.8±11.6 years; UC group: 50.4±11.6 years; p=0.40), gender distribution (p=0.85), proportion of ethnic group composition (p=0.93), and baseline PFT. Over 2 years, PFTs declined in all groups, including the nondiabetic group (Figure). TI was non-inferior to UC for mean change in FEV_1 from baseline to 24 months using Mixed Model Repeated Measure analysis with a prespecified non-inferiority margin of 50 ml/year. After a slightly larger initial decline at the first post-baseline assessment visit, annual rate of change (slope) in PFTs from month 3 to month 24 was not statistically different between the TI- and UC-treated subjects. Annualized change in FEV_1 between 3 to 24 months was -0.047 l/year for TI-treated subjects and -0.036 l/year for UC-treated subjects (difference between the treatment groups, 0.010 l/year [95% CI -0.003, 0.022]); DL_{CO} was -0.507 ml/min/mm Hg per year for TI-treated subjects and -0.455 ml/min/mm Hg per year for UC-treated subjects (difference between the treatment groups, 0.117 ml/min/mm Hg per year [95% CI -0.058, 0.292]).
Conclusion: Small declines from baseline in PFTs were observed in diabetic subjects treated with TI and UC and also in subjects without diabetes. Observed differences between TI and UC groups in the change from baseline in FEV_1 and DL_{CO} were small, noted at the first post-baseline assessment visit (3 months) and thereafter remained non-progressive over 2 years of continuous therapy.



921

Pulmonary safety of inhaled Technosphere insulin therapy in adults with diabetes using high-resolution computerized tomography of the chest

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MannKind Corporation, Valencia, United States.

Background and aims: Technosphere Insulin (TI) is a rapid-acting inhaled insulin with pharmacokinetics well suited for control of postprandial plasma glucose. Because TI is intended to be administered via the pulmonary route, the TI clinical program was designed to assess possible radiological changes associated with the chronic use of TI therapy.
Materials and methods: Adult subjects with diabetes were evaluated with high-resolution computerized tomography (HRCT) of the chest during the clinical trials. In controlled clinical trials, MKC-TI-005 (N=217; 174 TI group, 43 Technosphere inhalation powder [TIP] group) and PDC-INS-0008 (N=121; 60 TI group, 61 TIP group) subjects were randomized to receive TI or TIP without insulin. Chest HRCTs were obtained at baseline and at the end of the 12-week treatment period. After completion of these two trials, 206 subjects continued in the uncontrolled open-label extension trial (MKC-TI-010) and underwent annual chest HRCTs (or magnetic resonance imaging [MRI] in Germany) for up to 4 years. In addition, subsets of subjects with type 1 or type 2 diabetes participating in trial MKC-TI-030 (n=127; 55 TI group, 72 usual care [UC] group) were also randomized to an annual chest HRCT during the 24-month treatment period. All HRCT images were reviewed centrally following a prespecified adjudication protocol by an independent, blinded, board-certified radiologist. All images for any subject with HRCT findings other than normal underwent secondary joint review by an independent board-certified radiologist (different from the primary reviewer) and an independent board-certified pulmonologist, who were blinded to the treatment group.
Results: A total of 667 subjects had a baseline and at least one post-baseline chest HRCT or MRI examination. Of these, 494 subjects were treated with TI, 101 were exposed to TIP, and 72 were in the UC group with no exposure to TI or TIP. Chest HRCTs for 94% of the TI group, 92% of the TIP group, and 96% of the UC group showed normal findings or the findings were not clinically significant (Table). Radiological findings considered abnormal and clinically significant consisted of atelectasis, septal thickening, peri-bronchial thickening, bronchial dilatation or mild bronchiectasis, one or more new or non-enlarging nodules, and ground glass densities; these findings were seen with comparable frequency in all three treatment groups.

Conclusion: Overall, HRCT and MRI findings suggest that there were no clinically significant radiological changes from baseline in all three groups. Observed radiological findings were not suggestive of a safety signal with the long-term use of TI therapy.

Total Subjects with HRCTs (N=667)

HRCT Results	Technosphere Insulin n (%)	Technosphere Inhalation Powder n (%)	Usual Care n (%)
Normal	140 (28.3)	40 (39.6)	14 (19.4)
Abnormal, not clinically significant	325 (65.8)	53 (52.5)	55 (76.4)
Abnormal, clinically significant	29 (5.9)	8 (7.9)	3 (4.2)
	494	**101**	**72**

922

Fears of hypoglycaemia and complications in insulin-treated patients with diabetes in Japan: preliminary results of a multi-center study

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Background and aims: Insulin-treated patients with diabetes seem to have many kinds of fear in their daily lives. Especially, fears of hypoglycemia and diabetic complications are considered major concerns of them. The aim of this study was to investigate which fear is mostly concerned among the fears of hypoglycemia, severe hypoglycemia, blindness, and dialysis in insulin-treated type 1 and type 2 patients by a multi-center cross-sectional survey in Japan.
Materials and methods: The study design was cross sectional multi-center study using questionnaire. As of February 2008, 15 hospitals or clinics throughout Japan joined this study. The questionnaire was originally developed and consisted of occurrence of hypoglycemia (including severe and nocturnal hypoglycemia); fears of hypoglycemia, severe hypoglycemia, blindness, dialysis; and socio-demographic factors (gender, age, height, weight, duration of diabetes, duration of insulin therapy, details of the therapy, status of complications, HbA1c, and so on). The strength of fear was measured by a 5-point Likert scale with 1 never feel fear, and 5 strongly feel fear. In this study, hypoglycemia was defined as a low blood glucose level less than 50mg/dl by meter or any hypoglycemia-related symptoms and severe hypoglycemia was defined as a low blood glucose level that results in loss of consciousness or requiring the treatment from another person.
Results: By the end of February 2008, 554 patients and their physicians at outpatient department of 15 hospitals or clinics had completed a questionnaire. The response rate was 75%. The total number of subjects was 554 (193 type 1 and 361 type 2, mean age 57 years, male 52%, mean duration of diabetes 16.9 years, mean duration of insulin therapy 7.3 years, and mean HbA1c 7.3%). Fear scores were 3.7 for hypoglycemia, 4.3 for severe hypoglycemia, 4.7 for blindness, and 4.7 for dialysis on a scale of 1 to 5. One way analysis of variance showed no significant difference between the fears of blindness and dialysis. However, there was significant difference between complications and severe hypoglycemia ($p<0.05$). There was also significant difference between severe hypoglycemia and hypoglycemia ($p<0.05$). Additionally, using logistic regression analysis, two factors such as an experience of severe hypoglycemia in the past year and age were significantly associated with an increased likelihood of fear of hypoglycemia (odds ratio = 3.5 and 1.0, respectively). Other factors such as gender, type of diabetes, duration of diabetes, presence of diabetic neuropathy and level of HbA1c were not significantly associated with fear of hypoglycemia.
Conclusion: Insulin treated patients with diabetes in Japan had stronger fears of complications than of hypoglycemia (blindness = dialysis > severe hypoglycemia > hypoglycemia). However, fear of hypoglycemia was definitely not low. Additionally, an experience of severe hypoglycemia in the past year was a major factor for fear of hypoglycemia. Thus, it seems to be critical for patients and health professionals to take various measures to reduce severe hypoglycemia.
Supported by: Health Labour Sciences Research Grant from Ministry of Health, Labour and Welfare, Japan

PS 78 Continuous glucose monitoring

923

Continuous glucose monitoring versus self-monitoring of blood glucose in non-pregnant patients with type 1 diabetes mellitus: meta-analysis and systematic review

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Background and aims: Continuous glucose monitoring (CGM) provides information about the direction and magnitude of changes in blood glucose levels and thus theoretically facilitates optimal treatment decisions for patients with type 1 diabetes mellitus (T1DM). However, possible beneficial effects in terms of glycaemic control remain to be evaluated. The aim of this review was to compare the effects of CGM to self-monitoring of blood glucose (SMBG) on glycaemic control and the risk of hypoglycaemic episodes.
Materials and methods: A systematic literature search for controlled clinical trials was conducted in the electronic databases MEDLINE, EMBASE and CENTRAL up to November 2008 and a systematic review and meta-analyses were performed.
Results: From 18 studies fulfilling the inclusion criteria 6 did not provide sufficient data on HbA1c and 2 more had to be excluded because the authors did not report data separate for adults and children. Out of the 10 remaining studies that could be included in the analyses of the HbA1c difference during follow-up, 2 provided data on adult patients, 6 on children only, and 2 on both age groups. The juvenile subgroup (n=110) of one trial included adults as well and therefore was not included in our analyses. All trials were published after the year 2000, and the follow-up periods of those relevant for the meta-analyses ranged from 3 to 6 months. Based on their methodological quality, many studies were judged to be at risk for bias. *Adults with T1DM (n=318)*: CGM use resulted in a statistically significant but clinically debatable difference in the change of HbA1c of -0.38% [-0.69, -0.06] (p=0.02) compared to SMBG. There was evidence of heterogeneity among the studies (I^2=69%). Severe hypoglycaemia was comparable between the groups and occurred in 0 to 10% of CGM patients and in 0 to 9% of patients in the SMBG groups. *Children with T1DM (n=514)*: No significant difference between the intervention groups regarding the change in HbA1c during follow-up could be shown (-0.04% [-0.23, 0.16] (p=0.71)). The percentage of patients experiencing at least one severe hypoglycaemic episode was comparable in both groups: 0 to 7% (CGM) vs. 0 to 10% (SMBG). Since reporting on mild hypoglycaemia and blood glucose fluctuation was scarce and heterogeneous for both adults and children, it was not appropriate to conduct a meta-analysis or reach a conclusive interpretation.
Conclusion: Use of CGM in the therapy of adults with T1DM resulted in a significant but clinically debatable reduction of HbA1c compared to SMBG, seemingly with a comparable rate of severe hypoglycaemia. In children with T1DM, CGM provides similar glycaemic control when compared to SMBG. The rates of severe hypoglycaemic episodes were comparable. More and larger long-term studies are still needed to allow a firm conclusion about whether CGM use in the therapy of T1DM provides an additional beneficial effect compared to SMBG.
Supported by: Roche Diagnostics

924

Comparison of three different techniques for subcutaneous glucose monitoring in type 1 diabetic patients

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Background and aims: Continuous subcutaneous glucose monitors might be important tools in the treatment of diabetes, revealing 24-hour glucose

patterns leading to a better adjustment of insulin therapy, and also in the establishment of closed loop systems. The aim of the present study was to investigate and compare the accuracy between two marketed products and a standard microdialysis sampling technique with reference measurements from arterialized venous blood in type 1 diabetic patients.
Materials and methods: Seven type 1 diabetic patients (age: 32.7 ± 8.1 years; BMI: 25.0± 1.8 kg/m²; diabetes duration: 14.6 ± 7.0 years) were investigated over a period of 26 hours in an inpatient setting. For subcutaneous glucose monitoring, the Guardian RT system (subcutaneous glucose sensor; GRT), the GlucoDay S system (microdialysis based extracorporeal glucose sensor; GDS) and a standard microdialysis catheter (MD) for sampling of interstitial fluid were applied. For reference glucose measurements, arterialized venous samples were obtained in frequent intervals and analyzed using a Beckman Analyzer. GRT and GDS systems were calibrated according to manufacturers' instructions. Measurements from interstitial fluid were analyzed using a Cobas Mira analyzer and prospectively calibrated to reference six hours after catheter insertion.
Results: Whereas all MD systems could be applied without restrictions, 2 subjects of GRT and 1 subject of GDS were not included in the analysis due to malfunction of the devices. Mean glucose difference [2SD] between reference blood glucose vs. interstitial glucose readings were as follows: GRT -10.5 [41.8]%; GDS 20.2 [55.9]%; and MD 6.5 [35.2]%, respectively. Median absolute relative difference (MARD) was 15.0[5.6; 23.4]% (median[IQR]) for GRT, 19.7[6.1; 37.6]% for GDS and 8.7[4.1; 18.3]% for MD, respectively.
Conclusion: Individual glucose readings indicated substantial deviations from reference blood glucose. Although the systems appear adequate for retrospective data analysis or as an adjunctive measure to reference glucose measurements, subcutaneous measurement technologies require substantial improvement until therapeutic decisions can be drawn from individual glucose readings.
Supported by: the European Commission as part of the CLINICIP project

925

Accuracy of a novel intravascular fluorescent continuous glucose sensor in an 8 hour outpatient clinical feasibility study
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Background and aims: The results of the Nice-Sugar study have raised questions about the appropriate target levels for tight glycemic control (TGC) in ICU patients. The increase in mortality in the intensive control group may have been related to the fourteen-fold increase in episodes of severe hypoglycemia (< 40 mg/dL) compared with the conventional control group. A continuous glucose sensor (CGM), accurate in the hypoglycemic range, has been cited as a new technology that could permit successful implementation of TGC in the critically-ill in the future without increasing the risk of severe hypoglycemia. The purpose of the study was to assess the accuracy of a novel fluorescent intravascular CGM, the GluCath, in human subjects, especially in the hypoglycemia range.
Materials and methods: The sensor is based on quenched-fluorescence using a modified boronic-acid glucose receptor. The sensor chemistry is immobilized in a hydrogel matrix and placed on the distal tip of an optical fiber which can be inserted in a blood vessel. In the presence of glucose, the bond between the quencher and the fluorophore is weakened resulting in an increase of fluorescence. In contrast to electrochemical enzymatic sensors, the GluCath fluorescent sensor exhibits a nonlinear modulation response function giving the highest level of accuracy in the hypoglycemic range. Red blood cells are excluded by a microporous membrane surrounding the sensor and by the hydrogel, resulting in a measurement of plasma glucose. The sensor was evaluated in an 8-hour outpatient feasibility study. The sensor was placed in a peripheral vein at the antecubital fossa in five subjects with type 1 diabetes. Sensor insertion was performed using a 22 Ga needle and a retractable cannula.
Results: One sensor failed during insertion due to mechanical damage. Data from the other four sensors were taken at one-minute intervals and compared with hospital and laboratory blood glucose measurements from venous samples in the contralateral arm every 15 minutes. Data was analyzed retrospectively using a temperature-corrected factory calibration with a one-point in vivo adjustment made within thirty minutes of the sensor insertion. The GluCath results were compared with measurements made on an ABL Radiometer 805, a Yellow Springs Instrument analyzer 2300 StatPlus and the LifeScan SureStep Flexx Pro. Comparison of the GluCath results to the Radiometer values found the GluCath accuracy met the performance standard in ISO-15197: 100% (30/30) of all values </= 75 mg/dL were within ±20% of the reference values. The mean absolute relative difference (MARD) was 8.95%.
Conclusion: This is the first use in human subjects of a fluorescent glucose sensor. The sensor was found to be highly accurate compared with laboratory reference measurements, especially in the hypoglycemic range. Sensors of this type may be useful in the future in permitting safe and effective implementation of TGC protocols in the critically-ill and other hospitalized patients.
Supported by: Glumetrics

926

Intensification of therapy and better glucose control after the continuous use of real time glucose monitoring combined with insulin pump (PRT system) in type 1 diabetes mellitus
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Background and aims: JDRF- Study demonstrated that the continuous use of REAL- Time Continuous Glucose Monitoring (RT-CGM)can be associated with improved glycemic control in Type1 Diabetes. PRT is an Insulin Infusion Pump combined with a RT-CGM. The aim of our study was to evaluate the effect of PRT on glycemic control, glucose fluctuations and insulin requirements for a long period of time.
Materials and methods: A total of 11 patients, mean age 34.0±20.3 years and diabetes duration 16.7± 9.1 years (X± SD), wore the Sensor-Augmented Pump system continuously for 12 months. Eight of them continued the continuous use for 18 months. All subjects for educational purposes were only on RT-CGM during the 1th month (remained on MDI). The Pump therapy was added the 2th month. HbAlc, average monthly glucose, hypo/hyperglycemic excursions, total daily dose (TDD) and mean units/bolus were evaluated at baseline, 4, 6, 12 and 18 months respectively
Results: HbAlc(%) decreased from 8.14±1.70 at baseline, to 6.88±0.58 and 6.71±0.37 after 4 and 12 months (p=0.019). Glycemic control improved from the beginning and the average Daily and Monthly Glucose Values did not significantly differ after 1, 4, 6, and 12 months. The average Area Under the Curve (AUC) above 180 mg/dl*24h tend to be reduced after the introduction of Pump and remained lower at 12th month. Average AUC below 70mg/dl remained constant throughout the study period. Severe hypoglycemia occurred only once! No significant weight gain was noticed. There was a statistically significant decrease of TDD of Insulin from 53.27±7.90 in baseline to 45.22±14.19 and 43.87±14.30 units at 4 and 12 months. In addition the TDD of Bolus Insulin decreased gradually despite the increased number of boluses given by the patients in order to be proactive to high glucose excursions. (Table1).

Table I: 11+8 patients, 1 year and 18 months continuous use

	11 patients				8 patients				
	Baseline	4 months	12 months	p	Baseline	4 months	12 months	18 months	p
Mean Glucose (mg/dl)	142.2	142.6	140.1		148.8	149.8	143.5	147.3	
Std Deviation (mg/dl)	52.1	51.0	47.4		54.8	52.6	48.6	53.5	
AUC above 180 mg/dl (mg/dl*24h)	9.75	8.86	7.70		11.98	10.79	8.28	10.18	
AUC below 70 mg/dl (mg/dl*24h)	0.47	0.33	0.51		0.45	0.33	0.46	0.40	
HbA1c (%)	8.15	6.88	6.71	0.019	8.65	7.11	6.83	6.86	0.022
TDD (units)	53.27	45.22	43.87	0.022	54.00	43.85	44.14	46.44	ns
No Bolus	3.00	6.17	6.82	<0.001	3.00	6.17	6.70	6.85	<0.001
Mean Units/ Bolus	8.29	4.40	3.25	<0.001	8.52	3.48	2.93	2.69	0.001

These results are significantly certified in the group of 8 patients after 18 months of use. HbA1c and glucose variability remain stable. We observed a further statistically significant increase of the number of bolus/day between 4th and 18th month of use. (6.17±2.03 vs 6.85±1.66, p<0,05) and at the same time a decrease of the mean units/bolus (3.48±0.66 vs 2.69±1.31 p<0,05)
Conclusion: The continuous use of PRT provides a useful tool to normalize glycemic control in T1DM. It decreases HbA1c and AUC of high glucose excursions. To derive full potential benefit from RT-CGM, patients were driven by the system to the intensification of insulin therapy with more frequent bolus but with less units/bolus. As the time pass the patient learns to react correctly to the predictive alarms of high and low excursions and remains motivated to achieve his targets for a long period of time

927

Evaluation of the effect of different sensor insertion methods on the accuracy of a continuous glucose monitoring system
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Background and aims: Continuous glucose monitoring may offer the important advantages of decreasing the risk of severe hypoglycemia and improves the adjustment of insulin therapy in type 1 diabetes. Recently several useful continuous glucose sensing devices have been brought to market. Most of them measure glucose in subcutaneous adipose tissue (SAT) by applying an enzyme-tipped catheter sensor. Placing the catheter sensor in the SAT is usually performed with the help of the inserter provided by the sensor manufacturer. The sensor insertion is then typically followed by a run-in period during which sensor sensitivity might be attenuated and unstable. It has been shown that the duration of the run-in period may last up to ten hours before the sensor attains steady-state glucose sensing conditions. One explanation for this prolonged run-in period may be an increased tissue trauma caused by the commercial product sensor inserter. The aim of the present study was to compare a commercial product sensor insertion technology with a manual, less invasive sensor insertion method.
Materials and methods: After an overnight fast, four identical glucose sensors were inserted in the SAT of 15 healthy volunteers, whereby two of the sensors were placed using a commercial product sensor inserter (AUTO_1, AUTO_2) and two were inserted manually by lancing the skin with a 0.6 mm needle and placing the sensor into the pierced tissue (MAN_1, MAN_2). The glucose data obtained from each sensor were calibrated against the plasma glucose value obtained 30 minutes after sensor insertion and the glucose sensor data were recorded continuously over a period of 11 hours. To quantify possible sensitivity changes we calculated the time courses of the Normalized Glucose Sensitivity (NGS) for each sensor as the ratio between the observed sensor glucose and plasma glucose values, normalized to the median sensitivity value obtained during the last hour of the experiment (hour 10 to 11).
Results: The glucose concentration measured with the AUTO-sensors fell rapidly during the first 3h after insertion, and then declined more slowly during the remainder of the study. A comparable behavior was observed for the MAN-sensors, however the rate of decline during the first 3h was less pronounced. By the end of the experiment glucose concentrations of the AUTO- and MAN-sensors were 49.3 ± 4.2 mg/dl and 58.9 ± 2.5 mg/dl, respectively. Both concentrations were significantly lower than the corresponding plasma glucose concentration (78.6 ± 1.1 mg/dl, p<0.01). This pattern of decline was also reflected in the shape of the derived NGS time courses, where during the first 3h the NGS for AUTO- and MAN-sensor fell rapidly to a value of 73.5 ± 4.8% and 87.6 ± 3.0% of the initial NGS value, respectively. Thereafter the NGS of both sensors decreased more slowly with comparable rates. By the end of the study, at 11h, the final NGS values for AUTO- and MAN-sensors were 63.1 ± 5.2% and 75.1 ± 3.3% of the initial NGS value (p<0.01).
Conclusion: The results obtained from these experiments indicate that sensor insertion into SAT is associated with sensitivity attenuation and that this attenuation is less pronounced with manually inserted glucose sensors compared to those placed with a commercial product sensor inserter. Thus, the duration of the run-in period after sensor implantation may be shortened when less invasive insertion techniques are applied.

928

Time lag differences of two continuous glucose monitoring systems
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Background and aims: The potential time lag between a subcutaneous sensor measuring electrons resulting from the glucose oxidase reaction using interstitial glucose (IG) and the venous blood glucose (BG) is currently not well understood. The aim of this pilot study is to evaluate the total system time lag of two FDA approved continuous glucose monitoring (CGM) systems, the SEVEN® and the Navigator®, as compared to YSI laboratory measurements; in adults with type 1 diabetes.
Materials and methods: Fourteen subjects with type1 diabetes, 33 ± 6 (mean ± SD) years old, contributed frequently sampled venous (~15 minutes)YSI blood glucose readings during three 8-hour in-clinic sessions conducted on days 5, 10, and 15 of this 15 day study. All subjects wore both CGM systems concurrently during the entire 15 days (both sensors changed every 5 days). YSI and CGM data from the in-clinic control sessions were evaluated for the time lag between IG and BG. The total system time lag of both CGM systems was estimated using various regression method related statistical estimators, including correlation coefficient, transformed R-squared Acceptance Criteria (AC), Root Mean Squared coefficient of Variation (RMS (CV)), Root Mean Squared Error (RMSE), and Mean Absolute Relative Difference (MARD). The time lag was assessed by linearly interpolating and then time shifting the CGM curves to obtain the optimum statistics criteria. A non-parametric Wilcoxon test was used to test the difference between the two CGM systems in the time lag estimate. Time lags were also estimated for steady state, increasing, and decreasing rates of change of glucose. A bootstrapping re-sampling analysis was also used to provide a 95% confidence interval for each time lag estimate.
Results: The overall time lag based on correlation coefficient criteria is estimated as 4.5±5 minutes (median±IQR) for the SEVEN System, and15±7 minutes for the Navigator System. The overall range of the estimator's median estimated total time lag, in minutes, for the two CGM systems versus YSI was different: [2,6] and [14,15] for SEVEN and Navigator, respectively (p < 0.01, for every time lag estimator; Table). The estimated time lags based on different statistical measures are similar within each CGM system

Table: Time lag Estimates (in minutes)

	SEVEN		Navigator		Wilcoxon
Estimator	Median	IQR	Median	IQR	p-value
Pearson	2.0	4	15.0	7	0.0002
Spearman	4.5	5	15.0	8	0.0012
AC	4.5	5	15.0	7	0.0002
Pearson(ln)	4.5	5	15.0	7	0.0002
RMSE	6.0	8	15.0	9	0.0001
RMS(CV)	5.0	5	14.0	7	0.0001
MARD	5.5	11	14.5	9	0.0013

Conclusion: Our study findings suggest that the use of commonly accessible statistics for the application to time lag estimation of CGM systems, such as correlation statistics, offer estimates that are comparable to more sophisticated approaches. The clinical significance of this difference in the time lag (SEVEN's total lag time at least 2 fold less than that of the Navigator) needs to be further evaluated in a larger clinical study.

929

Continuous Glucose Monitoring (CGM) reduces glucose variability and improves glycaemic control in patients using Multiple Daily Injections (MDI) or insulin pumps
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Background and aims: We compared the effectiveness of CGM with real time display in patients with type 1 diabetes utilizing MDI vs Continuous Subcutaneous Insulin Infusion (CSII; Insulin Pump).
Materials and methods: CGM using DexCom SEVEN was studied in 64 patients with type 1 diabetes using MDI (26 patients) or CSII (38 patients), The

CGM data was masked during Week 1 and displayed during Weeks 2 and 3. We computed 30 parameters of glucose variability and glycemic control.
Results: Fifteen parameters significantly improved in response to CGM display ($P<0.05$), e.g., the relative percentage of glucose values within the target range (3.9-7.8 mmol/l) increased by 17% and 19% during the display period for CSII and MDI respectively. There were significant reductions in the mean glucose, standard deviation (SD), SD between daily means (SD_{dm}), SD between days - within time periods (SD_b), and Service's Mean Amplitude of Glycemic Excursion (MAGE) and Mean of Daily Differences (MODD). There were improvements in quality of glycemic control (Schlichtkrull's $M_{5.56}$ (reference glucose 5.56 mmol/l or 100 mg/dL), Wojcicki's J Index, a new Index of Glycemic Control (IGC), and Hill's GRADE score (Figure). Changes were linearly related to baseline A1C but these relationships were not significantly different between groups.
Conclusion: Use of CGM SEVEN system with continuous display in subjects with type 1 diabetes resulted in significant improvements in multiple measures of glucose variability and glycemic control. These improvements were indistinguishable in patients using MDI and CSII.

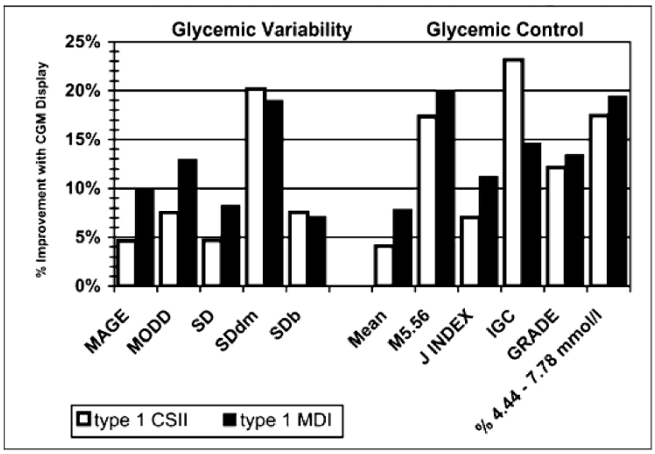

Figure Legend: Ordinate: % improvement with display of CGM during Week 3 relative to control Week 1 (no display). Abscissa: Measures of Glycemic Variability and Glycemic Control for patients with type 1 diabetes using CSII (white bars) or MDI (black bars). When CGM data were displayed in real time, there was significant improvement ($P<0.05$) in 15 parameters, but there were no significant differences between CSII and MDI ($P>0.05$). $M_{5.56}$, IGC, and GRADE are averages of related but distinct noninear transformations of glucose values giving greater influence to glucose values that fall progressively further outside the target range.
Supported by: DexCom, Inc., San Diego CA USA

930

New features and performance of a next generation SEVEN day continuous glucose monitoring system with apparent short lag time
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Background and aims: Since the introduction of the continuous glucose monitoring system for diabetes management, there have been significant technological advances. The purpose of this study was to establish the new features and evaluate the performance of SEVEN® system (DexCom, Inc.), the next generation version of seven (7) day, real-time continuous glucose monitoring (CGM) system, as well as to evaluate the lag time between the subcutaneous (ISF) glucose and parallel reference blood glucose (BG) in a controlled in-clinic setting. Accuracy was compared relative to Yellow Springs Instrument (YSI) and self-monitoring of blood glucose (SMBG) measurements.
Materials and methods: Fifty-three (53) subjects were enrolled at 3 US centers. 43 (81%) of the subjects had T1DM and 10 (19%) had insulin-requiring T2DM. Subjects inserted and wore 1 or 2 sensors for 7 days. A subgroup of patients (n=18) wore 2 sensors to track precision. Subjects participated in one 8h in-clinic session with blood draws every 15 minutes on study Day 1, 4, or 7 to collect YSI and SMBG reference measurements. For the remainder of the week, CGM was used as an adjunct to SMBG during out of clinic monitoring. Clarke-Error Grid Analysis (EGA) and Continuous Glucose Error Grid Analysis were used to quantify the clinical accuracy of CGM in reference to laboratory standard YSI. Various statistics measurements includes Pearson correlation coefficient (ρ), regression root mean squared Coefficient of Variations (RMSCV), and R-squared of transformed linear regression (R2),were used to estimate the time lags associated with subcutaneous (ISF) glucose sensing and parallel reference blood glucose (BG).
Results: User features, such as sensor life (89% lasted 7 days) and data capture (87% of sensors provided 76-100% of readings) were significantly improved relative to the current generation SEVEN system. The overall median Absolute Relative Difference (ARD) vs. YSI was 13.0%. The pooled %20/20 accuracy results relative to YSI was 76.1%. A and B regions of the Clarke Error Grid of CGM measurements was 73.8% and 22.1%, respectively. Precision ARD was 15.3% ± 6.2% (mean ± SD). The median ARD vs. SMBG was 12.1%. No difference was found between in-clinic and out of clinic monitoring. Glucose rate of change (trend arrow) and data input were made available for the users. The time lag obtained with various measures, between CGM and YSI blood samples were similar and estimated as 7 ± 10 minutes (median ± IQR). Subjects elected to check and view the glucose trend while wearing the SEVEN PLUS receiver 44 ± 21 (mean± STD) times per day. No serious adverse events or infectious complications were reported.
Conclusion: We conclude that use of this new generation of a 7-day continuous glucose monitoring device was accurate, safe, well-tolerated and frequently-used. This study is the first to show all characteristics of the new generation of SEVEN PLUS device. The performance of the system compares favorably to the current SEVEN system in terms of accuracy, precision, sensor life, and rate of data capture. Analysis of data suggests a short lag time, which may help patients better manage their diabetes.
Supported by: Dexcom Inc.

931

Safety of overnight closed-loop using FreeStyle Navigator® continuous glucose monitoring system (FSN) and model predictive control (MPC) algorithm: in silico assessment
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Background and aims: Hypo- and hyperglycaemia during closed-loop (CL) insulin delivery based on subcutaneous glucose sensing may arise due to (i) over- and underdosing of insulin by control algorithm and (ii) difference between plasma glucose (PG) and sensor glucose (SG), which may be transient (kinetics origin and sensor artifacts) or persistent (calibration error). Using extensive *in silico* testing, we assessed hypo- and hyperglycaemia risk during overnight CL and made comparison against incidence observed during open-loop (OL) studies in young people with type 1 diabetes (T1D) treated by CSII.
Materials and methods: Simulation environment comprising 18 virtual subjects with T1D and combining experimentally-derived characteristics of FSN sensing error was used to simulate overnight CL study with MPC algorithm. A 15 hour long experiment started at 17:00 and ended at 08:00 the next day. CL commenced at 21:00 and continued for 11 hours. At 18:00, protocol included meal (50g CHO) accompanied by prandial insulin. MPC algorithm advised on insulin infusion every 15min. Transient SG-PG differences were obtained by combining model of interstitial-plasma glucose kinetics with FSN error artifacts calculated as a difference between two sensor traces provided by simultaneously inserted sensors in 58 subjects with T1D over 194 night-time periods. Persistent SG-PG difference due to FSN calibration error was quantified from 585 sensor insertions yielding 1421 calibration sessions from 248 subjects with diabetes.
Results: Episodes of severe (PG<2.0mmol/l) and significant (PG<2.5mmol/l) hypoglycaemia, and hyperglycaemia (PG>16.7mmol/l) were extracted from 18,000 simulated CL nights. Severe hypoglycaemia was not observed when calibration error defined as (SG - PG)/PG was less than 40%. Hypo- and hyperglycemia frequency during OL was assessed from 21 overnight studies in 17 young subjects with T1D (M 8; age 13.5±3.6 yrs; BMI 21.0±4.0 kg/m²; duration diabetes 6.4±4.1 yrs; A1C 8.5±1.8%; mean±SD) participating in the Artificial Pancreas project at Cambridge. Severe and significant hypoglycaemia occurred once every 105 and 5.6 years during CL compared to once every 21 and 10.5 days during OL, respectively. Hyperglycaemia occurred once every 278 weeks during CL and once every 2.3 days during OL. Recalibrating FSN when difference between SG and PG exceeded 3mmol/l reduced

frequency of severe and significant hypoglycaemia during CL to 353 years and 7.4 years.
Conclusion: Using simulations with experimentally-derived FSN error, the risk of severe and significant hypoglycaemia reduced 2000 and 200 fold during overnight CL with MPC control compared to that observed during OL. Hyperglycaemia was 800 times less likely. Overnight CL with FSN and MPC control is expected to reduce substantially the risk of hypo- and hyperglycemia.
Supported by: JDRF and NIHR

PS 79 Glucose measuring devices

932

Improved system for non-invasive glucose monitoring at home
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Background and aims: OrSense has developed the NBM device, a general platform for non-invasive detection of blood analytes. The device is based on red/near infrared Occlusion Spectroscopy technology, which has been previously shown to measure blood glucose (BG) accurately. The NBM uses a multi-wavelength sensor shaped as a ring, located at the finger's base. It utilizes an enhanced optical signal resulting from a temporary over systolic occlusion produced by a finger based pneumatic cuff. OrSense's NBM device has recently entered the clinic as a device for hemoglobin and oxygenation determination. In the present study we evaluate the performance of a new sensor, which includes improved mechanical and optical design, as well as a new calibration scheme. The device was operated in a *profiler* mode, which reports the daily BG profile at the end of each daily session.
Materials and methods: The trial was carried out in a home-like setting. The NBM's probe was placed on each patients' thumb, where it performed non-invasive continuous measurements for up to 10 hours, with readings every 10 minutes. Four calibration points were used in each session in proximity to breakfast and lunch times (one calibration point just before each meal and another, one hour after each meal starts). Accuracy was assessed by comparing NBM data with paired self-monitoring blood glucose meter readings (FreeStyle, Abbot Inc.), taken at 30 minutes intervals. A total of 150 sessions were carried out on 9 patients (7F, 2M; 26-70 y/o; 4 Type 1, 5 Type 2; 6 subjects participating in 20-26 sessions each, and the 3 other in 2-4 sessions). 20 sessions were excluded from our analysis due to calibration failures as identified by our algorithm.
Results: Reference BG values were in the range 47 to 378 mg/dL. In all cases there was good patient compliance and no adverse effects were identified. The median relative absolute deviation (RAD) is 11.2%. Clarke error grid analysis based on 1926 paired data points shows that 98.3% of the results are in the A+B zone, with 68.8% in the clinically useful A zone, 29.5% in the clinically benign B zone and none in the potentially dangerous E zone. Deming regression slope is 1.0 (assuming variances ratio λ=1).
Conclusion: This study substantiates the potential use of OrSense's NBM device as a non-invasive sensor for continuous BG monitoring. The device was comfortable for subjects, safe and well tolerated. Further studies are underway, to examine use of this truly non-invasive continuous glucose-sensing device in other settings.

933

Wavesense algorithms account for haematocrit variations while determining blood glucose
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Background and aims: Studies have shown that controlling blood glucose can reduce the onset and progression of the long-term microvascular and neuropathic complications associated with the chronic course of diabetes mellitus. Blood glucose monitoring is a very useful metric to evaluate glycemic control at a point in time. Effective interpretation of readings is dependent on obtaining frequent and accurate glucose readings. While frequency of testing depends on patient motivation, measurement accuracy is influenced by a variety of known factors. It is understood that system errors attributable to sample characteristics such as: hematocrit (Hct), testing environment (ambient temperature, humidity) or manufacturing errors (lot-to-lot variability in reagent strips) can all affect blood glucose readings.
Hematocrit affects results of blood glucose testing because red blood cells in the sample can alter the ratio of blood glucose to plasma glucose, as well as the flow of plasma and delivery of oxygen into the test strip. Blood glucose meters typically tend to underestimate blood glucose levels at higher Hct, and overestimate the levels when the Hct is lower-than-normal. The positive and negative bias are relevant because Hct variations are quite common in the diabetic population. Renal dysfunction can cause anemia, while smoking or living at high altitudes can lead to an increase in red blood cells. Wavesense technology includes a suite of algorithms that corrects for several different sources of errors and variations, including hematocrit.

Materials and methods: A total of 416 adult patients previously diagnosed with either of Type 1 or Type 2 DM were tested using Wavesense Technology no code meters embedded with novel algorithms for improving accuracy. The studies were conducted at 4 different locations with known differences in climatic conditions. Results from fingerstick samples were correlated with the YSI 2300 Stat Plus.
Results: The results demonstrate that the bias does not change over a wide range of Hct (31 - 53.8) since the linear regression has a slope close to zero (Figure 1).
Conclusion: Wavesense algorithms account for the variations in Hct and thus strive to provide better readings that may be helpful in achieving tighter glycemic control in the management of DM.

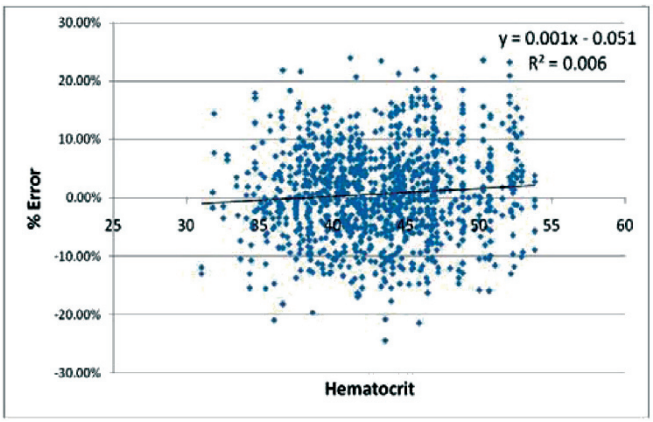

Figure 1: A slope &0.0014) near zero indicates that the bias does not change with Hct (n=471)

934

New optical method for blood glucose self-monitoring
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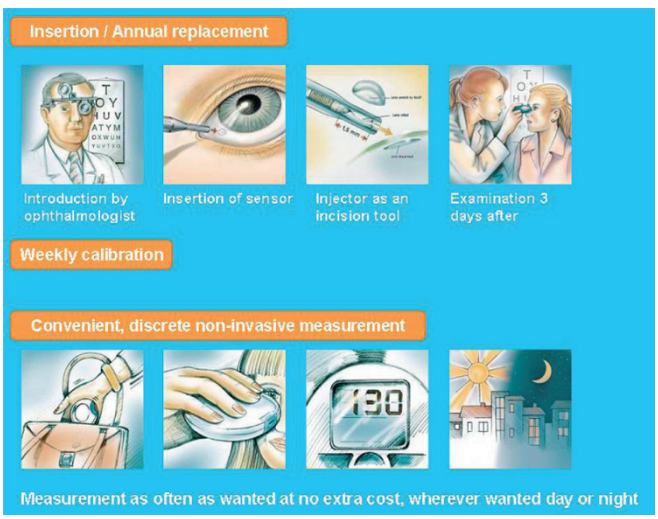
Background and aims: Blood sugar self- monitoring is a basic component of diabetes therapy. Since years new technologies capable of doing this without taking a droplet of blood via finger pricking are in high demand. So far all approaches failed mainly due to insufficient accuracy.
Materials and methods: The goal of the new concept is to overcome the hurdles of the alternative measuring technologies by using a glucose specific sensor placed at a body site, which basically lacks severe foreign body reactions. This is the eye used as a window to the body and benefiting from an immune privilege. More specifically, a small hydrogel chip with a chemical sensor in it is inserted below the conjunctiva in a painless minimally invasive procedure. The read out of the glucose concentration is done optically with the help of a small, handheld photometer.



Results: A clinical study was run with 11 insulin-dependent diabetics in 44 measurement series over 18 days. 1944 combined data points were registered. An excellent correlation with corresponding conventionally measured blood glucose concentration was found (r=0,93 p< 0,001). The Clarke Error Grid Analysis resulted in more than 99,5% of data points falling into combined zones A and B. 7 data points fall in zone D, however, they droped out of zone A by only 2 - 3 mg/dL. The mean average relative error was 10,3%. A lag time of 5 - 15 min was observed versus capillary blood measurements depending on the dynamics of blood glucose changes. Patients experienced the insertion as painless and simple. The implant was well tolerated over the whole wearing time.
Conclusion: The first clinical results impressively show the potential of the new technology to replace finger sticking for blood glucose measuring. It allows non-invasive measurement after the insert is placed below the conjunctiva in a convenient way, reversibly as often as wanted without extra costs.

935

A truly non-invasive SMBG device for home use
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Background and aims: For over a decade, the medical and industrial communities struggle to provide an answer for people with diabetes and replace painful finger- prick glucose monitoring with a non-invasive methodology. Generally, Non-Invasive (NI) devices measure physiological phenomena which are reflected in tissue parameters changes that are correlated with blood glucose. These devices (in development stages) produce either trend analysis or continuous glucose values. However, the actual glucose value derived from such correlation is different than the real glucose value, since factors, other than glucose, influence tissue parameters as well and cause inaccuracies in the reading. Previous publications suggest a unique way to minimize the impact of such disturbances through an approach of combining technologies. Three independent NI methods are used: Ultrasound, Electromagnetic and Thermal. The weighted average reading reflects blood glucose value with smaller impact of interferences, leading to more accurate, *real-time (prospective) spot* glucose readings.
Materials and methods: Clinical trials were performed at the Diabetes Unit, Soroka University Medical Center, Beer-Sheva, Israel. 30 subjects were evaluated (427 measurement pairs): 5 T1DM (2F, 3M), 25 T2DM (11F, 14M), aged 52 ± 29 years, BMI of 30.9 ± 9.1Kg/m^2. The measurements were performed externally on the earlobe, which has a large blood supply and is also a convenient site that doesn't interfere with daily activities. The calibration was individually performed against invasive basal and post-prandial blood glucose references using HemoCue® Glucose 201+ Analyzer from capillary fingertip blood. The calibration procedure is easy, takes about 1.5 hours and more importantly, valid for one month. The majority of the values during the trials were measured on a different day than the calibration process.
Results: Clarke Error Grid analysis of the weighed result shows 98% of the points in the clinically accepted zones A+B, of which 61% in zone A. $MARD_{mean}$ is 21.3% and $MARD_{median}$ is 15.9 %. Further work is currently done to improve the accuracy level even further.
Conclusion: We have shown that the present model of *GlucoTrack*™ gives promising results. The upgraded version is in its final stages and new set of clinical trials is scheduled for April 2009. A key benefit of the device is its long intervals between re-calibrations and the ability to perform frequent spot measurements without the need to continuously wear the device. This small, i-pod size, user friendly and easy to operate device is intended for home use and provides answers for the diabetic population. The device can mainly (however not only) be beneficial for non-insulin dependent type 2 DM, because it does not need to be worn continuously and does not require expendables. *GlucoTrack* will improve monitoring adherence and allow diabetics a frequent and painless way to monitor and track their BG, leading to tighter glucose control.

936

Laboratory investigation for the assessment of haematocrit interference on handheld blood glucose meters for patients' self blood glucose testing

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Background and aims: Recent reports have indicated that the majority of hospital-based point-of-care blood glucose meters show pronounced interference with hematocrit (HCT) variations. The purpose of this laboratory investigation was to explore to what extend hand-held glucose meters for patient self-monitoring (SBGM) may also be affected by this phenomenon.
Materials and methods: Seven SBGMs (AccuCheck Aviva, Ascencia Contour+, Ascencia Breeze 2, Precision Xceed, FreeStyle Freedom, OneTouch Ultra 2, and FineTouch) were tested with laboratory samples with different hematocrit values (20, 30, 40, 50, and 60 %) and at three different glucose concentrations (50, 170, 370 mg/dl). Each individual sample was tested 6 times with each meter. In addition, two point-of-care devices and one reference method were tested (HemoCue, StatStrip, Cobas). For the interference analysis, the value determined with 40 % hematocrit was used as the reference value. Mean deviations were calculated for all meters over all three blood glucose ranges. No analysis of precision or accuracy was undertaken, as the study was performed with artificially modified laboratory samples and not with capillary whole blood, the natural specimen for these devices.
Results: The mean percent deviation results for all glucose ranges are provided in the Table. Different degrees of interference were observed for many of the SBGMs at either low or high hematocrit values (or both). Only the FineTouch device showed a stable measurement performance together with one point of care device (StatStrip) and the reference method (Cobas).
Conclusion: Small hematocrit variations (especially increases) showed pronounced effects with some meters. Although this study was not performed in capillary blood samples, an appropriate education and clear labelling appears to be strongly recommended for these meters. Only one point-of-care meter (StatStrip, Nova Biomedical) and one handheld SBGM (FineTouch, Terumo) were not substantially influenced by hematocrit interference. As variation in hematocrit can occur in daily life (e.g. in patients with dehydration or during hemodialysis), this can be considered to be an advantage of these meters in practical routine use.

Deviations from the reference measurement (at 40 % hematocrit) by device

Hematocrit	20 %	30 %	40 %	50 %	60 %
AccuCheck Aviva	2.7 %	2.2 %	0.0 %	-12.4 %	-24.1 %
Ascentia Contour	6.4 %	2.2 %	0.0 %	-9.1 %	-9.4 %
Ascencia Breeze2	38.9 %	20.3 %	0.0 %	-17.3 %	-34.7 %
FreeStyle Freedom	20.5 %	2.3 %	0.0 %	-7.1 %	-8.5
Precision Xceed	12.8 %	8.7 %	0.0 %	-15.3 %	-31.2 %
OneTouch Ultra 2	10.3 %	6.6 %	0.0 %	-22.3 %	-35.2 %
FineTouch	-1.7 %	-8.1 %	0.0 %	3.5 %	2.7 %
StatStrip (POC)	0.4 %	1.7 %	0.0 %	-3.1 %	-5.0 %
HemoCue (POC)	4.8 %	7.6 %	0.0 %	-10.7 %	-19.1 %
COBAS	-3.6 %	0.0 %	0.0 %	-1.8 %	2.4 %

Supported by: Terumo, Eschborn, Germany

937

Waste of test strips compared among four blood glucose monitoring systems

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Background and aims: Applying insufficient blood or getting an error message can result in wasting of a test strip. Some waste of strips is inevitable to confirm questionable results and avoid errors. However, test strips represent most of the cost in blood glucose monitoring. The Abbott FreeStyle Lite Blood Glucose Monitoring System does not require coding, uses a very small blood sample (0.3 µL), is designed to start the test only after an adequate sample has been applied, and allows the user to apply more blood for up to 1 minute. A study was conducted to assess whether a device with these features is associated with less wasted strips than other systems when used by diabetic patients at home.
Materials and methods: 180 diabetic subjects who routinely performed blood glucose monitoring were enrolled at three clinics to use two systems which they had no prior experience with. The two systems consist of FreeStyle Lite system and one of the following: LifeScan OneTouch Ultra2 (sample size required: 1 µL), Roche Accu-Chek Aviva (0.6 µL) and Bayer Contour (0.6 µL). The Ultra2 and Contour are not designed for blood reapplication; more blood may be applied to the Aviva within 5 seconds of the first application. Each system was used by the subject at home 3 times a day for 2 weeks. The test results, number of strips used and error messages were recorded in the subject diary; 165 subjects completed the study.
Results: More than 6,000 tests were performed in this study. Approximately 1,000 tests (range: 916-1,182) were completed with each system in the paired testing. ANOVA analysis was used to compare the average % strips wasted of the 4 systems in the study. The results showed that significantly more strips were wasted with the OneTouch Ultra2, Accu-Chek Aviva and Contour than with the FreeStyle Lite system. All statistical tests were two-sided with alpha=0.05.

Study Site	Strips wasted among systems, % (strips wasted / total strips tested)				Chi-square p-value
	FreeStyle Lite	OneTouch Ultra2	Accu-Chek Aviva	Contour	
A	3.1% (33/1145)	5.9% (70/1182)			0.0009
B	3.3% (30/916)		5.4% (50/926)		0.025
C	2.7% (32/1175)			6.5% (81/1162)	<0.0001
	Strips wasted among subjects				
A	2.8%	5.5%			0.0057
B	3.0%		4.9%		0.12
C	2.5%			6.1%	0.0007

Paired t-test was used to compare the average % strips wasted among subjects. The results indicated that subjects on average experienced less wasted strips with the FreeStyle Lite system than with OneTouch Ultra2 and Contour, but not significantly different from Accu-Chek Aviva. One subject had a higher % strips wasted with FreeStyle Lite system than the rest of the subjects. If this subject was excluded, the results show that the average % strips wasted of subjects for FreeStyle Lite system was significantly less than that for Accu-Chek Aviva ($p = 0.02$ instead of $p=0.12$).
Conclusion: Overall, strip waste was 2.5-3.3% with the FreeStyle Lite system versus 4.9-6.5% with the other three systems. For the patients, reducing waste of strips by approximately half, as seen with the FreeStyle Lite system, should be encouraging. For the payers of diabetic supplies, a 2 to 3% decrease in expenditures could be viewed favorably.

938

A review of adverse events associated with false glucose readings measured by glucose dehydrogenase pyrroloquinolinequinone (GDH-PQQ)-based glucose monitoring systems in the presence of interfering sugars

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Background and aims: GDH-PQQ, a commonly used glucose test strip enzyme, is not glucose-specific. As such, it measures other sugars (maltose, galactose, xylose) in addition to glucose if they are present at detectable concentrations. This may result in falsely elevated "glucose" readings in patients treated with agents containing or metabolized to interfering sugars. Such agents include icodextrin-containing peritoneal dialysis (PD) solutions, some intravenous immune globulins (IVIG), abatacept (ORENCIA®) and ADEPT® Solution. Over the past 10 years there have been several case reports in the literature and from global regulatory agencies describing events of severe hypoglycemia and death due to overtreatment with insulin guided by GDH-PQQ-measured false glucose readings. We report a quantitative review of the medical literature and the US Food and Drug Administration Manufacturer and User Facility Device Experience (MAUDE) database of adverse events related to this issue.

Materials and methods: MAUDE, Ovid, and PubMed databases (1998-2009) were searched for published reports of adverse events due to discrepancies between glucose monitoring systems and reference values. Search terms included blood glucose, maltose, galactose, xylose, icodextrin, peritoneal dialysis, maltodextrin, abatacept and IVIG. The bibliographies from key articles were searched manually. Reports were independently assessed by multiple reviewers and summarized. Cases found in both the MAUDE and literature databases (n=3) were counted once.
Results: The search revealed 76 reports related to falsely elevated glucose readings due to the non-specificity of GDH-PQQ test strips (57 in MAUDE database, 19 in literature). Approximately half the reports (47%) were from outside the US. Of the 76 reports, 11 (14%) resulted in death, 45 (59%) in severe hypoglycemia, 11 (14%) in non-severe hypoglycemia, and 9 (12%) were unclassified. Four cases of severe hypoglycemia resulted in permanent impairment. Agents or disorders associated with events were icodextrin-containing PD solution (60 events, 79%), IVIG (10 events, 13%), a "maltose-containing substance" (3 events, 4%), galactosemia (2 events, 3%) and maltodextrin (1 event, 1%). Results from the MAUDE database revealed that 34 (60%) events occurred in an inpatient setting, 14 (25%) in an outpatient setting, and 9 (15%) were not classified. For the year 2008, the MAUDE database contained 2 fatal cases and 4 cases of severe hypoglycemia associated with this issue.
Conclusion: A significant number of adverse events, including death, related to glucose non-specificity of GDH-PQQ test strips have been reported globally over the past 10 years despite mitigations by manufacturers and global health authorities. This important drug-device interaction should be recognized by healthcare providers and patients, and actions must be taken to further enhance patient safety.

939

System accuracy evaluation of 27 blood glucose monitoring systems according to DIN EN ISO 15197
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Background and aims: Self monitoring of blood glucose (SMBG) is used for metabolic control and for a therapy adjustment by the patient himself. As insulin therapy is controlled by the measured values, high-quality blood glucose monitoring systems are required. This study intended to verify if the accuracy requested by DIN EN ISO 15197 is achieved in 27 SMBG devices currently available in Europe.
Materials and methods: In this study 27 CE-labelled SMBG devices of 18 manufacturers, were evaluated for system accuracy according to EN ISO 15197 versus a reference method: glucose oxidase (YSI 2300 STAT PLUS) or hexokinase (Gluco-quant / Hitachi 917) depending on the method used by the manufacturer. Two measurements performed from 100 samples with a defined distribution of glucose concentrations were included in the evaluation. According to EN ISO 15197 the minimum acceptable accuracy limits for blood glucose self-testing systems are as follows: 95 % of the results shall fall within ± 15 mg/dl of the results of the reference measurement at glucose concentrations 75 mg/dL.
Results: The tested samples had glucose concentrations between 20 and 500 mg/dL. Data of 3 of the 27 systems were evaluated versus the hexokinase reference, 24 systems were evaluated versus glucose oxidase. Sixteen of 27 tested systems complied with the system accuracy requirements of EN ISO 15197 with >95 % of the results showing the minimum acceptable accuracy. Eleven systems did not meet the system accuracy requirements. The percentage of results complying with the ISO criteria ranged from 73.5 to 100 % for the different systems and reached 94.6 ± 6.6 % [mean ± SD] on average.
Conclusion: In a system accuracy evaluation according to EN ISO 15197 only 16 of 27 SMBG systems met the minimum acceptable accuracy limits. SMBG systems providing inaccurate results bear the risk of false therapeutic decisions by the patient with the risk of severe health injury. In conclusion the EN ISO 15197 describes a reliable and highly standardized method to assess the quality of SMBG systems and thus, as the results demonstrate, could be useful to have systems analyzed by independent organisations.
Supported by: Roche Diagnostics

PS 80 Pump treatment

940

Evaluating the feature of glycaemia excursion detected by continuous glucose monitoring system during temporary continuous subcutaneous insulin infusion
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Background and aims: The aim of this study was to find out glycemia excursion feature via continuous glucose monitoring system(CGMS) in diabetes patients on early stage of continuous subcutaneous insulin infusion(CSII) treatment.Discuss the relationship between bedtime glucose level and nocturnal hypoglycaemia. Summarized the distribution feature of insulin dosage.
Materials and methods: 257 diabetes patients (156 males and 101 females , age from 12~88 years) with 0.1~31 years diabetic history were recruited. Their HbA1c level was(10.97±2.11)%. Glycated albumin level was (31.88±7.51)%. All patients changed to CSII therapy while a 72 hours period CGMS was performed. Participants were divided into hypoglycemia group and no hypoglycemia group according to the hypoglycemia events read from CGMS data.
Results: 1. From CGMS profiles we find 243 hypoglycemia events occurred in 118 participants at early stage of CSII treatment. The HbA1c, fasting C-peptide values,HDL-C and BUN were statistical difference between the two groups($P< 0.05$). Mean blood glucose(MBG) were significantly lower and standard deviation of blood glucose(SDBG) were significantly higher in hypoglycemia group($P< 0.05$). Multiple regression results indicated that MBG and fasting C-peptide were negatively correlated with hypoglycemia but SDBG and duration of diabetes were positively correlated with hypoglycemia. When bedtime blood glucose was below 5.9mmol/L, the youden index was max which was analysed by ROC. The sensitive was 54.8%, specificity was 77.8%. Positive predictive value was 44.3%, negative predictive value was 84.2%. So the cut-point of bedtime blood glucose to predict nocturnal hypoglycaemia might be 5.9mmol/L.2. The average total daily dosage(TDD) was (0.78±0.23)u·kg^{-1}·d^{-1}, basal rate was (0.34±0.09)u·kg^{-1}·d^{-1}, bolus dose was (0.45±0.16)u·kg^{-1}·d^{-1} during CSII. The insulin sensitive factor(ISF) was 1.77±0.49, insulin-carbohydrate ratio(ICR) was 9.55±2.67. TDD and bolus were correlated with BMI, urine glucose and 120minute C-peptide. Basal rate was correlated with BMI, urine glucose, HbA1c and 30minute C-peptide. The TDD and basal rate were higher in which the diabetic duration was over 5 years. The nocturnal basal rate was lower in whose age was over 60 years old.
Conclusion: 1.Hypoglycemia detected by CGMS were occurred in about 1/3 type 2 diabetes patients at the early stage of CSII therapy. 2. Beside of MBG and β-cell function, glycemic excursions were related closely to hypoglycemia. 3.There was a higher risk of nocturnal hypoglycaemia if the bedtime blood glucose concentration was below 5.9mmol/L. 4.At the beginning of CSII a low nocturnal basal rate would be appropriate to avoid hypoglycemia in elder patients. 5. The TDD would be large if the body weight was increased or the glycemia control was poor.

941

Continuous subcutaneous insulin infusion improved glycaemic control and health-related quality of life (HRQoL) in type 2 diabetes mellitus
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Background and aims: Limited information exists about the use of continuous subcutaneous insulin infusion (CSII) in type 2 diabetes mellitus (T2DM). This 16-wk, open-label, uncontrolled, pilot study evaluated insulin dosing requirements and patterns aimed at achieving normal or near-normal glycemic control with CSII in patients with T2DM. HRQoL and treatment preference were also assessed.
Materials and methods: Fifty-six insulin pump-naïve patients (28 male, 28 female) were enrolled (age 57±10y, DM duration 13±6y, A1C 8.4±1.3%, FPG 9.3±2.9mmol/l, weight 99±19kg, BMI 34±5kg/m², mean±SD)). Baseline DM therapy was either Cohort A: ≥2 oral agents (OA) (n=18), Cohort B: basal insulin ± OA (n=17; mean daily insulin dose 32±20U) or Cohort C: multiple

daily injections ± OA (n=21; mean daily insulin dose 99±65U). All antihyperglycemic agents except metformin were discontinued and CSII (Animas® 2020 insulin pump with insulin glulisine) was initiated with one daily basal rate and bolus doses at meals. Insulin dose was titrated to safely achieve the best possible glycemic control, and additional basal rates were added at the investigator's discretion. The Insulin Delivery System Rating Questionnaire (IDSRQ) was used at Baseline and Wk 16 to assess HRQoL and patient perception of insulin delivery system in Cohorts B and C. In Cohort A, a modified version of the IDSRQ, the Diabetes Medication System Rating Questionnaire (DMSRQ), was used. The score for each of the 7 questionnaire subscales ranged from 0 to 100.

Results: Glycemic control improved after 16 wks of CSII: A1C 7.2±0.9% (-1.2±1.2%, p<0.001) and FPG 7.2±1.9mmol/l (-2.1±3.4mmol/l, p<0.001). In patients with baseline A1C >8.5% (n=23, mean baseline A1C 9.6±0.9%), A1C was reduced by 2.1±1.1% (p<0.0001). At Wk 16, the mean daily basal, bolus, and total insulin doses were 51±30U, 45±32U, and 95±59U, respectively, and ~90% of patients were treated with ≤2 basal rates/day. Body weight increased by 1.9±3.3kg (p<0.001). Mild hypoglycemia was experienced by 59% of patients, with no severe hypoglycemia. Change from baseline at Wk 16 for the DMSRQ/IDSRQ are presented below.

Conclusion: CSII significantly improved glycemic control in this heterogeneous group of T2DM patients. In patients using insulin by injection at baseline, the majority of HRQoL measures improved significantly after 16 wks of CSII. Each Cohort perceived increased clinical efficacy with CSII and preferred CSII to baseline therapy despite weight gain. Future trials are needed to further define the role of CSII in managing T2DM.

Change from baseline at Wk 16 for the DMSRQ/IDSRQ

DMSRQ/IDSRQ Subscale	Cohort A (n=18)	Cohort B (n=17)	Cohort C (n=21)	All Cohorts (n=56)
Treatment Satisfaction	-5.8 (16.4), p=0.15	12.7 (16.8), p<0.01	16.4 (14.6), p<0.001	8.0 (18.5), p<0.005
Treatment Interference with Daily Activities	4.5 (19.1), p=0.33	-2.2 (19.4), p=0.65	-3.2 (15.8), p=0.36	-0.4 (18.0), p=0.86
Clinical Efficacy	15.6 (19.0), p<0.005	19.3 (24.0), p<0.005	24.8 (13.2), p<0.001	20.2 (18.9), p<0.001
Diabetes Worries	2.6 (8.2), p=0.20	-11.8 (17.9), p<0.05	-7.5 (18.2), p=0.07	-5.5 (16.4), p<0.05
Diabetes Social Burdens	-3.2 (17.0), p=0.44	-6.7 (14.4), p=0.07	-10.5 (11.8), p<0.001	-7.0 (14.4), p<0.001
Psychological Well-Being	0.4 (9.6), p=0.85	6.4 (10.7), p<0.05	5.0 (10.7), p<0.05	3.9 (10.5), p<0.01
Overall Treatment Preference	18.1 (17.4), p<0.001	21.1 (16.5), p<0.001	33.8 (17.5), p<0.001	24.9 (18.3), p<0.001

942

Fasting plasma glucose rather than HbA_{1c} more linked to lipid profiles and IL-10 in subjects with type 2 diabetes treated via long-term continuous insulin infusion therapy

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Background and aims: Continuous subcutaneous insulin infusion (CSII) treatment has been reported to be effective in amelioration of hyperglycaemia and dyslipidaemia in type 2 diabetes. However, the significance of maintaining normal fasting plasma glucose (FPG) rather than HbA_{1c} level obtained via CSII has not been investigated in the aspect of clinical and laboratory beneficial outcomes.

Materials and methods: We discontinued oral antidiabetic drugs (OADs) and lipid lowering agents and applied CSII therapy to subjects with type 2 diabetes who had failed to control hyperglycaemia with OADs and/or insulin injections (number, 20 with 25% of male; age, 61.2 ± 7.8 years; duration, 13.3 ± 7.7 years; HbA1c 8.8 ± 2.5%). Three months after CSII treatment we divided the subjects into two groups, i.e., normal FPG (N-FPG, < 7 mmol/l) and high FPG (H-FPG, ≥ 7 mmol/l) groups and compared the changes in BMI, blood pressure and laboratory findings, including serum lipids and cytokines between two groups.

Results: Baseline characteristics were not significantly different between N-FPG and H-FPG groups (Before CSII in Table 1). The means of HbA_{1c} and free fatty acids decreased in both groups 3 months after CSII therapy ($p < 0.01$ for both). Although the means and medians of HbA_{1c} after CSII treatment were the same in two groups, means of FPG, HDL-cholesterol, and IL-10 were significantly different between two groups (After CSII in Table 1). Moreover, means of BMI and HDL-cholesterol significantly increased ($p < 0.01$ and $p < 0.05$, respectively) and the diastolic blood pressure level decreased ($p < 0.05$) in N-FPG group after CSII treatment as compared with those at baseline, but these findings were not observed in H-FPG group. Instead, the mean triglyceride level increased in H-FPG group after CSII treatment when compared with the level at baseline ($p < 0.05$). The means of IL-6 and sICAM-1 after CSII treatment appeared lower in N-FPG than in H-FPG group but did not reach to the statistical significance. FPG showed correlation with triglycerides ($r = 0.546$, $p = 0.013$) and HbA_{1c} correlation with IL-10 ($r = 0.71$, $p = 0.03$).

Conclusion: Excursion of FPG level seemed to be linked to lipid profiles or cytokine levels, indicating frequent examination of FPG may be recommended together with a periodic determination of HbA_{1c} for optimal diabetic care, although HbA_{1c} has been known as a useful parameter in the assessment of glycaemic control.

Table 1. Comparison between normal and high fasting plasma glucose groups

* p<0.001, **p<0.05 vs N-FPG After CSII	Before CSII			After CSII	
a, p<0.01 b, p<0.05 vs the same group Before CSII	ALL	N-FPG	H-FPG	N-FPG	H-FPG
BMI (kg/m²)	22.9±3.4	23.0±2.8	22.8±3.8	25.1±2.6[a]	23.8±3.1
BP diastolic (mmHg)	79.4±9.9	74.6±9.7	82.5±9.0	69.3±9.9[b]	73.6±12.7
FPG (mmol/l)	7.8±3.5	8.1±3.0	7.6±3.9	5.1±1.2	10.9±3.4*
HbA_{1c} (%)	8.8±2.5	9.0±2.7	8.7±2.5	6.8±0.9[a]	6.8±1.2[a]
Free fatty acid (mmol/l)	0.64±0.30	0.67±0.36	0.62±0.27	0.20±0.062[a]	0.29±0.18[a]
Triglycerides (mmol/l)	1.4±0.8	1.4±0.7	1.2±0.7	1.4±0.9	1.8±1.2[b]
HDL-cholesterol (mmol/l)	1.3±0.3	1.4±0.3	1.3±0.3	1.7±0.5[b]	1.3±0.2**
IL-6 (pg/ml)	2.3±1.6	2.5±1.9	2.2±1.5	2.0±1.0	2.7±1.4
IL-10 (pg/ml)	15.7±6.8	10.5±1.4	19.9±6.5	8.9±3.7	18.8±7.5**
sICAM-1 (ng/ml)	192.8±67.4	162.3±41.1	217.1±78.4	174.8±67.0	209.1±44.9

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943

Continuous subcutaneous insulin infusion in patients with type 2 diabetes safely improved glycaemic control using a simple insulin dosing regimen

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Background and aims: Limited information exists about the use of continuous subcutaneous insulin infusion therapy (CSII) in patients with type 2 diabetes (T2DM). This 16-week, open-label, uncontrolled, multicenter pilot study was designed to evaluate insulin doses and dosing patterns aimed at achieving normal or near-normal glycemic control with CSII in patients with T2DM.

Materials and methods: Fifty-six insulin pump-naïve patients (28 male, 28 female) were enrolled (age 57±10y, DM duration 13±6y, A1C 8.4±1.3%, FPG 9.3±2.9mmol/l, body weight 99±19kg, BMI 34±5kg/m², mean±SD). Baseline diabetes therapy was either ≥2 oral agents (OA) (n=18), basal insulin ± OA (n=17; mean daily insulin dose 32±20U) or multiple daily injections ± OA (n=21; mean daily insulin dose 99±65U). All antihyperglycemic agents except metformin were discontinued and CSII (Animas® 2020 insulin pump with insulin glulisine) was initiated with one daily basal rate and bolus doses at each meal. Insulin dose was titrated to safely achieve the best possible glycemic control, and additional basal rates were added at the investigator's discretion. CGM (DexCom SEVEN®) was conducted for a week at baseline and repeated during the final week of the study.

Results: Glycemic control improved significantly after 16 wks of CSII: A1C 7.2±0.9% (-1.2±1.2%, p<0.001) and FPG 7.2±1.9mmol/l (-2.1±3.4mmol/l,

p<0.001). At Wk 16, 45% and 28% of patients had A1C values <7.0% and <6.5%, respectively. In patients with baseline A1C >8.5% (n=23, mean baseline A1C 9.6±0.9%), A1C was reduced by 2.1±1.1% (p<0.0001). Mean pre-prandial and postprandial glucose values from self-monitored 7-point glucose profiles declined significantly from baseline at Wk 16 (pre-prandial -1.2±2.6mmol/l, p=0.002; postprandial -2.1±3.1mmol/l, p<0.001). CGM revealed significantly more values within the 3.9-10.0mmol/l range during the last week of the study compared with baseline (Baseline=59%; end-of-study=71%, p<0.001). At Wk 16, the mean daily basal, bolus, and total insulin doses were 51±30U, 45±32U, and 95±59U (0.94U/kg), respectively, and ~90% of patients were treated with ≤2 basal rates per day (1 basal rate 70%; 2 basal rates 18%; >2 basal rates 12%). Body weight increased by 1.9±3.3kg (p<0.001). Total cholesterol (TC) and triglycerides (TG) improved significantly during the course of the study (TC: Baseline=4.7±1.1mmol/l, Wk 16=4.5±1.2mmol/l, p<0.05; TG: Baseline=2.4±1.7mmol/l, Wk 16=1.8±1.1mmol/l, p<0.001). There was no change in LDL or HDL cholesterol. Mild hypoglycemia was experienced by 59% of patients during the 16-wk study period with no episodes of severe hypoglycemia.
Conclusion: Glycemic control was significantly improved with CSII using a minimal number of basal rates in a heterogeneous group of T2DM patients. Future well-controlled, randomized trials are needed to further assess the benefits of CSII in T2DM.

944

Intensified insulin therapy vs CSII: the influence on family cohesion and adaptability of type 1 diabetics
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Background and aims: Type 1 diabetes mellitus appears at a young age and is treated with intensified regimes of insulin therapy or continuous subcutaneous insulin infusion (CSII), following the patient and changing not only his/her life, but also that of the family. With this study, we attempt to establish possible differences between families of type 1 diabetes patients following intensified regimes of insulin therapy and those using continuous subcutaneous insulin infusion as to the adaptability, the cohesion and the type of family as defined by Olson.
Materials and methods: 72 patients following intensified insulin therapy (Group A), and 46 patients using CSII (Group B) of comparable age and gender filled out an approved questionnaire (FACES-III) of 40 questions determining the level of family adaptability (rigid, structured, flexible, very flexible) and cohesion (disengaged, separated, connected, very connected), as well as its type according to international literature where 4 types of family are specified (extremely imbalanced, moderately imbalanced, moderately balanced, balanced). HbA1c and somatometric characteristics were recorded in both groups. Statistical analysis was carried out using SPSS 15.0. A p-value<0.05 was considered significant.
Results: There was no statistically significant difference between groups A and B as to HbA1c (7.62 vs 7.47, p=0.394), age (34.97 vs 36.06, p=0.403) and diabetes duration in years (13.83 vs 13.65, p=0.900), while there was a difference as to BMI (28.02 vs 25.35, p=0.001). Concerning adaptability, a statistically significant difference was found between the two groups (p=0.041), with patients using CSII scoring better on the fourth level (very flexible) of adaptability (73.5% vs 50%). There is also a significant difference in cohesion, with patients using CSII having a higher percentage (55.9% vs 20.8%) on the level of very-connected families (p=0,004). Patients using CSII outnumber patients in intensified insulin therapy in the fourth atype of family (64.7% vs 33.3%, p=0,009), with the former showing higher rates in the third family type (50.0% vs 26.5%, p=0.012). The level of adaptability, as well as the level of cohesion and the type of family, affects the regulation of diabetes mellitus. A higher level of adaptability is correlated with lower insulin values for the group in intensified regime as well as the group using CSII, with similar results concerning cohesion for the first (p=0.014) as well as the second (p=0.002) group of patients. The same applies for the effect of family type (intensified insulin therapy group: p=0.044; insulin pump group: p=0.030).
Conclusion: Families of patients with type 1 diabetes following a continuous subcutaneous insulin infusion show higher adaptability and cohesion compared τo families of patients using an intensified insulin regime. The findings are similar concerning the type of family. Family type, adaptability and cohesion play a crucial role in the regulation of diabetes mellitus irrespective of the kind of treatment followed by the patient.

945

Retrospective case-controlled long-term study on continuous subcutaneous insulin infusion vs multiple daily injections for functional insulin treatment in type 1 diabetes
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Background and aims: In intensified insulin treatment, pump therapy is sometimes preferred to multiple insulin injections. Our aim was to compare treatment outcomes in FIT (functional insulin treatment, discriminating between correctional, prandial and basal insulin use) educated patients with type 1 diabetes who later on have chosen on their own an insulin pump (CSII) vs. those who have chosen multiple daily injections (MDI).
Materials and methods: Retrospective case-controlled study where for each CSII patient two MDI controls have been selected, matched for diabetes duration, FIT duration, associated diseases at time point of FIT training and if possible, gender. Patients have been selected from participants of an annual structured outpatient FIT UPDATE module (educational weekend developed for FIT patients). Thirty type 1 diabetic FIT patients with CSII (females n=23, 77%), and 60 with MDI (6.3±1.4 daily injections, females n=40, 67%) comparable in age (CSII: 45.2±12.2 vs. MDI: 44.6±13.0 yrs), diabetes (24.2±13.3 vs. 23.4±10.2 yrs) and FIT (13.0±6.4 vs. 14.0±5.9 yrs) duration, and associated diseases were included in the study, their glycemic control was traced and extracted from electronic and paper patient files. The impact of diabetes treatment was measured with ADDQoL (Bradley), DTSQ extended for FIT State version and DES (Anderson) and with a specifically developed ranking scale for advantages and disadvantages of pumps and/or injections.
Results: There was no significant difference in HbA_{1c} values between the two groups at any point of observation. HbA_{1c} values remained good in both patient groups on the level comparable to that one of the intensified treatment of DCCT group for the mean observation period of CSII treatment of 5.0±2.8 years, however already slightly higher as after the initial main educational intervention of FIT training. Mean HbA_{1c} level during the period of the controlled study was 7.4±0.7 (CSII) vs 7.2±1.0% (MDI), p=0.36. Initial pre-pump HbA_{1c} level was slightly (p=0.09) higher in the CSII group (7.5±0.8 vs.7.3±1.0%), but it improved with CSII initiation reaching its nadir of 7.2±0.5 after 7-12 months following the initiation of the pump treatment. Thereafter, HbA1c values tended to increase and after 19-24 months of CSII reached the values comparable to those in the pre-pump period. No significant differences for frequency of severe hypoglycaemia were found. Behavioural differences between groups were given for reported number of daily blood glucose self-measurements (CSII: 5.6±0.9 vs MDI: 5.1±0.8, p=0.01). The differences in treatment satisfaction were significant only for item 13 of the DTSQ extended for FIT, dealing with satisfaction with basal insulin where pump patients displayed somewhat higher satisfaction. Additional questions for basal insulin dealing with stability of blood glucose values between meals and with fasting values did not reach statistical significance nor the other subscores did. The results of ADDQoL, and DES did not show any significant differences among the two groups.
Conclusion: No significant differences were found between outcomes of CSII and MDI in FIT educated patients beyond an initial HbA_{1c} improvement of 6-12 months. Functional insulin use with in average more than 6 daily injections results in FIT-educated patients in similar outcomes independently of the mode of insulin delivery.

946

Integrated real-time continuous glucose monitoring/insulin pump system (PRT) usefulness in 122 children with type 1 diabetes. A 3-year follow-up study
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Background and aims: Real-time continuous glucose monitoring and the insulin pump have been combined into the sensor-augmented pump system

(PRT) (Medtronic MiniMed, Sesto San Giovanni, Italy). The objective of the study was to evaluate the clinical effectiveness and safety of PRT in a large series of children with type 1 diabetes using insulin pump therapy.
Materials and methods: This is a multicenter observational study. A questionnaire was sent to all paediatric diabetologic centres in Italy (n=65); data was analyzed only regarding patients aged 18 or less and using PRT for 6 months or more.
Results: A total of 48 centres (73.85%) answered the questionnaire. The total number of patients with type 1 diabetes followed by the centres is 12.549, of whom 1437 (11.4%) have been using insulin pump therapy for more than 6 months. Of all patients using an insulin pump, 129 have been using PRT for at least 6 months, with a mean follow-up of 1.4±0.7 yrs (range 0.5-3 yrs). Their age was 13.5±3.8 yrs, with disease duration of: 6.3±3.4 yrs). After 0.5-3 yrs of using PRT, HbA1c showed a significant improvement (8±1.5 vs 7.4±0.8%, p = 0.002). Insulin requirement showed a significant decrease (0.88±0.25 vs 0.79±0.23 U/kg/day, p =0.003). BMI did not change during the observational period. Mean usage of PRT per month was 8.1 day/month and any significant correlation between sensor use and HbA1c has been observed (r2=0.0005, p=0.239). No DKA was observed during the follow-up, while episodes of severe hypoglycemia significantly decreased (p=0.04).
Conclusion: The increased availability of continuous glucose sensors is likely to have a significant impact on pediatric diabetes therapy and education in the near future. Selection of patients capable and motivated to use sensor-augmented pump with proper age-appropriate education could be the key factors for the long-term success of these new technological advances in diabetes therapy as we have seen in our large group of children using PRT.

PS 81 Improving insulin

947

A new approach for oral delivery of insulin
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Background and aims: Oral administration of insulin is the most physiological and comfortable way. However, insulin a 51 amino acid peptide, is less absorbed by the gastrointestinal tract and is degraded by proteolytic enzymes. Thus, in order to circumvent these difficulties, we have encapsulated insulin in nanoparticles (NPs) composed of alginate, coated with chitosan-PEG (polyethylene glycol 4000)-albumin.
Materials and methods: The biological, metabolic and toxicological effects of insulin NPs were investigated after oral delivery in diabetic rats induced by streptozotocin.
Results: (1) NPs, less than 600 nm in size, were able to encapsulate more than 85% of insulin. They protect insulin againt pepsin. In vitro, at pH 1.2 (gastric pH), they released 25% of their insulin content and 70% at pH 6.8 (intestinal pH). (2) When administered orally in fasted diabetic rats, insulin NPs (25, 50, 100 I.U./kg) reduced dose-dependently glycemia from 2h, with a maximal effect at 10h (-53%, p<0.001) and maintained this effect up to 48 h. Insulin NPs also improved the glycemic profile after an oral glucose overload (2 g/kg) and increased (9 times) plasma insulin level. (3) The pharmacological bioavailability calculated against a s.c. administration of free insulin was about 21% for a dose of 50 I.U./kg insulin. (4) After a daily administration of 50 I.U./kg insulin NPs during 4 days, the water and food intakes, urine volume and proteinuria were significantly reduced. (5) After a 15 days daily administration at the dose of 50 I.U./kg, there was none hematological nor tissular toxicity. (6) Finally, encapsulated insulin labelled with FITC was absorbed through the intestinal epithelium and the Peyer's patches.
Conclusion: NPs composed of an alginate core coated with chitosan-PEG-albumin induced a prolonged anti-diabetic effect after oral administration. This effect may be explained by the protection of insulin against proteolytic enzymes in the GIT and the facilitation of its absorption due to chitosan which transiently opens the tight junctions.

948

***In vivo* validation of a double encapsulation of insulin for an oral administration**
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Background and aims: The purpose of this study was to develop a new formulation of oral insulin in order to improve the treatment for diabetic patients. Normally, peptide hormones like insulin are given by parenteral injections routes because they are destroyed by the acid and proteolytic enzymes in stomach and intestine. Our project focuse on insulin double encapsulation for oral administration.
Materials and methods: The first system of encapsulation of insulin is based on Poly(Lactide-co-Glycolide) Acid (PLGA) nanoparticles. This should allow the uptake and the transport across mucosal intestinal barrier. These nanoparticles are put in a gastroresistant capsule made of methacrylic acid and ethyl acrylate (1/1). The gastroresistant property of the capsule was observed by x-ray tomography after gavage of baryum chloride capsule to rats. Concerning the nanoparticles, microscopic visualizations were done by TEM and CSEM, the encapsulation rate of insulin was evaluated by the Bradford's test and the insulin conformation can be analysed by Circular Dichroisme. *In vivo*, the biofunctionnality of the nanoparticles was evaluated after subcutaneous injections. The absorption of insulin was first performed by the visualization of insulin-FITC loaded nanoparticles injected in the duodenum. In a second way, we measured the kinetic of the glycemia and the biofunctionnality of NP in streptozotocin-induced diabetic rats after intra-duodenal injections of insulin nanoparticles and intraperitoneal injections of insulin. The bioavaibility was evaluated after quantification of the insulinemia after intra-peritoneal injections of insulin and after intraduodenal injections of the same quantity of encapsulated insulin.

Results: The gastroresistance of the capsule was confirmed as 5h after gavage the capsule was still intact in the stomach of the rats. After synthesis, the size of nanoparticles is 160 ±10nm and the rate of encapsulation is around 95%. Circular dichroïsm indicated that insulin conformation was preserved during particles synthesis. Sub-cutaneous injections of insulin nanoparticles induce the same decrease of glycemia than subcutaneous injections of insulin showing the biofunctionnality of the system. *In vivo*, fluorescence microscopy carried out on portions of the small intestine revealed the presence of concentrated fluorescence into the mucosa 30min after the administration of insulin-FITC loaded nanoparticles. So nanoparticles can efficiently protect insulin in the intestine and can be absorbed. Intraduodenal injections of insulin nanoparticles induce a significant decrease of glycemia from 5,2 ±0,5g/L to 0,9 ±0,2g/L 9h after injection (t test; p≤0,001). In comparison with intra-peritoneal injections, results showed that nanoparticles' biodisponibility is at less 20%.
Conclusion: PLGA insulin-loaded nanoparticles are efficient and the biological effect of insulin is preserved. These polymeric particles allow the absorption of insulin through intestinal mucosa into the bloodstream. Thus this new delivery insulin formulation seems to be an interesting approach.

949

Combination of intestinal goblet HT29 and Raji-B cells with absorptive Caco-2 cells to predict intestinal absorption of insulin encapsulated into nanoparticles

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Background and aims: Oral delivery of therapeutic proteins like insulin is desired due to its convenience. Pharmacokinetics and biodistribution of insulin after oral administration can be improved with the incorporation into nanoparticles. Solid lipid nanoparticles (SLN) are an alternative in the production of pharmaceutical systems. The use of chitosan to coat SLN constitutes an important advance to enhance the permeability of drugs towards the intestinal epithelium. The Caco-2 line is a well-established in vitro cell model for predicting the absorption of orally administered drugs in humans. The prediction of intestinal drug absorption by an appropriate in vitro cell model based on Caco-2 cells incorporating different intestinal cells like HT29 mucus-producing goblet cells or Raji B M-cell phenotype cells, in order to mimic as close as possible the intestinal epithelium, would avoid costly and time-consuming animal experiments. In this work the aim was to investigate the permeability of insulin loaded into SLN and chitosan-coated SLN across different Caco-2/HT29 and Caco-2/Raji co-culture cells monolayer, attempting future correlation with in vivo physiologic absorption.
Materials and methods: Insulin was entrapped into SLN by W/O/W multiple emulsion and further coated by chitosan to take advantage of its mucoadhesive properties. For permeability experiments, Caco-2, HT-29 and Raji B were seeded single and in the co-culture system using different proportions onto Transwell® permeable supports 3,0 µm, polycarbonate membrane. Transepithelial electrical resistance (TEER) of the cell monolayers was measured using an EVOM epithelial voltohmmeter to check monolayer integrity and were cultured for 21-28 days before the initiation of an experiment. Transwell® insulin absorption experiments were run at 37ºC from apical to basolateral chamber. Insulin was measured by HPLC.
Results: Permeability assays realized in Transwells® showed insulin permeability increase with increase of HT29 cell content for all formulations (insulin in solution, insulin loaded SLN and insulin loaded chitosan-coated SLN). Permeability results for Caco-2/HT29 90:10 co-culture was more consistence with the human intestine cell distribution. The effect of number of Raji cells on insulin absorption also demonstrates the implication of M-like cells on drug absorption. Although latter onset, probably due to delay of release from nanoparticle matrix, chitosan coating of SLN demonstrated absorption enhancing effect. This may occur due to mucoadhesion and opening of the tight conjunction between the epithelium cells that can improve the insulin permeability into the co-culture monolayer. TEER values were measured during the experiment to evaluate the integrity of the cell monolayer. During all experiments, TEER values decreased, but the integrity of the cells was maintained.
Conclusion: Results demonstrate that Caco-2/HT29 and Caco-2/Raji co-culture cell model are reliable systems to correlate in vitro insulin absorption with in vivo animal model. Absorption of insulin entrapped into chitosan-coated SLN seems to be a promising alternative for the development of a formulation for oral insulin administration.
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950

Management of impaired glucose tolerance using buccal spray insulin

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Background and aims: Postprandial hyperglycaemia occurs as the consequence of a reduced first phase insulin response after a meal and has been associated with an increased risk of cardiovascular events and mortality. Subjects with postprandial hyperglycaemia often present with dyslipidaemia, hypertension, abdominal obesity, microalbuminuria, endothelial dysfunction and markers of inflammation. Correction of the first phase insulin response using fast insulin is an attractive option. The objective of this phase II study was to investigate the safety and efficacy of treatment with buccal spray insulin (Oral-lyn™)on post-prandial plasma glucose and insulin levels in subjects with impaired glucose tolerance (IGT).
Materials and methods: A total of 20 Caucasian subjects, mean age 48.7 ±14.3 SD years, a body mass index of 32± 6 Kg/m2, 13 females and 7 males, with confirmed IGT were included in the study. Subjects were randomized to take 4, 6 or 12 Oral-lyn™ puffs, split in two equal doses each, one before and the second 30 minutes after a standard 75 gr oral glucose tolerance test (OGTT). Glucose excursions and insulin levels were measured at baseline and 30, 60, 90, 120, 180 min afterwards.
Results: There were no significant differences in plasma glucose levels during OGTT with 4 or 6 puffs compared to OGTTs performed without insulin administration. Treatment with 12 Oral-lyn™ puffs (equal to 12 IU) was followed by a significant 29.6% decrease in mean plasma glucose at two-hours (from 175.3± 14.0 to 124.0±39.1 mg/dl), and a 26.8% decrease at three-hours (from 124.7±50.5 to 91.3±26.0 mg/dl), altogether p=0.01. Considering all time points of the OGTT up to 180 min, there was a mean reduction of 15.8% in plasma glucose levels. There was a trend for increased insulin (µU/L) levels at all measurements. Finally, no adverse events were observed during the study period and no hypoglycaemic episodes were complained at any timepoints.
Conclusion: This proof of concept study demonstrates that treatment with buccal spray insulin is a simple and valuable therapy for reducing postprandial hyperglycaemia in obese subjects with IGT. Importantly, this treatment was safe and none of the study subjects experienced hypoglycaemia. The use of insulin through an alternative route such as the buccal mucosa can represent a novel approach for treatment of postprandial hyperglycaemia.

951

Comparison of glycaemic variations in Japanese patients with type 1 diabetes receiving long-acting soluble insulin as assessed by continuous glucose monitoring (CGM)

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Background and aims: Japanese patients with type 1 diabetes receiving the long-acting soluble insulin preparations insulin glargine and insulin detemir were compared for glycemic variation by using continuous glucose monitoring (CGM).
Materials and methods: A total of 16 patients with type 1 diabetes (males/females, 5/11) were enrolled in the study. Their median age was 55.0 years (range, 35.0-60.8), median BMI 22.5 kg/m^2 (range, 19.5-23.6), median HbA1c 7.2% (range, 6.7-8.8), and median urinary CPR 1.1 µg/day (range, 0.7-5.5). Of these, 2 patients received one insulin injection, and 14 received two insulin injections. The subjects were crossed over from insulin glargine or insulin detemir or vice versa with no change in the number of insulin preparations injected or in the timing for such injections, and were monitored by CGM while on either preparation for glycemic variation in a non-blinded fashion during the 4-day hospitalization, with the timing for another hospital stay scheduled more than 1 month after change of insulin preparations. CGM data obtained on day 3 of their hospitalization were used for analysis. Mean glucose levels, their standard deviations (SD), M values, duration of hypoglycemia (< 70 mg/dL), duration of hyperglycemia (> 200 mg/dL), range of postprandial glucose increase and time to peak glucose values (time to peak glucose values after each meal and a mean time to peak glucose values after 3

meals) were compared between those given insulin glargine and those given insulin detemir by using Mann-Whitney's U test. All statistical analyses were performed by using SPSS 16.0. The present study was approved by the Ethics Committee of the Jikei University School of Medicine.
Results: There was no significant difference between those receiving insulin glargine and those receiving insulin detemir with their mean glucose level being 158.5 mg/dL (133.5-200.5) and 154.0 mg/dL (129-178.5), their SD being 53.5 mg/dL (41.5-80.3) and 50.5 mg/dL (41.5-64.5), and their M values being 36.0 (26.8-76.0) and 34.5 (20.8-66.0). Those receiving insulin glargine or insulin detemir were not significantly different, either, with regard to the duration of hypoglycemia (< 70 mg/dL) or duration of hyperglycemia (> 200 mg/dL). In addition, there was no significant difference between those receiving insulin glargine and those receiving insulin detemir with regard to the range of postprandial increase in glucose levels after morning meals, evening meals as well as the mean range of increase after 3 meals, and the time to peak glucose levels, while those on insulin detemir tended to show a narrower range of postprandial glucose increase (P = 0.10) and a shorter time to peak glucose levels (P = 0.067). After completion of the study, asked to choose between the two insulin preparations, more patients tended to opt for insulin detemir over insulin glargine, with 12 out of 16 (75%) choosing insulin detemir (P = 0.077).
Conclusion: A comparison of glycemic variations in type 1 diabetic patients receiving insulin glargine or insulin detemir by CGM demonstrated that both insulin preparations were almost equally efficacious. It was also shown that insulin detemir could potentially offer better glycemic control after midday meals than insulin glargine and that more patients tended to choose insulin detemir over insulin glargine after completion of the study.

952

Technosphere insulin suppresses endogenous glucose production earlier than a rapid-acting analogue (lispro) and an inhaled insulin (Exubera®)
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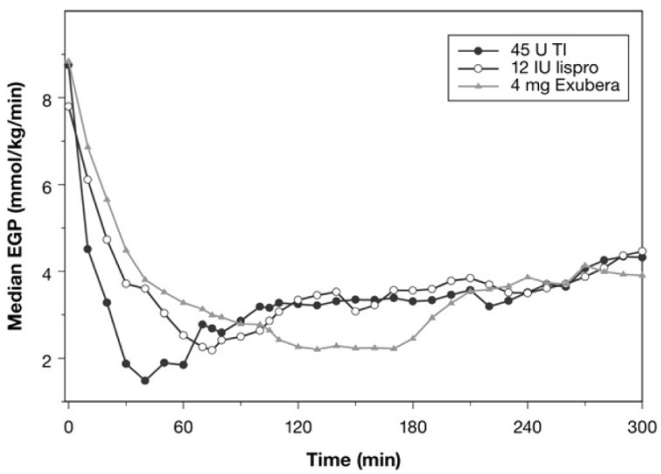
Background and aims: Available insulins are unable to replicate normal hepatic glucose suppression, presumably due to slow absorption. Technosphere Insulin (TI) is a rapid-acting insulin generating peak insulin levels within 12 to 14 minutes of dosing. We conducted a study to determine whether the unique pharmacokinetic profile of TI resulted in a more rapid suppression of endogenous glucose production (EGP).
Materials and methods: We compared 45 U TI administered by inhalation with 12 IU s.c. insulin lispro and 4 mg inhaled Exubera (EXB) in an open-label, single-dose, three-way crossover study incorporating a meal challenge (nutritional energy drink [12 fl oz] enriched with U13-C-glucose) in 18 insulin-treated subjects with type 2 diabetes and normal pulmonary function. A continuous glucose infusion enriched with 6,6-$^2H_2$ glucose was used to assess EGP. Prior to the meal, subjects' blood glucose was adjusted to 90 mg/dl using an individual continuous low-dose i.v. insulin infusion, which was fixed 90 minutes before dosing. If necessary, glucose was infused to maintain blood glucose at ≥90 mg/dl.



Results: EGP suppression occurred markedly earlier with TI, followed by insulin lispro and EXB (40, 75, and 130 minutes post-dose of the median EGP-time profiles, respectively). Significant differences between insulin lispro and EXB were observed up to 40 minutes compared with TI (p<0.002) and up to 2 hours for the EXB-TI comparison (p<0.05). Median total areas over the EGP curve were comparable across groups (1,938, 1,842, and 2,294 µmol/min). Median postprandial blood glucose AUCs were 53,343, 50,608, and 54,598 mg/dl•min for TI, insulin lispro, and EXB, respectively.
Conclusion: EGP was suppressed earlier following TI administration compared with s.c. insulin lispro and inhaled EXB, which suggests that treatment with TI may result in a more physiologic EGP suppression.

953

Accelerated insulin pharmacokinetics and improved glycaemic control in patients with type 1 diabetes mellitus by coadministration of prandial insulin with recombinant human hyaluronidase
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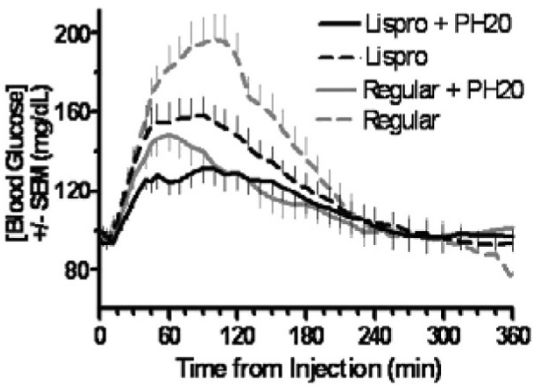
Background and aims: Recombinant human hyaluronidase (rHuPH20) is a locally and transiently acting enzyme that catalyzes depolymerization of hyaluronan, thus reducing the barrier to local bulk fluid flow and increasing the permeation of coinjected drugs. This prospective Phase 2 study compares the pharmacokinetics (PK) and postprandial glucose (PPG) response to SC insulin lispro (lispro) and regular human insulin (regular) ± coinjected recombinant human hyaluronidase (rHuPH20) following a liquid meal in T1DM.
Materials and methods: Patients fasted (10 h) and refrained from SC insulin for 12 h before dosing. Starting 2 h before a liquid meal (60 g CHO) challenge, patients were titrated to 100 mg/dL glucose target with IV glucose and/or insulin followed by a 30 min intervention-free equilibration. The same dose of insulin lispro (mean 5.7 ± 3.0 U, optimized for each patient) ± rHuPH20 (0.2 µg/U insulin) was injected SC immediately before the meal, and plasma insulin and glucose concentrations were monitored for 8 h. This program was then repeated for regular (mean 6.2 ± 3.5 U) ± rHuPH20.
Results: Of a planned 20 patient 4-way crossover study, final PK data are available for the first 12 and final glucose data for all 21 completers (14 male, 7 female, age = 41.3 ± 10.6 SD yr, BMI = 24.4 ± 2.9 SD kg/m^2). PK for both lispro + PH20 and regular + Ph20 showed accelerated but overall comparable exposure as compared to each without PH20. Early $t_{50\%\ max}$ decreased from 19.9 to 12.6 min (p=.0002) for lispro and 40.1 to 14.8 (p=.033) for regular; t_{max} decreased from 43.8 to 27.9 min (p=.002) for lispro and 96.7 to 52.1 (p=.086) for regular; late $t_{50\%\ max}$ decreased from 98.6 to 68.6 min (p=.0001) for lispro and 195.0 to 111.2 (p=.007) for regular. Corresponding glycemic response curves are shown in the figure. AUC>140 mg/dL (0-4 h) was reduced from 3217 to 1298 min*mg/dL (p=.044) for lispro and 6606 to 1854 (p=.006) for insulin; peak glucose (0-4 h) fell from 179 to 152 (p=.006) for lispro and 211 to 168 (p=.004) for insulin. All injections were well tolerated and there were no serious, significant or moderate adverse events. The most common adverse event (mild) was low blood glucose concentration, which was similar between treatment groups.
Conclusion: Coinjection of rHuPH20 accelerated insulin PK and led to improved PPG control for both lispro and regular insulin.



Supported by: Halozyme, Inc.

954

Pharmacokinetics of Technosphere insulin unchanged in patients with chronic obstructive pulmonary disease

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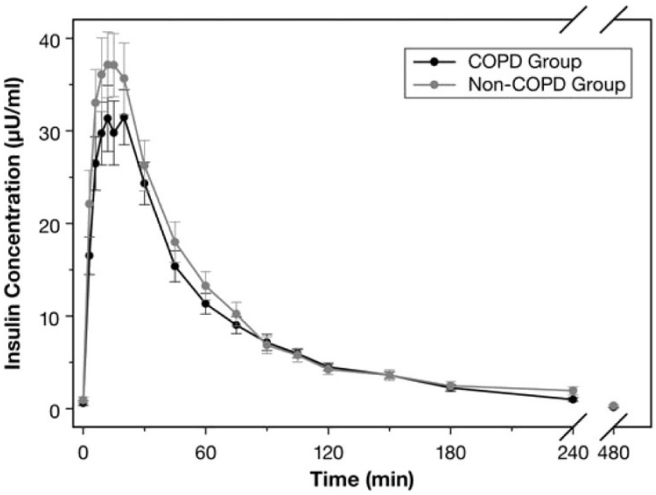
Background and aims: Inhaled insulin has historically been shown to decrease the rate and extent of insulin exposure by approximately 20% to 50% in subjects with chronic obstructive pulmonary disease (COPD).
Materials and methods: The pharmacokinetics (PK) of Technosphere Insulin (TI), a rapid-acting insulin for pulmonary delivery, was evaluated in an open-label, single-dose, euglycemic glucose clamp study in 37 non-diabetic, non-smoking healthy subjects (N=19; baseline mean ± SD age 51±14 years; BMI 29±3 kg/m^2; forced expiratory volume in 1 second [FEV_1] 3.5±1) and subjects with COPD (N=17; aged 60±9 years; BMI 29±5 kg/m^2; FEV_1 2.6±0.8).
Results: Thirty-four subjects were age-, gender-, and BMI-matched. One COPD subject was excluded. Each subject received a single dose of 30 U of TI via inhalation. Serial blood samples were drawn for insulin and C-peptide concentration determination until 480 min post-dose. Insulin concentrations were C-peptide corrected to account for endogenous insulin, and C-peptide corrected values were used for PK parameter estimation. There was no statistically significant difference in absorption in the two groups. Mean peak insulin was 34.7 and 39.5 μU/ml (p=0.285) with a median time to maximum insulin concentration of 15 and 12 min (p=0.241) in the COPD and non-COPD groups, respectively. Mean insulin exposure from time 0 to 240 min post-dose ($AUC_{0\text{-}240}$) was 2037 and 2270 μU/ml•min (p=0.469) for the COPD and non-COPD groups, respectively. Dosing was well tolerated.
Conclusion: These results indicate that the characteristic absorption pattern of TI is not significantly altered in the COPD population.



955

Lung deposition and absorption of insulin from Technosphere insulin

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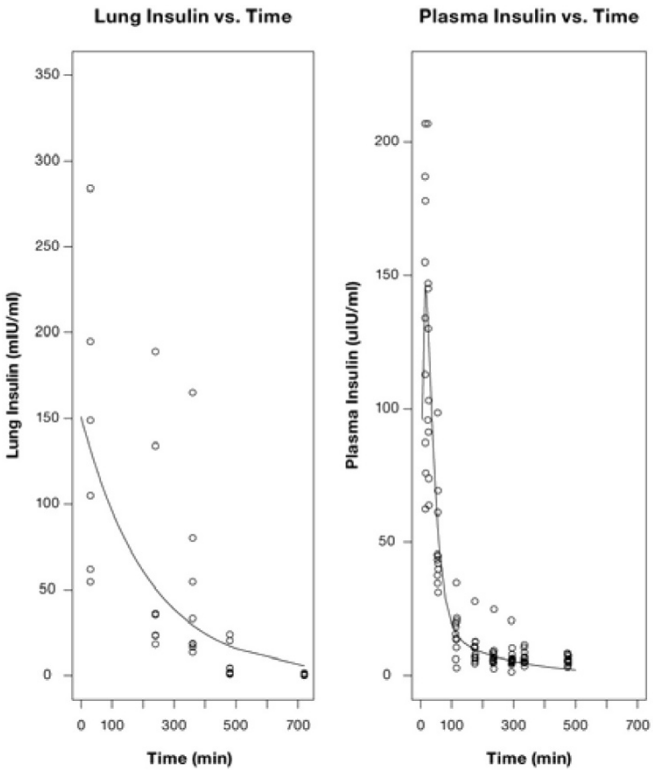
Background and aims: A clinical trial in 12 healthy subjects determined the pulmonary concentrations of insulin and fumaryl diketopiperazine (FDKP) using bronchoalveolar lavage (BAL) before and after administration of a rapid-acting insulin, Technosphere Insulin (TI) Inhalation Powder.
Materials and methods: Each subject received a 60 U dose and had two bronchoscopies at 0.5 and 6 h, or 4 and 8 h, or 0.5 h before dosing and 12 h after dosing. At each bronchoscopy, two independent BAL samples were collected. Serum samples for serum insulin, FDKP, and serum C-peptide were collected at baseline and up to 475 min post-dose. Urea concentrations in BAL samples and blood urea nitrogen were measured and used for correcting BAL concentrations (see figure below).
Results: We previously reported a phase I trial using ^{99m}Tc-labeled TI inhalation particles. These particles were evenly distributed with approximately 28% to 48% (mean 39.4%) of the ^{99m}Tc TI delivered to the deep lung. In this study, subjects were dosed with 60 U of TI. Concentrations of insulin and FDKP in BAL fluid were corrected for urea BAL to serum concentration ratios. The amount of insulin and FDKP remaining in the lung epithelial lining fluid was highest (21.5 mIU/ml) in the first post-dose BAL samples (0.5 h). By 4 h, levels were 31% of maximum; at 12 h, insulin and FDKP values in BAL were 0.3% and 0.4% of maximum, respectively. Accumulation of insulin and FDKP in the lungs after chronic dosing is unlikely.
Conclusion: Preliminary pharmacokinetic modeling of insulin (see solid line figure below) suggests a two-compartment lung absorption model, one with fast absorption reflected in the plasma profile of insulin. The other compartment reflects reduced absorption, which is presumably due to proteolysis and mucociliary clearance of insulin.



Supported by: MannKind Corporation

PS 82 Technical aspects of insulin delivery

956

Comparison of glycaemic stability with long acting insulin analogue vs CSII in type 1 diabetic patients during the fasting period of functional insulin therapy

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Background and aims: The aim of our study was to compare the pharmacodynamics properties of the two modes of basal insulin administration during a 36-h fast in 24 type 1 diabetic patients training for functional insulin therapy.
Materials and methods: Only basal insulin was delivered during the 36-h fast and glycemic stability was evaluated by continuous glucose monitoring (Glucoday®). The basal insulin dose was determined for each patient using the Howorka formula. In case of hyper or hypoglycaemia, insulin correction (1 or 2 UI of lispro or aspart) or carbohydrate collation (10 or 20 g) were respectively provided in order to recover normoglycaemia (80 - 120 mg/dL). Fifteen patients (8 Men 7 Women, age = 39.6 ± 12.5 years, weight = 76.4 ± 13 Kg, HbA1c = 7.67 ± 1%, diabetes duration = 20.5 ± 9.8 years) were treated with one long acting analog insulin injection (Glargine group). Nine diabetic patients (5 Men 4 Women, age = 43.3 ± 12.3 years, weight = 70 ± 13 Kg, HbA1c = 7.93 ± 0.9%, diabetes duration = 21.77 ± 7.7 years) were treated with continuous subcutaneous insulin infusion (CSII) of fast acting analog (Lispro or Aspart) at a constant basal infusion rate (CSII group). The analysis of the interstitial glucose curves (Glucoday®) recorded during the fast has allowed to determine the following asymptomatic glycaemic variability indexes: standard deviation of mean interstitial glucose concentration, MAGE (Mean Amplitude Glycaemic Excursions) and MODD (Mean Of Daily blood glucose Differences).
Results: During the fasting period, the basal insulin dose was similar in the two groups (0.28 ± 0.08 UI/Kg/day in the Glargine group vs 0.29 ± 0.07 UI/Kg/day in the CSII group, p=NS). However, the number of insulin corrections necessary to maintain normoglycaemia was much higher in the CSII group (n = 3.2 ± 1.2 vs 2.1 ± 1.8, p = 0.05), but the number of « carbohydrate intake » necessary to maintain normoglycaemia was similar in the two groups (n = 2.7 ± 1.8 vs 2.7 ± 1.5 , p = NS). Mean interstitial glucose level and standard deviation of the mean interstitial glucose level (CSII = 99 ± 9 mg/dl vs glargine = 99 ± 15 mg/dl), MAGE index (CSII = 78 ± 38 mg/dL vs glargine = 75 ± 31 mg/dl) and MODD index (CSII = 44 ± 22 mg/dl vs glargine = 37 ± 26 mg/dl) were not significantly different between the two groups.
Conclusion: Fasting glycaemia regulation is as effective with subcutaneous long acting insulin injection as it is with CSII at a constant basal infusion rate.

957

The optimal type of bolus following a 'Mediterranean' meal in children and adolescents with type 1 diabetes using insulin pump therapy

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Background and aims: Few is known about the best type of bolus before meals in patients with type 1 diabetes (T1DM) using insulin pump therapy. Last year we observed that the best bolus for a 'pizza' meal (pizza 'margherita') is 30/70 dual wave bolus extended over 6 hours. After this, we start to consider that for a 'Mediterranean' meal (more carbohydrate and less proteins and lipids) a dual wave bolus might be better than a simple bolus. The aim of our study is to compare a simple bolus with different kind of dual wave bolus in order to identify the optimal one in case of a Mediterranean meal.
Materials and methods: We evaluated 26 children and adolescents, aged 5-23 yrs (mean 15.40±4.84 yrs) with T1DM from 1 to 19 yrs (9.67±4.91 yrs), BMI 22.01±4.41 kg/m2, in therapy with subcutaneous insulin infusion (insulin requirement 0.76±0.14U/kg/day). Each patient participated in a study to compare postprandial glucose values following 6 meal bolus regimens for a daily meal (1600-2000 kcal according to children age). Each patient utilized the following 6 aspart regimens on three consecutive days, and glucose values were recorded with SMBG: a) a single-wave bolus (100% of insulin given immediately) injected 15 min before the meal; b) a single-wave bolus (100% of insulin given immediately) injected just before the meal; c) 4-h dual-wave bolus (50% of insulin given immediately and 50% given over a 4-h period) injected 15 min prior the meal; d) 4-h dual-wave bolus (50% of insulin give immediately and 50% given over a 4-h period) injected just before the meal; e) 4-h dual wave bolus (70% given immediately and 30% given over a 4-h period) injected 15 min before the meal; and f) 4-h dual wave bolus (70% given immediately and 30% given over a 4-h period) injected just before the meal. Total CHO was kept constant for each meal; insulin dose was calculated according to glycaemic value and CHO, using ISF and INS:CHO ratio. personalized for each patient.
Results: The results are shown in the table.
Conclusion: 70/30 double wave bolus extended over 4-h period following a Mediterranean meal injected 15 min before provided significantly less postprandial hyperglycemia during the 4-h period. Single-wave bolus could be used only if given 15 min before meal, even if we observed a rise in glycaemic values in the last two hours of the study.

Glycaemic values according the different bolus types in the children studied.

Type of bolus	T' 0	T' 30	T' 60	T' 90	T' 120	T' 180	T' 240	T' 300	T' 360
Single-wave bolus 15 min before	157±83	145±67	139±60	139±70	133±50	122±67	138±59	161±60	148±62
Single-wave bolus just before	143±77	144±71	136±82	119±75	122±70	144±51	156±65	180±65 p=0.02	193±80 p=0.01
Double-wave bolus 50/50 in 4h 15 min before	114±64	163±52 p=0.03	181±63 p=0.02	172±72 p=0.03	163±75 p=0.03	131±68	121±70	105±44	98±26
Double-wave bolus 50/50 in 4h just before	125±45	140±55	149±73	147±58	144±63	139±69	124±77	144±104	152±111
Double-wave bolus 70/30 in 4h 15 min before	113±52	146±57	144±47	121±46	137±66	123±55	122±58	113±63	105±43
Double-wave bolus 70/30 in 4h just before	109±37	114±42	114±50	114±54	116±41	127±66	152±60 p=0.02	181±72 p=0.01	189±76 p=0.01

958

Intraperitoneal insulin delivery gives higher circulating IGF-I activity than CSII in type 1 diabetes

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Background and aims: In type 1 diabetes the circulating IGF-system is altered with low IGF-I and changes in IGF-binding proteins (IGFBPs). These alterations may be of importance for development of diabetes complications. We hypothesized that the IGF-system is affected by the route of insulin administration and that intraperitoneal (IP) insulin infusion has a more pronounced effect than subcutaneous insulin administration.
Materials and methods: We compared 10 patients with type 1 diabetes and implantable insulin pumps administrating IP insulin to 20 age-and sex matched patients on continuous subcutaneous insulin infusion (CSII) treatment, age 53.1± 9.1 years vs 52.8±9.0 (mean±SD), diabetes duration 24.2±11.3 years vs 30.8±6.0 and HbA1c 8.6±1.4 % vs 7.9±0.8 % (DCCT standard)(ns). Circulating levels of IGF-I bioactivity and IGFBP-1 were measured with validated in-house assays. IGF-I bioactivity was measured with an IGF-I specific kinase receptor activation assay (KIRA).
Results: Fasting levels of bioactive IGF-I was 1.83±0.76 µg/L in patients with IP insulin infusion compared with 1.16±0.24 µg/L in patients with CSII (p=0.02). The corresponding concentrations of IGFBP-1 were 48.5(77.3) and 80.6(42.2) µg/L [median (IQR)](p=0.07). Regression analysis showed a sig-

nificant inverse correlation between bioactive IGF-I and IGFBP-1 (r=-0.59, p=0.001).
Conclusion: The IGF-I bioactivity is higher in patients with intraperitoneal pumps compared to CSII supporting the theory that the route of insulin administration is of importance for the IGF-system. Intraperitoneal insulin administration may therefore be beneficial by normalising the alterations of the IGF system in type 1 diabetes.

959

Time delay to occlusion detection of various insulin pumps
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Disetronic Medical Systems AG, Burgdorf, Switzerland.

Background and aims: Patients on pump therapy have only a small subcutaneous depot of insulin and therefore encounter an increased risk of ketoacidosis if basal insulin delivery is interrupted. To limit this risk all insulin pump models are equipped with occlusion alarm mechanisms that, however, differ in sensitivity. A new, additional occlusion detection algorithm was developed and implemented into Accu-Chek Spirit Combo to detect occlusions earlier. The algorithm analyses the change in pressure over time to detect occlusions before the specified absolute force threshold is reached. The aim of this study was to compare the occlusion detection performance of the new pump to other commercially available insulin pumps.
Materials and methods: The response to an infusion set occlusion was tested in two to five insulin pumps of each model according to the standardized procedure specified in IEC 60601-2-24 section 51.101b. Plastic cartridges and infusion sets with 110 cm tubing were used (Accu-Chek TenderLink, Paradigm Silhouette® and Paradigm Quick-set®).
Results: First results demonstrate that the time to occlusion alarm was dependent on the basal rate and significantly differed between pump models and settings (Table 1).
Conclusion: Accu-Chek Spirit Combo and Animas® 2020 (sensitivity set to "high") detected occlusions on average 2 to 5 times earlier at the lower basal rate tested. Time delays for MiniMed Paradigm® 522/722 were much longer compared to the other models or settings used. Measured time delays were compatible with the information of the respective user manuals. The marked different delay times in occlusion detection should be considered when selecting the insulin pump model for a patient since these differences may become relevant during the night and between meals when no boluses are given.

Table 1: Time to occlusion alarm depending on insulin pump and basal rate delivery

	Basal Rate 0.05 IU/h	**Basal Rate 1.0 IU/h**
	Mean ± SD (hh:mm)	
Accu-Chek Spirit Combo (n=5)	11:07 ± 5:04	0:51 ± 0:16
MiniMed Paradigm® 722 (n=2) & 522 (n=3)	60:58 ± 13:38***	1:58 ± 0:25***
Animas® 2020 (n=5) *Occlusion detection set to* "**low**"	36:22 ± 6:41***	1:22 ± 0:13**
Animas® 2020 (n=5) *Occlusion detection set to* "**high**"	12:47 ± 4:08	0:34 ± 0:09
One Touch® Ping™ (n=5) *Occlusion detection set to* "**low**"	35:51 ± 22:59*	1:00 ± 0:23
One Touch® Ping™ (n=5) *Occlusion detection set to* "**high**"	22:36 ± 13:21	0:41 ± 0:16

* *P* <0.05; ** *P* <0.01; *** *P* <0.001 (t-Test) as compared to Accu-Chek Spirit Combo.

960

The influence of needle length on glycaemic control and injection-related complaints in obese diabetic subjects
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Background and aims: A correct injection technique is important for optimal glucose control with insulin injections. Recent studies show that 5mm needles are safe and associated with unchanged HbA1c levels and reduced discomfort for patients compared with 8 or 12mm needles. Obese patients are usually advised to use 8-12mm needles when injecting insulin. This study investigated the effects of using 5mm x 31G needles vs 8mm x 31G needles in obese DM patients.
Materials and methods: In this multicenter, open-label, cross-over study, 130 patients with either type 1 or type 2 DM, injecting insulin with a pen and a BMI ≥ 30kg/m^2 were randomized into 2 groups. Group A used a 5mm needle in the first period and an 8mm needle in the second period; group B used the reverse order - each period was 3 months. The effects on HbA1c, fructosamine, 1.5 Anhydroglucitol (1,5 AG) and patient-reported hypoglycemic events, bleeding, bruising, backflow of insulin and pain were compared.
Results: 126 patients, mean BMI 36.4 (range 30-62), completed the study. Baseline parameters of HbA1c, amount of insulin, and BMI of the 2 groups were similar. There was no significant change in HbA1c while using either needle length, in either group. For all patients, mean HbA1c decreased from 7.6 to 7.5 (p=0.03) when using the 5mm needle, and stayed 7.6 % with the 8mm needle. Group B showed a minor decrease of insulin with the 5mm needle. The 1,5 AG and fructosamine assays are pending. Table 1 shows HbA1c in relation to needle length before enrollment to the end of the first treatment period. Differences were not significant. They support the primary findings of little HbA1c difference due to needle length within the two trial periods.

HbA1c at baseline and after 3 months, needles classified to length 5 and length 8

Needle length before inclusion	First Period	Baseline HbA1c	HbA1c after 3 months
5mm (n=65)	Length 5 (n=31)	7.5 ± 1.1	7.4 ± 1.1
	Length 8 (n=34)	7.3 ± 0.9	7.5 ± 0.8
8mm (n=54)	Length 5 (n=31)	8 ± 1.2	7.7 ± 0.9
	Length 8 (n=23)	7.8 ± 0.9	7.5 ± 0.9

There were no differences in hypoglycemic events, bruising and pain in either group during both periods. Patients reported less bleeding (p=0.04) with the 5mm needle, and less insulin backflow with the 8mm needle (p=0.01). The 5mm needle was preferred by 46% of patients, the 8mm needle by 41%; 13% had no preference. Neither needle length before inclusion or BMI were correlated to preference. No correlation (with both needle lengths) was found between backflow of insulin and BMI, WHR, HbA1c, amount of used insulin units, skinfold or injection site. There was a strong correlation (p<0.01) between bleeding and bruising. Results of hypoglycemic events, bleeding, bruising, backflow of insulin and pain with the first-period questionnaire were strongly correlated (p<0.01) with the outcomes of the second-period questionnaire.
Conclusion: 5mm needles can be safely used in obese DM patients without negative effects on HbA1c and without differences in local injection-related complaints, compared to 8mm needles. Injection complaints in obese patients appear more patient-dependent than needle-length dependent. Patients should receive information about the various needle lengths available and if they experience problems when injecting insulin, a trial of a different needle may be helpful.
Supported by: BD

961

The best insulin injection pen device for caregivers: results of simulation injection trials using 5 insulin injection devices
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Aim: In Japan, trained caregivers, generally family members, are allowed to administer insulin injections to diabetic patients who cannot self-inject insulin. An increased elderly and diabetic population has caused an increase in the number of diabetics who cannot self-inject insulin, requiring a greater number of caregivers who possess this skill. Here, we assessed the problems when insulin was injected by caregivers, and performed subjective evaluations of insulin injection devices.
Methods: We evaluated 5 devices_Opticlik®, SoloStar®, MirioPen® without an antiskid tool (Mirio(-)), MirioPen® with an antiskid tool (Mirio(+)), and

FlexPen®. The 22 respondents performed a sham injection to themselves (self-injection) and others (other-injection). Thereafter, respondents evaluated the feel and ease of the use of the pen devices through questionnaires. (1) Thickness of the insulin pen device, (2) Length, (3) Weight, (4) Stability of the needle on injecting into prosthetic skin , (5) Ease in seeing the dial at injection: the ease with which the dial and its movement can be seen, (6) Ease of pushing at injection: the usability and the presence of sound with successful injection button at the time of insulin injection, (7) Recapping the needle after injection: the ease of recapping the needle and any awkward arm movements required for recapping, (8) Needle disposal after recapping: the series of movements required for the disposal of needle after recapping it for destroying the needle, (9) Comprehensive evaluation were scored by respondents. For (4), (5), (6), (7) , (8) , (9) , a score of 5 meant good, 4(slightly good), 3 (normal), 2 (slightly bad), 1 (bad).

Results: Scores of comprehensive evaluation in self-injection were (Mean± S.D.) : 2.909 ± 1.192 for OptiClik, 3.273 ± 0.985 (SoloStar) , 3.227 ± 0.813 (Mirio(-)), 2.818 ± 0.958 (Mirio(+)), 3.545 ± 0.912 (FlexPen). Scores of comprehensive evaluation in other-injection were 3.364 ± 0.790 (OptiClik) , 2.864 ± 0.774 (SoloStar) , 3.091 ± 0.868 (Mirio(-)), 2.818 ± 0.958 (Mirio(+)), 2.591 ± 0.854 (FlexPen). FlexPen was selected to as the best device for self-injection but as the worst device for other-injection. Opticlik was selected to as the second worst device for self-injection but as the best device for other-injection. Moreover, for other-injection, FlexPen was considered to be too long, less stable, difficult to see the dial, difficult to recap, and comprehensively inferior.

Conclusion: We identified problems that were not apparent during studies evaluating conventional self-injection. We consider that the viewpoint from a caregiver is necessary for developing an insulin device specifically meant for insulin injection by other-injection.

Supported by: Tokyo Metropolitan Gov't Clinical Research Foundation

962

Risk of repeated use of needles for insulin pen needle in patients with diabetes mellitus

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Background and aims: Repeated use of needles for insulin pen needles is a serious violation of insulin injection technique. At repeated use, needle is exposed to significant deformation that increases injection morbidity and, probably, risk of lipodystrophy in sites of insulin injections. In literature, there are evidences that repeated use of insulin needles increases risk of infection development. Aim of study was to estimate danger of repeated use of needles BD Micro-Fine *Plus* for insulin pen needles on degree of contamination of needles with bacterial microflora, intensity of pain and presence of local reaction in sites of insulin injection.

Materials and methods: Blind, randomized study. 45 patients with diabetes mellitus of 1 and 2 types, age over 18 years old, on regimen of triple injections of short-acting insulin action and signed informed consent form were included in the study. The patients were randomized in 3 groups - 15 people in each. First group of patients used needle one time, second group - for 4 days (12 injections), and third group - within 7 days (21 injections). Changing of needles was carried out by medical staff. Duration of observation in all groups was 7 days. After use, microbiologic washouts were obtained from needles on aerobic, anaerobic flora and fungi. Intensity of pain after injection was estimated using the Visual-Analogue Scale (VAS) on 1, 4 and 7 day of study. Presence of local reaction in insulin injection site was determined by doctor on 1, 4 and 7 day of the study. Statistical processing of the received results was carried out using programs SPSS version 11.0 for Windows, for detection of differences between the groups, the nonparametric method of assessment - Mann-Whitney criterion (U-criterion) was used. Differences considered statistically significant at p <0,05 (95 % level of significance).

Results: Growth of microbe flora (Staphylococcus.koar - (Hly +) was revealed in 26,6 % of DM patients, who used a needle one time. Maximum quantity of needles contaminated by microbe microflora was registered in 3^{d} group (33,3 %) (Staphylococcus.koar - (Hly +) and Gram+ bacilli. Intensity of pain was significantly higher in 2nd group, than in 1st one (p=0,45) on fourth day of study, and in 3^{d} group pain was considerably more intense than in 1st group (p=0,03) on 7 day of the study. Hyperemia foci in injection sites on 4 and 7 days of the study were revealed only in 2nd and 3^{d} groups (13,3 and 26,6 %, correspondingly).

Conclusion: Already after single use, risk of microbe contamination of an insulin needle increases. Repeated use of needles amplifies risk of needle contamination. Patients using insulin needles several times suffered from pain caused by insulin injections more often.

963

Microneedle-based intradermal injection of lispro or human regular insulin accelerates insulin uptake and reduces post-prandial glycaemia

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Background and aims: Previously, intradermal (ID) insulin injection has demonstrated increased insulin uptake and action under glucose clamp conditions. This study investigated the effects of injection route, timing, and insulin type on the pharmacokinetics (PK) and prandial pharmacodynamics (PD) of insulin lispro (IL) and regular human insulin (RHI) under standardized meal conditions.

Materials and methods: After blood glucose stabilization at 120 mg/dL, 29 Type 1 diabetic males (mean age 35 yrs, BMI 25.7 kg/m^2, HbA_{1c} 7.4%) received 0.125U/kg of insulin at 2 or 17 mins before a standardized high-carb liquid meal (82g CHO), in a 5-arm randomized crossover study (Table 1). ID injections were administered using 1.5 mm x 34G microneedles or 8 mm x 31G needles for subcutaneous (SC) delivery.

Results: PK: Both insulin types showed markedly faster uptake and increased C_{max} when given ID compared to SC. Although total bioavailability over 4 hrs was similar between routes, ID delivery increased early phase absorption by 40-50% during the first 90 mins post-dosing. Likewise, the median T_{max} was reduced by one-half or greater for each insulin type. PD: The post-prandial glycemic (PPG) excursion (measured by primary PD endpoint: BG-AUC, 0-1.5h) for ID RHI was significantly reduced vs SC injection when both were given at -17 mins premeal (p<0.0001). Injection timing was also a significant factor, as ID RHI had a lower PPG when injected -17 mins pre-meal vs -2 mins. Overall, the mean BG response curve for ID IL tended to be lower and more level than that observed for SC IL when administered immediately pre-meal. However, the PPG for ID injection was not significantly different (NS) vs SC IL (P=0.1). Interestingly, the 4 hr PPG excursion for ID RHI given at -2 min was also NS from SC IL as measured by BG-AUC, although the BG max was slightly higher and occurred later. SC RHI at -17 mins had the highest BG responses, while ID RHI at -17 mins had one of the better PD outcomes. ID injection was well tolerated.

Conclusion: This study demonstrates that microneedle-based ID administration has a substantial impact on insulin PK, with a faster onset time observed for both insulin types. The reduction of prandial BG seen for ID regular insulin and the BG trends observed for ID insulin lispro may offer promise for improved outcomes with prandial insulin therapy.

Table 1. PK/PD Outcomes (Mean±SD)

	ID IL -2 min	SC IL -2 min	ID RHI -2 min	ID RHI -17 min	SC RHI -17 min
PD					
$BG\text{-}AUC_{0\text{-}1.5h}$ (h*mg/dL)	218±37	231±41	234±42	208±43	242±43
$BG_{1.5h}$ (mg/dL)	156±45	167±54	178±45	161±46	194±53
$BG\text{-}Max_{0\text{-}1.5h}$ (mg/dL)	173±35	185±41	190±40	171±40	201±48
$tBG\text{-}Max_{0\text{-}1.5h}$ † (min)	50	60	70	80	90
PK					
$INS\text{-}AUC_{0\text{-}1.5h}$ (h*µU/mL)	60±18	47±18	53±22‡	56±19‡	38±16‡
$INS\text{-}AUC_{0\text{-}4h}$ (h*µU/mL)	100±29	99±30	119±57‡	NA	NA
INS-MAX (h*µU/mL)	59±18	47±17	49±28	47±20	35±18
tINS-MAX† (min)	30	58	45	30	105

†Median times

‡Adjusted for background baseline

Supported by: BD Technologies

PS 83 Starting insulin in type 2 diabetes

964

Clinical outcomes at 24 months following insulin initiation in Europe: data from the INSTIGATE study

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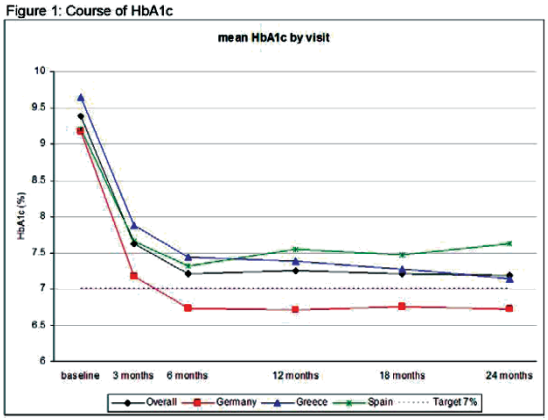
Background and aims: INSTIGATE is a prospective observational study in Europe, investigating patients with type 2 diabetes who initiate insulin during usual care. Follow up data to 24 months (m) were collected in Germany (De), Greece (Gr) and Spain (Es). An objective of the study is to describe metabolic control and clinical outcomes following insulin intitiation. This abstract describes diabetes therapy and observed clinical outcomes over the 24m following insulin initiation.
Materials and methods: Data on medication, clinical outcomes, health related quality of life and resource use were collected when patients initiated insulin and at regular intervals for 24m.
Results: Of 726 patients entering the study (De 256; Gr 263; Es 207), 564 (De 155; Gr 237; Es 172) had at least a 12m visit and 498 (De 119; Gr 227; Es 152) were followed to 24m.
In De patients were prescribed more intensive insulin regimens, at baseline 76% of patients (118/155) were on prandial based regimens, mean 3.1 injections per day. In contrast in Gr and Es at baseline either basal only or premix only were more common; 80% (190/237) in Gr and 88% (152/172) in Es, mean daily injections of 1.8 and 1.4, respectively. There were few changes in insulin regimens over the first 24m; in Gr 25 patients (11%) and in Es 12 (8%) changed their insulin regimen, there were more changes in De where 34 patients (29%) changed regimen. At baseline mean total daily dose (IU/Kg) was highest in Gr 0.41 (De 0.28, Es 0.27), by 24m mean dose in De was highest at 0.59 (Gr 0.54, Es 0.34). Mean HbA1c fell in all countries in the 6m following insulin initiation, and remained relatively stable over the subsequent 18m follow up. Only in De did mean HbA1c fall below 7% (Figure 1). Increase in weight was variable; mean weight change (Kg) in the 24m following insulin initiation was +0.23 in Es, +2.96 in Gr and +3.81 in De. Data was also collected on hypoglycaemia. The % of patients reporting at least one episode of hypoglycaemia in the first 6m of insulin therapy were Es 20%, De 23% and Gr 29%; but rates fell over time. Between 18 and 24m 8% in Es, 10% in De and 23% in Gr reported at least one episode. The number of episodes requiring 3rd party assistance or hospitalisation was low, particularly in De where in the 24m following insulin initiation there were no episodes requiring hospitalisation (Es 3, Gr 2) and only 4 episodes requiring 3rd party assistance (Es 42, Gr 65).
Conclusion: There was a consistent lowering of HbA1c in the 6m following initiation thereafter HbA1c remained stable. There was relatively little change in insulin regimens over time. It is evident that across different countries approaches to diabetes management with insulin therapy vary; due to local guidelines and health care systems, which impact patient outcomes.

Figure 1: Course of HbA1c



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965

Prandial-basal insulin regimens to improve mealtime glycaemia in type 2 diabetes: comparison of two approaches in starting and intensifying insulin

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Background and aims: Many type 2 diabetes (T2D) patients initiate insulin with basal insulin, then need prandial insulin coverage to achieve blood glucose (BG) targets. A premix insulin with 50% basal/50% prandial insulin (insulin lispro mix 50, 50% insulin lispro/50% insulin lispro protamine suspension [ILPS]; LM50) may be a practical alternative. This 36-wk international study tested noninferiority between the addition of once-daily basal insulin with up to 3 injections of premeal prandial insulin, (glargine + lispro; G+L) if needed, vs addition of once-daily premix insulin (LM50) with progressive escalation to 2-3 injections if needed, in patients with T2D and inadequate glycemic control on 2 or more oral antihyperglycemic drugs (OAD).
Materials and methods: Adult T2D pts with HbA_{1c} ≥7.5 and ≤12.0% on at least 2 OAD (metformin, thiazolidinedione, or sulfonylurea) were randomized to begin 10 U of dinnertime LM50 or 10 U of morning insulin glargine, each continuing on OAD. Protocol-driven dose titrations and addition of insulin injections (LM50 or lispro) targeted pre-meal BG 4.44-5.56 mmol/L and HbA_{1c} <7% at 12- and 24-wks. Insulin injections were not added after wk 24. The primary outcome was change in HbA_{1c} from baseline to endpoint (36 wks or LOCF). Noninferiority was established if the upper bound of the two-sided 95% CI (confidence interval) for treatment difference was <0.3%. Other measures included 7-pt BG profile, hypoglycemia, insulin dose and weight.
Results: Both groups had similar baseline age (59 vs 60 yrs), duration of diabetes (11 vs 12 yrs), weight, OAD use and HbA_{1c} (results in table).
While endpoint HbA_{1c} and proportion of pts with HbA_{1c} <7% had no statistically significant difference between groups, noninferiority was not met (primary objective CI upper limit 0.3%). Majority of pts (286/469~61%) had >1 injection at study end (median=2 in both). Eleven pts replaced dinnertime LM50 with LM25 (25% insulin lispro/75% ILPS). Endpoint 7-pt BG profiles remarkably improved in both groups from baseline, and profiles were similar at all time points except fasting and evening 2-h pp. Endpoint hypoglycemia rate was significantly higher for G+L at endpoint, but not over the entire study (p=0.22). A statistically significant difference was noted in daily insulin dose at endpoint. There was no significant difference in weight over the study (p=0.80).
Conclusion: Starting once-daily insulin glargine then adding mealtime insulin lispro, compared to starting with once-daily LM50 then advancing to 2-3

Total Subjects* N=383	**G+L N=195**	**LM50 N=188**	**p-value**	**Point estimate (95% CI)**
HbA_{1c}, % (SD)				
Baseline	9.17 (1.17)	9.31 (1.18)	p=0.11	
Change	-1.93 (1.38)	-1.76 (1.42)	p=0.10	
Endpoint	7.49 (1.02)	7.66 (1.09)	p=0.10	
Difference (change from baseline to endpoint)				0.17 (-0.03 - 0.37)†
Pts with HbA_{1c} <7%	39%	35%	p=0.48	

*Per-protocol population. Data is expressed as Mean (SD) unless otherwise stated.
†Supportive analysis using ITT population (95% CI: (-0.01-0.36) consistent with per-protocol analysis.

Total Subjects‡ N=479	**G+L N=240**	**LM50 N=239**	**p-value**
SMBG, mmol/L (SD)			
Endpoint fasting	6.53 (1.91)	6.98 (2.01)	p=0.01
Endpoint evening 2-h postprandial (pp)	9.84 (2.40)	9.27 (2.42)	p=0.01
Hypoglycemia rate			
Endpoint episodes/pt/30 days	2.19 (3.60)	1.57 (2.98)	p=0.02
Daily insulin dose (U/kg)			
Endpoint	0.51 (0.28)	0.57 (0.36)	p=0.017

‡ITT population. ITT, Intent-to-treat. ANCOVA model was used for all continuous variables, nonparametric analysis for hypoglycemia rate and logistic regression for % pts with HbA_{1c} <7.0%.

daily injections, had no statistically significant difference in endpoint HbA_{1c}, however, noninferiority of LM50 to G+L was not met. Lower fasting BG and higher endpoint hypoglycemia rate with G+L and lower dinnertime ppBG with LM50 were seen. Both groups demonstrated clinically meaningful ability to improve glycemic control supporting 2 alternative basal-bolus insulin approaches for patients with T2D.
Supported by: Eli Lilly and Company

966

Once-daily insulin glargine requires a significantly lower dose than insulin detemir twice daily to achieve good glycaemic control in patients with type 2 diabetes failing oral therapy

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Background and aims: Basal insulin is recommended for the initiation of insulin therapy in type 2 diabetes (T2D). However, studies comparing the two basal insulin analogues insulin glargine (GLAR) and insulin detemir (DET) are limited. This 24-wk multinational, open-label, parallel-group, randomized trial compared the efficacy and safety of GLAR once-daily (od) vs DET twice-daily (bid) in patients (pts) with T2D inadequately controlled on oral glucose-lowering drugs (OGLDs). The primary objective was to demonstrate the non-inferiority of GLAR vs DET with respect to the percentage of pts reaching HbA_{1c} < 7% without symptomatic hypoglycaemia confirmed by plasma glucose (PG) ≤ 56 mg/dL (3.1 mmol/L).
Materials and methods: 964 insulin-naïve pts, aged 40-75 yrs, T2D duration ≥ 1 yr, BMI < 40 kg/m^2, with inadequately controlled T2D (HbA_{1c} 7.0-10.5%) on OGLDs (including metformin [MET] ≥ 1 g/day) were randomized to either GLAR (n=478; at dinner or bedtime) or DET (n=486; at breakfast and before dinner), added to stable doses of MET. For both insulins the starting daily dose was 0.2 U/kg, which was then titrated every 2 days by 2 U to obtain fasting PG (FPG) < 100 mg/dL (5.6 mmol/L). For DET, there also was a predinner titration target < 100 mg/dL.
Results: Baseline mean (± SD) age was 58.4 ± 8.3 yrs, T2D duration 9.9 ± 5.8 yrs, FPG 173.8 ± 42.0 mg/dL and HbA_{1c} 8.7 ± 0.9%. GLAR od was non-inferior to DET bid; 27.5 and 25.6% of pts reached HbA_{1c} < 7% without confirmed hypoglycaemia (difference 1.85% [95% CI: -3.78; 7.48]). Change from baseline to endpoint HbA_{1c} was similar with GLAR and DET (-1.46 ± 1.09 and -1.54 ± 1.11%; p=0.149), endpoint HbA_{1c} levels were 7.2 ± 0.9 and 7.1 ± 0.9%, respectively. Endpoint FPG was lower with GLAR vs DET (108 ± 24 vs 119 ± 32 mg/dL) and decreased significantly more with GLAR vs DET (-64 ± 45 vs -58 ± 45 mg/dL; p<0.001). There was a lower rate of daytime symptomatic hypoglycaemia confirmed by PG ≤ 56 mg/dL (3.1 mmol/L) in the GLAR vs DET group (1.06 ± 3.13 vs 1.64 ± 5.42 events/patient yr; p=0.046). Frequencies of asymptomatic, overall symptomatic, nocturnal symptomatic and severe hypoglycaemia were comparable between treatment groups. Compared with the GLAR group, pts treated with DET gained significantly less weight (0.6 vs 1.4 kg, difference 0.77 kg [95% CI: 0.38; 1.16]; p<0.001) and required significantly higher daily insulin doses (76.5 vs 43.5 U, difference 33.1 U [95% CI: 27.8; 38.3]; p<0.001). Significantly more pts in the DET group terminated the study early (10.1 vs 4.6%; p=0.001), the most common reason was adverse events (4.5 vs 1.5%), in particular skin reactions (9 vs 0 pts).
Conclusion: GLAR od was non-inferior to DET bid with respect to the percentage of pts reaching the current glycaemic goal of HbA_{1c}< 7% without symptomatic confirmed hypoglycaemia. GLAR od and DET bid resulted in similar improvements in HbA_{1c}. However, pts treated with DET bid required higher daily insulin doses, had less weight gain and more drop outs vs the GLAR od group.
Supported by: sanofi-aventis

967

A quantitative assessment of patients' barriers to insulin

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Background and aims: The International Diabetes Management Practices Study (IDMPS) is a 5-year study aimed at assessing therapeutic management of diabetic patients in Africa, Asia, Latin America and the Middle East. Within the third wave of the study (2008), a patient questionnaire was administered to assess preferences and perceptions of diabetes treatments, with a focus on identifying patient barriers to insulin treatment.
Materials and methods: The questionnaire was administered using indirect and direct methods. Discrete choice modeling was used to assess how product attributes (formulation [oral / injection], dosing [number of daily doses], blood sugar maintenance, risk of hypoglycemia symptoms, and presence of side effects [dermatological, GI, and cardiac]) influence patients' preferences for diabetes treatment. These attributes were translated into hypothetical scenarios within choice sets for inclusion in the patient questionnaire. A multinomial logit model was used to analyze the discrete choice data and to find the odds ratio for each parameter. This model allowed for analysis of the probability of selecting a chosen alternative given a choice set as well as the derivation of relative attribute importance, an indication of how influential product attributes are in the respondents' choices. Sub analyses were performed to explore the impact of factors such as diabetes understanding, comfort with needles and geographic location.
Results: The IDMPS questionnaire was administered to 14,033 diabetes patients in 18 countries. The majority of respondents were female (54%), with a high proportion suffering from Type 2 diabetes (T2DM) (n=11,883; 85%). More than half (56%) of respondents reported receiving diabetes education. Additionally, 48% of patients self-monitor their blood glucose levels. Across subgroups, formulation was a primary driver of patient preference while risk of hypoglycemia symptoms did not heavily influence treatment decisions. Patient preferences vary significantly by diabetes type, with T2DM patients assigning much higher relative importance to formulation than Type 1 patients (30.86% vs.4.99%, respectively; p<0.0001). Experience with insulin treatment also has a significant impact on the importance placed on formulation. Insulin treated T2DM patients placed less importance on formulation than insulin naïve T2DM patients (3.09% vs. 47.48%, respectively; p <0.0001). Furthermore, patients treated with insulin placed greater importance on side effects compared to insulin naïve patients (31.59% vs. 13.75%, respectively; p=0.0298). Diabetes education also appears to have a significant effect on the priority given to formulation between T2DM patients who received diabetes training and those who did not (33.68% vs. 28.21%, respectively; p<0.0001).
Conclusion: The insulin barriers perceived by patients with diabetes evolve with their experience of the disease. Formulation is the primary driver of preference for insulin naïve patients. However, patients become increasingly concerned with more clinically relevant barriers (eg side effects, blood glucose levels) as they gain experience with insulin. This finding indicates that patients using insulin understand the importance of achieving an optimal balance between safety and efficacy. Education is important when selecting a treatment option, as the optimal regimen will change over the course of disease duration.
Supported by: sanofi-aventis

968

Pharmacodynamic and pharmacokinetic effects of subcutaneous insulin glulisine versus insulin aspart prior to a standard meal in obese subjects with type 2 diabetes

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Background and aims: This multinational, randomized, double-blind, two-way crossover trial (7-day washout) compared the pharmacodynamic (PD)/pharmacokinetic (PK) profiles of insulin glulisine (GLU) versus insulin aspart (ASP) followed by a standard meal in insulin-naïve, obese subjects with Type 2 diabetes (T2D). The primary endpoint was glucose excursion (AUC_{0-1h}) during the first hour after the beginning of the test meal.
Materials and methods: Overnight-fasted subjects received a 0.2 U/kg dose of GLU or ASP subcutaneously within 2 min before starting the meal. Blood

samples were taken every 15 min, starting 20 min before the meal and ending 6 hr post-meal. After a 1-week washout period, the same procedure was repeated using the alternative insulin preparation.
Results: Of the 37 subjects who were randomized (13/24 females/males; mean ± SD age, 60.3 ± 8.3 yr; BMI, 33.7 ± 3.3 kg/m^2; T2D duration, 7.3 ± 4.9 yr; HbA_{1c}, 7.1 ± 0.8%), 30 were eligible for PD/PK analyses. $AUC_{0\text{-}1\,h}$ and maximal glucose concentration ($glucose_{max}$) were significantly lower with GLU than with ASP (Table). For the total study period, however, plasma glucose concentration was similar between GLU and ASP (Table). Insulin levels throughout the study (geometric [arithmetic] means: 2002 [2007] vs 1289 [1333] pmol.hr/L; p<0.0001), and peak insulin concentration (534 [570] vs 363 [385] pmol/L; p<0.0001) were significantly higher with GLU than with ASP. Among the 37 patients randomized, hypoglycaemia events (<3.9 mmol/L with or without symptoms) were reported in 13 subjects on GLU and 16 subjects on ASP; there were no cases of severe hypoglycaemia.
Conclusion: In this study, GLU was associated with lower glucose levels during the first hour post-meal, owing to a faster subcutaneous absorption rate than for ASP in obese subjects with T2D.

Table

PD parameters	GLU	ASP	p value	Estimate for mean ratios (90% CI)[GLU/ASP]*
$AUC_{0\text{-}1\,h}$, mg.hr/dL†	149	158	0.0455	94% [90%, 99%]
$AUC_{0\text{-}6\,h}$, mg.hr/dL†	738	750	NS	98% [95%, 104%]
$Glucose_{max}$, mg/dL†	170	181	0.0337	94% [90%, 99%]
$T_{Glucosemax}$, min‡	60	60	0.3328	-5 [-20, 5]§
Time to fraction of total glucose AUC (10%), min‡	40	40	NS	-2 [-6, 2]§

*Based on untransformed data; †mean, ‡median; §difference between treatment medians. Superiority concluded if p value <5%. If non-significant, bioequivalence concluded if 90% CI for mean ratios within [80%; 125%]

Supported by: sanofi-aventis

969

Diabetes treatment satisfaction in a 20-week, randomized, controlled trial (TITRATE STUDY)

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Background and aims: To assess treatment satisfaction using the Diabetes Treatment Satisfaction Questionnaire (DTSQ) in a treat-to-target trial with insulin detemir in combination with OAD therapy using two FPG titration targets
Materials and methods: The TITRATE® study was a 20-week, randomized, controlled trial in subjects with type 2 diabetes who failed to achieve glycemic targets with 1-3 OADs. After a 2-week screening period, subjects were randomized 1:1 to two FPG titration targets (either 3.9-5.0 mmol/l or 4.4-6.1 mmol/l) for basal insulin initiation and dose titration. Results presented elsewhere showed both targets were safe and effective for lowering HbA1c, with the more aggressive FPG target providing superior glycaemic control. Here we assessed treatment satisfaction using the validated DTSQ. Subjects completed this 8-question assessment at randomization (Week 0) and at end of study (Week 20).
Results: Subjects in both groups trended toward improved satisfaction with their current diabetes treatment at Week 20 compared to Week 0. Within each treatment group, the greatest improvements were observed for item #s 1 (How satisfied with current treatment?), 2 (How often were blood sugars unacceptably high recently?) and 8 (How satisfied to continue present treatment?). Improvements were also noted for item #s 4 (How convenient is current treatment?), 5 (How flexible is current treatment?), 6 (Satisfaction with understanding of diabetes) and 7 (How likely to recommend current treatment?). There were no significant differences in improvements between treatment groups. Subjects reported less satisfaction on Item #3 (How often were blood sugars unacceptably low recently?), where they indicated an increase at Week 20 as compared to Week 0. This was the case to a greater extent for those subjects in the FPG 3.9-5.0 mmol/l treatment group (p=0.025 for FPG 3.9-5.0 mmol/l group vs. FPG 4.4-6.1 mmol/l group), even though the overall rates of hypoglycemia between the two groups were low and not statistically significantly different.
Conclusion: Compared to baseline, overall subject satisfaction was improved at end of study for 7 of the 8 DTSQ questions despite subjects beginning insulin therapy, and being asked to aggressively self-titrate their insulin doses. The exception (incidence of low blood sugar) is not surprising given the extent of improved glycemic achieved. Indeed, from a baseline HbA1c of > 7.9 %, > 50% of patients achieved the ADA-recommended HbA1c target of < 7% with mean HbA1c of 6.9% at the end of the study. These results, in formerly insulin-naïve subjects, highlight the importance of patient empowerment and education to enhance achievement of glycemic goals and patient satisfaction.
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970

Comparing insulin glargine (GLAR) with insulin detemir (DET) - effects on HbA_{1c} on weight change and insulin dose in patients with type 2 diabetes

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Background and aims: Basal insulin therapy offers additional improvements in HbA_{1c} for patients with type 2 diabetes (T2D) who have inadequate glycaemic control on oral therapy. Selective comparisons of clinical trials indicate that DET is associated with less weight gain vs GLAR.
Materials and methods: We did a meta-analysis of GLAR and DET randomized controlled trials. Studies with a duration of ≥4 wks in insulin-naïve patients starting basal GLAR/DET were included, regardless of comparators. We used a weighted average method to combine the study results and a random effect model to take into account effects in the studies that may differ. Outcomes were weight, HbA_{1c} change and the weight/HbA_{1c} ratio, in addition to insulin dose and insulin dose/HbA_{1c} ratio. GLAR was taken qd in 20 studies included in this analysis; DET (four studies) was taken qd or bid. 50% of patients were taking DET bid or required therapy intensification. For studies where SDs were not reported, maximum SDs from other studies were used. Estimates were determined using a PROC NLMIXED (SAS v9.2) bivariate model. Two models were developed: one without covariates and one with baseline covariates (baseline values of HbA_{1c}, BMI and insulin dose).
Results: In the unadjusted model (Table), HbA_{1c} change was similar for GLAR and DET (-1.4 vs -1.4%), as was weight gain (2.3 vs 1.7 kg) and weight/HbA_{1c} (1.7 vs 1.2 kg/%) (p=NS for all variables). However, patients required a significantly higher DET vs GLAR dose in order to achieve the same HbA_{1c} (Table).
Conclusion: This analysis reveals that the weight/HbA_{1c} ratio for GLAR is similar to that of DET. DET-treated patients in this analysis required a higher insulin dose than GLAR-treated patients to achieve the same HbA_{1c}. Caution should be taken when interpreting these results, as direct comparison is limited owing to the small number of DET studies.

Table

	GLAR (n=4295)		DET (n=1086)		p value
	Estimate (SE)	95% CI	Estimate (SE)	95% CI	
HbA_{1c} and weight (unadjusted)					
HbA_{1c}, %	-1.4 (0.1)	-1.6, -1.2	-1.4 (0.2)	-1.9, -1.0	NS
Weight, kg	2.3 (0.2)	1.9, 2.8	1.7 (0.4)	0.5, 2.8	NS
Weight/HbA_{1c}, kg/%	1.7 (0.2)	1.4, 2.1	1.2 (0.3)	0.2, 2.1	NS
HbA_{1c} and final insulin dose (unadjusted)					
HbA_{1c}, %	-1.4 (0.1)	-1.6, -1.2	-1.4 (0.1)	-1.9, -1.0	NS
Insulin dose	36.6 (2.8)	30.8, 42.4	51.5 (6.1)	32.1, 71.4	0.013
Insulin dose/HbA_{1c}	26.7 (1.5)	23.6, 30.0	36.1 (4.3)	22.5, 49.6	0.018

Supported by: sanofi-aventis

971

Safety and efficacy of insulin glargine compared with NPH insulin in older adults with type 2 diabetes mellitus

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Background and aims: Hypoglycemia is a deterrent to the use of insulin in older people with type 2 diabetes mellitus (T2DM). In randomized controlled trials (RCTs), adding insulin glargine to T2DM patients poorly controlled on oral hypoglycemic drugs (OHDs) has been similarly effective as adding NPH insulin at controlling hyperglycemia and has caused less hypoglycemia. We hypothesized that glargine is similarly effective as NPH but safer when treating T2DM in older adults.

Materials and methods: We compared the safety and efficacy of adding glargine or NPH to treatment for older adults already on OHDs. The first patient-level analysis was conducted by combining 4 international, multisite RCTs with similar patient selection criteria (ie, age, body mass index [BMI], baseline HbA_{1c}) and treat-to-target study designs. We compared HbA_{1c} reduction and hypoglycemic events at the end of 24 wk in patients ≤65 y (glargine [n=831] vs NPH [n=859]) vs >65 y (glargine [n=215] vs NPH [n=236]). Hypoglycemia was defined by symptoms confirmed with self-monitored blood glucose <70 mg/dL. Combined data were analyzed using fixed- and random-effects models and controlled for age, treatment, study, and baseline HbA_{1c} and BMI.

Results: The older patients had lower BMI (P<0.01). Both insulins achieved similar reductions in HbA_{1c} levels in the younger patients, but the older patients on glargine had significantly greater HbA_{1c} reductions (Table). Daytime hypoglycemic events and insulin therapies were similar in both age groups, whereas the rate of nocturnal hypoglycemic events was less in glargine-treated patients, including those aged >65 y (P<0.01).

Conclusion: These data suggest that when compared with NPH, adding glargine to OHDs in older adults with poorly controlled T2DM was better at lowering HbA_{1c} and was safer. Glargine may be a preferred therapeutic choice for older adults with T2DM.

Baseline Characteristics: Mean (SD)						
	≤65 y		>65 y		Total	
Characteristic	Glargine (n=831)	NPH (n=859)	Glargine (n=215)	NPH (n=236)	Glargine (n=1046)	NPH (n=1095)
Age, y	53 (7.6)	54 (7.2)	69 (3.2)	69 (3.4)	56 (9.6)	57 (9.2)
HbA_{1c}, %	8.9 (1.0)	8.9 (1.0)	8.9 (1.0)	8.8 (0.9)	8.9 (1.0)	8.9 (1.0)
BMI, kg/m²	29 (5.1)	29 (5.1)	28 (4.2)	28 (4.4)	29 (5.0)	29 (5.0)
Key Outcome Measures at 24 wk:						
Δ HbA_{1c} (%), Mean (SD)	−1.4 (1.2)	−1.3 (1.2)	−1.2 (1.2)*	−0.9 (1.3)	−1.3 (1.2)	−1.3 (1.2)
Daytime Hypoglycemia <70 mg/dL, Events Per Patient-Year, Mean (SE)	3.76 (0.30)	4.23 (0.32)	3.18 (0.49)	3.68 (0.56)	3.64 (0.26)	4.11 (0.28)
Nocturnal Hypoglycemia <70 mg/dL, Events Per Patient-Year, Mean (SE)	1.72 (0.17)*	3.30 (0.25)	1.99 (0.43)*	3.45 (0.52)	1.77 (0.15)*	3.34 (0.22)
Daytime Severe Hypoglycemia, Events Per Patient-Year, Mean (SE)	0.03 (0.01)	0.05 (0.02)	0.01 (0.01)	0.05 (0.02)	0.03 (0.01)	0.05 (0.02)
Nocturnal Severe Hypoglycemia, Events Per Patient-Year, Mean (SE)	0.03 (0.01)	0.08 (0.02)	0.08 (0.06)	0.14 (0.08)	0.04 (0.01)	0.09 (0.02)

*P<0.01 glargine vs NPH.
Severe hypoglycemia: hypoglycemic event requiring the assistance of another person, and plasma glucose level <50 mg/dL for 3 studies and <56 mg/dL for the fourth study or with prompt recovery after oral carbohydrate, intravenous glucose, or glucagon administration.

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972

Long-acting insulin analogue therapy initiation and follow-up by healthcare practitioners in patients with type 2 diabetes mellitus uncontrolled with oral antidiabetic drugs: the LIGHT Study

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Background and aims: Diabetes mellitus has emerged as an important medical condition worldwide. As the disease progresses and oral antidiabetic drug (OAD) treatment fails, patients generally require insulin. Several clinical studies have been conducted in patients with type 2 diabetes (T2DM) investigating long-acting insulin analogues (IA). However, few large observational studies have been published to describe IA initiation in real-life conditions.

Materials and methods: The LIGHT observational study conducted in France in 2007/2008 involved 491 French general practitioners and 270 diabetologists. It described IA initiation (insulin detemir and glargine) in combination with OADs (T0) and at 3-month follow-up (T3) in patients aged ≥40 years old and with poor glycaemic control on OADs alone. Each investigator included the first two patients who conformed to inclusion criteria.

Results: 2541 patients with T2DM (mean age: 64 years; male: 56%; BMI: 29.8 ± 5.2 kg/m²) were included. At T0, 12.6% were treated by monotherapy (44% metformin [Met], 36% sulphonylurea [SU], 15% glinides, 3% glitazones [TZD] and 2% α-glucosidase inhibitors [αGI]), 59.7% by bitherapy (67% Met + SU, 8% Met + TZD and 11% Met + glinides) and 27.7% by tritherapy (45% Met + SU + TZD, 31 % Met + SU + αGI). OAD use was ongoing for 9.0 ± 5.5 years; 48% of patients were diagnosed with diabetes more than 10 years ago and 17% less than 5 years ago. HbA_{1c} at baseline was different in patients with mono (8.9%), bi (8.8%) or tritherapy (9.0%)***, but was not dependent on the OAD or OAD combination prescribed. For 95% of patients at least one OAD was continued at IA initiation; in 96% the IA was prescribed alone; in 4% along with another insulin. The insulin analogue dose and the glycaemic parameters are described in Table 1. At T3, 92.3% of the 1863 patients included were taking IA alone, 4.4% in combination with another insulin. IA was prescribed once a day in 95.5% of patients. A 0.5 kg weight decrease was obtained in the insulin detemir group (p<0.0001 vs. baseline), which was statistically higher in patients with highest BMI at baseline (p<0.0001, up to 1.5 kg weight loss when BMI ≥35 kg/m²).

Conclusion: This study shows that 3-month treatment with IA in insulin-naïve patients with T2DM on OADs resulted in a significant improvement of glycaemic parameters (HbA_{1c} and fasting plasma glucose). This improvement and the IA doses were of the same magnitude for insulin detemir and insulin glargine, and were both associated with a slight increase in the incidence of hypoglycaemia. In addition, insulin detemir showed a 0.5 kg weight decrease.

***p<0.05

Table 1

n: patients still receiving analog at T3		All patients n=1802	Detemir insulin n=1423	Glargine insulin n=368
HbA_{1c} (%)	T0	8.82 ± 1.21	8.79 ± 1.19	8.86 ± 1.25
HbA_{1c} (%)	ΔT0/T3	-1.296(*)	-1.278(*)	-1.322(*)
HbA_{1c} <7% (*n*(%))	T3	488 (26%)	394 (28%)	92 (25%)
Fasting plasma glucose (g/L)	ΔT0/T3	-0.55(*)	-0.55(*)	-0.51(*)
Insulin dosage (UI/kg/day)	T3	0.30 ± 0.17	0.30 ± 0.17	0.31 ± 0.17
Hypoglycaemia (*n*(%))	T0 - At least one episode 6 months before T0	247 (14%)	208 (14.8%)	33 (9%)
Hypoglycaemia (*n*(%))	T3 - At least one episode 4 weeks before T3	273 (14.7) (NS)	207 (14.7%)(NS)	56 (16%)(**)
Incidence of hypoglycaemia (event/pt/month)	T0	0.1 ± 0.9	0.1 ± 0.5	0.1 ± 1.6
Incidence of hypoglycaemia (event/pt/month)	T3	0.4 ± 1.2 (*)	0.3 ± 1.1 (***)	0.4 ± 1.4 (*)

*p<0.0001, **p<0.005, ***p<0.05. All the statistical comparisons were performed from baseline.

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973

Improved symptom distress and wellbeing following initiation of insulin glargine in suboptimally controlled type 2 diabetes patients on oral agents. An observational study

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Background: Insulin initiation is often delayed in type 2 diabetes (T2DM) patients, resulting in suboptimal glycaemic control. Switching to insulin is likely to reduce hyperglycaemia, but little is known about the concomitant impact on Quality of Life. We prospectively studied the effects of starting once-daily insulin Glargine on patient-reported diabetes symptom distress and emotional well-being in a cohort of suboptimally controlled insulin-naïve T2DM patients.
Materials and methods: Data were collected in an observational study with a pre-post design, in 363 Dutch primary care practices at baseline, 3 and 6 month follow-up. Patients were included if they were switched from oral medication to once-daily insulin Glargine or insulin Glargine was added to their previous treatment (n=1063), due to poor metabolic control. Treatment was in accordance with the Dutch College of Family Physicians standard. Diabetes symptom distress was assessed using the Diabetes Symptom Checklist revised (DSC-r) and emotional well-being with the WHO-5 Well-being Index (WHO-5). Patients who provided complete data on the DSC-r and WHO-5 for at least two measurement points, were included in the present analysis (N=652). At baseline mean±SD age was 63±11 years, time since diagnosis 6.9±5.7 years, 48.6% was female and 54.2% had a lower education. Mean±SD weight was 87±18 kg, 25% had ≥1 complication and 25.3% had ≥1 co-morbidity.
Results: HbA1c decreased from 8.4±1.7 at baseline to 7.6±1.0 at 3 and 7.3±1.0 at 6-month follow up ($p<0.001$). Diabetes symptom distress (DSC-r) total score significantly decreased from 0.56±0.55 (median: 0.38, 75th percentile: 0.85) to 0.41±0.46 at 3 month and 0.36±0.45 ($p<0.001$) at 6 months follow-up. Similarly, emotional well-being improved as indicated by an increase in the WHO-5 score from 58.3±25.0 at baseline to 65.5±21.3 and 68.0±21.2 ($p<0.001$) respectively. The WHO-5 and DSC-r score were moderately correlated (Spearman's rho = -0.633, $p<0.001$). Patients with initial satisfactory wellbeing (WHO-5≥50) reported relatively low symptom distress at baseline (DSC-r 0.36±0.40, median 0.24, 75th percentile 0.47), yet their DSC-r score improved significantly during the follow-up period (-0.12±0.32, median -0.06, 75th percentile 0.03, $p<0.001$). While negative affect is known to be associated with higher symptom reporting, patients with WHO-5 scores indicative of depression (<28) showed the greatest improvement on the DSC-r, with a decrease of 0.41±0.55 (median -0.38, 75th percentile -0.01) points over the 6-month period ($p<0.001$). Overall, the greatest symptom distress reductions were observed on the subdomains 'Hyperglycaemia' and 'Fatigue' of the DSC-r, which decreased from 0.79±0.92 to 0.41±0.66 ($p<0.001$) and from 1.12±1.11 to 0.66±0.84 ($p<0.001$) respectively.
Conclusion: Improving glycaemic outcomes by means of once-daily insulin Glargine in suboptimally controlled T2DM patients results in a sustained reduction of diabetes symptom distress and subsequent improvements in emotional well-being, particularly in those previously reporting depressive affect.
Supported by: sanofi-aventis

PS 84 Type 1 diabetes - clinical aspects

974

Network approach to type 1 diabetes: association patterns between diabetic complications and metabolic, clinical and life style risk factors in a set of 4197 patients

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Background and aims: Diabetic complications are influenced by numerous genetic and environmental factors. Accurate knowledge of the complex interdependencies between these factors is critical for pinpointing the best targets for research and treatment. Therefore, the aim of this study was to describe the association patterns between clinical and biochemical features of type 1 diabetes (T1DM) complications.
Materials and methods: Baseline clinical data on 4,197 patients with T1DM were collected at the 92 FinnDiane Study Centers in Finland. The study visit included a clinical examination, registering of complications, and questionnaires on education, smoking, alcohol consumption, working status (disabled vs. employed/unemployed), asthma, rheumatoid arthritis and thyroid disease. Blood and 24h urine samples were also collected. The mean age and T1DM duration were 38 and 22 years, respectively and 52% were male. A total of 875 patients (21%) had nephropathy (AER > 300 mg/24h or end-stage renal disease) at the time of study. Vitality status was obtained prospectively from the national population registry after an average of 6.5 years of follow up (25,714 patient years). The dataset comprised 28 clinical and 25 biochemical variables that were regarded as the nodes of a network; the associations between the variables were quantified by pair-wise correlation coefficients (continuous data only) or by linear regression modeling (continuous and binary data combined). The resulting structures were visualized by a force-directed node-positioning algorithm.
Results: Heavily connected cliques of dependent variables such as HDL-related lipid markers, indicators of kidney failure and indices of body mass were observed. The links between the cliques showed biologically relevant interactions: an inverse relationship between HDL cholesterol and the triglyceride clique ($P < 10\text{-}16$), a connection between triglycerides and body mass via C-reactive protein ($P < 10\text{-}16$) and estimated IDL cholesterol as the connector between lipoprotein metabolism and macroalbuminuria ($P < 10\text{-}16$). The regression-correlation network showed strong associations between blood pressure, working ability and complications.
Conclusion: There was modularity in the dataset and several biologically relevant connections were visible, which supported the use of networks in clinical data analysis. In particular, accelerated vascular aging was seen in blood pressure correlations and IDL emerged as a putative lipoprotein covariate in the pathogenesis of diabetic nephropathy.
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975

TSH receptor autoantibodies in patients with type 1 diabetes mellitus

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Background and aims: Autoimmune thyroid disease (AITD) is the most prevalent autoimmune disorder associated with type 1 diabetes (T1D). It is known that AITD may be presented as hypothyroidism, subclinical dysfunction of the thyroid gland, euthyroid state with positive antithyroid auto antibodies or rarely hyperthyroidism i.e. Graves' disease. The course of autoimmunological diseases, including AITD, is mostly clinically silent therefore autoimmunological markers of AITD should be seeked to discover patients at risk, predict and monitor AITD. The third thyroid autoantigen - besides TPO and TG - is the TSH receptor. The purpose of the study was to determine TSH receptor autoatibodies (TSHR Ab) levels in the group of type 1 diabetic pediatric patients with or without thyroid dysfunction.

Materials and methods: In the group of 84 children and adolescents with T1D, age 2 - 18 yr (mean age: 11,3 ± 4,47), girls (58,3%), with T1D duration from 1 up to 11 yr. (mean T1D duration: 2,89 ± 2,35) anti TSH receptor autoantibodies (TSHR Ab), thyroid peroxidase antibodies (TPO Ab) and thyroblobulin antibodies (TG Ab) levels were measured in serum. TSHR Ab > 14 U/l, TPO Ab > 50 U/mL, TG Ab > 70 U/mL were considered positive. Radioreceptor assay (RRA) for quantitative determination of TSHR Ab was performed. TPO Ab and TG Ab levels were measured by ELISA. In all children clinical, hormonal examination and thyroid ultrasound, if necessary, were performed to establish thyroid function.
Results: None of 84 children had TSHR Ab. (+) TPO Ab, (+) TG Ab or both (+) TPO Ab and TG Ab determination were present in 24 of 84 patients (28,6%). Girls (77,8%) had more frequently raised antibodies than boys. (+) TPO Ab were present in 13 children (15,5%) - in 2 of them subclinical hypothyroidism was recognised and 1 patient was treated with L-thyroxine due to hypothyroidism. (+) TG Ab were present in 11 children (13,1%) - in 2 of 11 subclinical hypothyroidism was recognised, only 6 patients (7,2%) were positive for both TPO Ab and TG Ab - in 2 of 6 children subclinical hypothyroidism was determined. Subclinical hypothyroidism is recognised in 4 children with (-) TPO Ab and TG Ab levels. The hyperthyroidism was determined in 1 patient with (+) TPO Ab.
Conclusion: 1) TSH R Ab-based screening is not probably useful at the beginning of autoimmune thyroid disease in type 1 diabetic patients 2) further studies should be conducted to study the benefits of TSHR Ab determination particularly among those type 1 diabetic patients who have longer T1D duration.

976

High prevalence of sleep apnoea syndrome in a type 1 diabetic adult population

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Background: Sleep Apnoea Syndrome (SAS) is an established cardiovascular risk factor. Its occurrence is high among obese or type 2 diabetic patients. No studies have addressed the question of SAS prevalence in type 1 diabetes.
Objectives: To prospectively evaluate the prevalence of SAS among a type 1 diabetic adult population, and whether SAS could be associated with any clinical characteristics in these patients.
Patients and methods: 40 type 1 diabetic patients were consecutively included for a screening oximetric procedure seeking sleep breathing disorders. According to the result of oximetry (normal, doubtful or abnormal), an overnight polysomnography was systematically proposed to subjects with doubtful or abnormal oximetry and randomly to 1/10 patients with normal oximetry.
Results: Among the 40 subjects (age 43 ± 13 years, Body Mass Index 24.7 ± 3.0 kg/m2, disease duration 23 ± 14 years, HbA1c 7.8 ± 0.9%), forty percent of patients exhibited SAS proven by polysomnography (apnea+hypopnea index> 15/h). Age of subjects was positively correlated with variability of oxygen saturation (higher variability reflecting repetitive occurrence of apneas) (R=0.61, p<0.001). In patients presenting with SAS, those suffering from macrovascular disease and retinopathy had lower nocturnal mean oxygen saturation. Mean SaO2 90.8 ± 2.9 vs. 94.4 ± 1.3% p=0.05 and 92.1 ± 2.4 vs. 94 ± 2.7% p=0.06 for patient with vs. without macrovascular disease and retinopathy respectively.
Conclusion: We demonstrated an unexpected high prevalence of SAS among type 1 diabetic patients. Sleep apnea was associated with age and the amount of oxygen desaturation with macrovascular angiopathy and retinopathy this suggestion SAS diagnosis as a key issue in type 1 diabetes.
Thanks to Agiradom for the logistic sustainement

977

Gastric emptying as prognostic factor for life expectancy in patients with type 1 diabetes

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Background and aims: Diabetic gastropathy is with an incidence of up to 58% an underestimated complication in patients with type 1 diabetes. To our knowledge only one existing study assessed the prognostic factor of gastric emptying disorders in diabetes. The aim of the present study was to investigate the influence of gastric emptying disorder on life expectancy in patients with type 1 diabetes and a long diabetes duration.
Materials and methods: 50 patients with long-standing diabetes type 1 were examined by gastric emptying scintigraphy. Following ingestion of a standardized semisolid test meal labeled with 37 MBq Tc-99m bound to 0.5 grams Dowex 2X8, gastric emptying was continuously recorded in supine position for a duration of 90 minutes with a dual-head gamma camera. After 30 minutes of postprandial walking, recording was resumed for additional 20 minutes. Subjects were followed up with a mean duration of 94±30 months. We assessed and analyzed gastric emptying rates in relation to HbA1c, diabetes duration, diabetic neuropathy and diabetic nephropathy. Kaplan Meier analysis and student's t-test were used if applicable.
Results: At baseline 29 patients (58%) had normal gastric emptying rates, 21 (42%) presented with gastric emptying disorders (accelerated in 4 subjects, delayed gastric emptying in 17 subjects). Between these groups diabetes duration and diabetic peripheral neuropathy was not statistical significant. HbA1c was comparable (8.9 ±1.4% vs. 8.3±0.8%). At time of follow up 12 patients have deceased (50% with normal gastric emptying, 25% with accelerated and 25% with delayed gastric emptying at baseline). 38 patients were still alive, of whom 60% showed a normal gastric emptying and 40% had a gastric emptying disorder (14 patients with delayed and 1 patient with accelerated gastric emptying rates).
Conclusion: Our data imply that patients with type 1 diabetes and accelerated gastric emptying have a decreased survival in comparison with patients with delayed gastric empting, suggesting accelerated gastric empting has a negative prognostic impact on survival.

978

The influence of gastric emptying patterns on blood glucose in type 1 diabetes

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Background and aims: Diabetic gastropathy is a common complication of long-standing diabetes. Few studies describe the influence of gastric emptying disorders on blood glucose levels.
What is the influence of gastric emptying patterns on blood glucose levels in long-standing type I diabetes mellitus? We investigated the correlation between gastric emptying patterns and blood glucose in 26 patients with long-standing type I diabetes.
Materials and methods: We invited 26 patients with long-standing type I diabetes (mean diabetes duration: 32 ± 10 years, mean age: 50 ± 13years) for gastric scintigraphy to evaluate gastric emptying rates. After ingestion of a standardized semisolid test meal (mashed potatoes, 21 g carbohydrates) labelled with Tc-99m, gastric emptying was continuously recorded in supine position during 90 min with a dual-head gamma camera. After 30 min of postprandial walking, recording was resumed for 20 min. Individual gastric emptying rates of the patients with diabetes were compared with the emptying rates of 25 healthy controls. Blood samples for glucose-assessment were performed before ingestion of the testmeal and continuously every 15 minutes during the investigation.
Results: In 13 patients the emptying rates (mean ± SD %) were normal (56±18 % vs. 31±13 % in controls), accelerated in 8 pts (24±11 % vs. 50±10 %) and delayed in 5 pts (74± 7 % vs. 31±13 %). The duration of diabetes was

similar between the groups (31 ± 11 vs. 32 ± 10 vs. 30 ± 7 years). At baseline glucose values were similar between the groups but at time point 90 minutes the blood glucose was significant different. Patients with normal gastric emptying rates showed a slow and smaller increase of glucose; the blood glucose profiles of patients with accelerated gastric emptying had an initial increase with higher values but after 90 minutes a decrease of glucose. Patients with delayed gastric emptying showed a continuous increase and the peak glucose values 150 minutes after ingestion of the testmeal (table with blood glucose values).

Conclusion: The different gastric emptying patterns influence the postprandial blood glucose profiles. The changed emptying rates due to diabetic gastropathy modify meal appearance of glucose. Our results should be taken in account in the management of type 1 diabetes in order to optimise prandial insulin replacement.

Emptying patterns	Glc (mg/dl) 0 min	30 min	60 min	90	120 min	150 min
Normal	205	231	254	266	257	286
Accelerated	225	255	299	315	289	267
Delayed	206	258	275	290	326	363

979

Reproducibility of the glucose response to moderate intensity exercise in people with type 1 diabetes exercise

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Background and aims: For people with type I diabetes achieving optimal blood glucose levels whilst exercising can be challenging; erratic blood glucose levels including hypoglycaemia can be problematic and a deterrent to participation in exercise. Prior to developing intervention strategies to improve blood glucose control during exercise it is vital to assess the typical variation of blood glucose during this period. Therefore the aim of the study was to determine the reproducibility of the blood glucose response to four repeated sessions of forty minutes of moderate intensity exercise in people with type I diabetes, and thus establish the typical variation of blood glucose in this experimental design.

Materials and methods: From a target population of 113 individuals, 14 participated in this quasi experimental study. Pre experiment, a sub maximal incremental walking treadmill test was used to determine 50%VO2max for each participant. All participants were currently using a basal bolus insulin regimen and were experienced in adjusting insulin dose and carbohydrate counting. All participants were habitual exercisers. The participants undertook the experiment over 2 consecutive weeks. On days 1 and 3 of each week the subjects undertook a 40 minute bout of exercise at 50% VO2max. In week 1 participant's self-managed their diabetes. In week 2 they used an exercise self management protocol devised by the research team to adjust insulin dose and carbohydrate intake, targeted to blood glucose levels. In each week from day 1 - 4 the blood glucose levels were recorded using a continuous blood glucose monitor at 5 minute intervals. The participant's heart rate was also monitored. To assess reproducibility all blood glucose data during the 40 minute exercise period were paired and analysed to assess the difference in mean values, standard error of measurement (SEM) and co-efficient of variation (CV%).

Results: Mean demographics of the participants at baseline were: 38 years of age, BMI 25 kg/m² and HbA1c 7.5%. There was a significant (p= 0.0003) difference (95%CI, 0.77 to 2.6 mmol·l^{-1}) between blood glucose levels between days 1 + 2 (7.2±2.4 vs. 8.8±3.8 mmol·l^{-1}), and a significant (p=0.04) difference (95%CI, 0.03 to 1.6 mmol·l^{-1}) between days 3 + 4 (10.7±2.6 vs. 9.8±4.2 mmol·l^{-1}). In terms of reproducibility the SEM between days one and two was 2.4 mmol·l^{-1} (CV= 33.2%), and between days 3 and 4 the SEM was 2.7 mmol·l^{-1} (CV= 33.7%).

Conclusion: This study has demonstrated a relatively large co-efficient of variation and thus poor reproducibility. However the magnitude of the CV% is comparable with other published literature which has examined the decline in blood glucose as a result of a bout of moderate exercise. This finding has implications for intervention studies attempting to alter the blood glucose response during exercise in people with type I diabetes.

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980

A 6-month treatment with diazoxide at bedtime in newly diagnosed subjects with type 1 diabetes does not influence measured parameters of beta cell function, but improves glycaemic control

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Background and aims: Continuous beta cell rest with K-ATP channel openers, such as diazoxide, is shown to have long term beneficial effects on residual insulin secretion in type
1 diabetes. However, disturbing side effects have hampered the possibility for implementation in clinical use. In a double blind study we tested whether lower and intermittent dosing of diazoxide could produce beneficial effects in the absence of side effects.

Materials and methods: Thirty-eight subjects (11 female and 27 male aged 18- 40 years, BMI 24±4 kg/m2), with newly diagnosed type 1 diabetes were randomized to 6 months of treatment with either placebo or diazoxide, 100 mg at bedtime. Glucose, HbA1C and C-peptide (fasting and glucagon-stimulated) were measured at baseline, during intervention and 3 and 6 months after intervention.

Results: Two subjects in the diazoxide, and one in the placebo group were excluded due to perceived side effects (rash, dizziness, sleep disturbance respectively). Other adverse effects were absent. During intervention there was no negative influence by diazoxide on insulin dosage. The insulin dose in the diazoxide treated group was at base-line 33 ± 20 U/24h and the increment during intervention 1 ±11 U. In the placebo group the insulin dose at baseline was 34 ±.30 U, with a decrement during intervention at 2 ± 37 U (difference between groups non-significant). Diazoxide treatment led to improved glycemic control following the intervention period. Thus, there was a significant reduction in HbA1c with a decrement of -2.16 after 3, and -2.19 % 6 months after the end of intervention. In contrast, in the placebo group, there was no clear improvement of glycemic control (HbA1c decrement of -0.40 and -0.71 % respectively). The differences between groups were significant, p = 0.046 at 3 months, p= 0.039 at 6 months after intervention. Fasting and stimulated C-peptide decreased markedly during and after the study in both arms of the study. There was no indication of improved beta cell function after treatment with diazoxide 3 and 6 months after intervention. At these time-points the ratio between fasting C-peptide and fasting glucose was reduced by 24 and 40 % respectively from baseline after treatment with diazoxide, compared to 17 and 31 % after placebo. Following glucagon stimulation, the decrement of C-peptide/glucose ratio was 16 and 33 % in the diazoxide group, and 10 and 27% in the placebo treated group. Differences between the groups were not significant.

Conclusion: Intermittent beta cell rest with diazoxide in newly diagnosed type 1 diabetes is well tolerated but does not improve measured parameters of beta cell function. Improvement of other aspects of the beta cell function could possibly explain the diazoxide-associated improvement of metabolic control.

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PS 85 Insulin therapy - type 1 diabetes

981

Effectiveness of a day without carbohydrates

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Background and aims: Nowadays, multiple insulin doses (MID) is one of the most common insulin treatment used in type-1 diabetes. This insulin therapy combines long-acting insulin, as basal insulin, with short-acting insulin, depending on the amount of carbohydrates included in each meal. However, doses of long-acting insulin are established in relation to patient body weight but there aren't others methods to individualize these insulin requirements. To evaluate if a day without carbohydrates (DWC) could be an effective method to evaluate long-acting insulin needs in patients with type-1 diabetes.
Materials and methods: We have included 24 individuals with type-1 DM, older than 18 years, with poor glycaemic control (HbA1c >7.5%) and treated with MID, using glargine as long-acting insulin. During a day, patients maintain long-acting insulin injection but they had to eliminate short-acting insulin and couldn't eat carbohydrates in their diet. Moreover, in this day, patients had to do glycaemic controls before and 2-hours after every meal and, depends of the result obtained, add short-acting insulin or eat glucose supplements if glycaemic values were >130mg/dl or <70mg/dl respectively. To improve the knowledge of glycaemic fluctuations during this day we had monitorized glycaemic values using a Continuous Glucose Monitorized Sensor (CGMS) of Medtronic Minimed during a 4-days period, including the DWC. We considered a long-acting insulin dose as no correct if, during this day, patient had to add short-acting insulin to correct hyperglycaemia or eat carbohydrates to treat hypoglycaemia. In this case, patient should repeat the DWC with a new long-insulin dose, calculated depending on the quantity of insulin added or carbohydrates consumed during the first DWC. We collect data about HbA1c, weight, BMI, and short and long-acting insulin doses, frequency of hypoglycaemia, physical activity and carbohydrates intake in their usual days, before and 4-months after the DWC. Results obtained were compared with a control group of type-1 DM patients that undergo the same protocol without the DWC (N=21).
Results: Four months after the DWC, HbA1c levels of the DWC group had decreased from 8.1±0.9 to 7.5±0.7 ($p<0.05$) compared to the control group (from 8.1±0.6 to 8.0±0.7, p=ns). Moreover, patients had increased usual doses of long-acting insulin from 24.7±8.0 to 27.2±10.5 units/day ($p<0.05$) compared to the control group (from 26.8±5.6 to 26.4±6.5 units/day, p=ns) and had reduced usual doses of short-acting insulin from 20.9±6.9 to 19.3±8.2 units/day (p=ns) compared to the control group (from 23.2±10.3 to 23.1±7.8 units/day, p=ns). The frequency of mild hypoglycaemia before and four-months after the DWC was similar in the DWC group (from 0.9±1.1 to 0.8±0.9 episodes-patient/week, p=ns) and in the control group (from 1.3±1.5 to 1.2±1.1 episodes-patient/week, p=ns). We didn't found episodes of severe hypoglycaemia or presence of ketonuria.
Conclusion: A day without carbohydrates method is an effective tool to evaluate long-acting insulin needs in patients with type-1 diabetes. With this method we have got an improvement in glycated hemoglobin without increase the frequency of hypoglycemic episodes.
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982

Comparative efficacy and safety of technosphere insulin and a rapid-acting analogue both given with glargine in subjects with type 1 diabetes mellitus in a 52-week study

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Background and aims: Technosphere Insulin (TI) is a rapid-acting inhaled insulin with pharmacokinetics well suited for control of postprandial plasma glucose (PPG). This study compared the efficacy and safety of TI and a rapid-acting analog (RAA) both given at meals with insulin glargine, a long-acting analog (LAA).
Materials and methods: Subjects with type 1 diabetes mellitus and HbA_{1c} >7.0% and ≤11.0% were randomized to a 52-week course of basal LAA plus prandial TI (n=301) or prandial RAA (n=288). Prespecified efficacy endpoints included change in HbA_{1c}, 1-h PPG, 2-h PPG, and fasting plasma glucose (FPG) following a standard meal challenge. Adverse events were monitored to compare safety profiles.
Results: At the end of the 52-week treatment period, both groups experienced comparable reductions in HbA_{1c}, and there were no significant differences in LAA doses between treatment groups. FPG levels and 1-h PPG values were significantly lower with TI than with RAA, and 2-h PPG levels were similar between both groups. The TI group had weight loss, whereas the RAA group gained weight, with the difference statistically significant. Finally, the TI group had a statistically significant reduction in the incidence of mild/moderate (odds ratio [OR]: 0.474; confidence interval [CI]: 0.0271, 0.831; p=0.0091) and total hypoglycemia (OR: 0.488; CI: 0.278, 0.856; p=0.0124).
Conclusion: Compared with RAA in subjects with type 1 diabetes mellitus, TI in combination with LAA resulted in comparable HbA_{1c} reductions; more favorable 1-h PPG and FPG; significantly less weight gain; and significantly less risk of hypoglycemia.

Parameter	TI+LAA	RAA+LAA	*p* Value, 95% CI, or OR
ΔHbA_{1c} from baseline (%)	-0.17 ± 1.09	-0.47 ± 0.91	CI 0.11, 0.38
ΔFPG from baseline (mmol/l)	-2.5 ± 5.8	-1.3 ± 5.7	p=0.0052
1-h PPG (mmol/l)	9.2 ± 4.3	11.2 ± 4.6	p=0.0203
ΔWeight from baseline (kg)	-0.5 ± 4.1	+1.4 ± 3.9	p<0.0001
Total hypoglycemia (%)	86.01	92.65	OR 0.488; p=0.0124
Mild/moderate hypoglycemia (%)	85.67	92.65	OR 0.474; p=0.0091
Severe hypoglycemia (%)	32.76	37.50	OR 0.812; p=0.2387
Incidence of hypoglycemia with blood glucose ≤2.7 mmol/l (%)	76.11	83.82	OR 0.615; p=0.0232

Supported by: MannKind Corporation

983

Reduced incidence and frequency of hypoglycaemia in an integrated analysis of pooled data from clinical trials of subjects with type 1 diabetes using prandial inhaled Technosphere insulin

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Background and aims: Technosphere Insulin (TI) is a rapid-acting insulin with a pharmacokinetic profile well suited for earlier control of postprandial plasma glucose (PPG). This integrated analysis includes the pooled data from three phase II/III clinical trials in subjects with type 1 diabetes mellitus inadequately controlled (HbA_{1c} >7.0% and ≤11.0%) with standard insulin regimens.
Materials and methods: Subjects were randomized to one of three treatment regimens to achieve predefined glycemic goals: TI (n=614) plus a basal insulin; s.c. insulin (n=599), which included insulin glargine plus aspart; or "usual care," with insulin adjustments according to investigator discretion. A structured titration regimen was not enforced. When experiencing hypoglycemic-like symptoms, subjects were instructed to confirm the event with a blood glucose reading. Subjects experiencing a severe hypoglycemic episode were required to report the details of third-party assistance (if needed), the presence of neurologic symptoms, and the specifics of treatment.
Results: Mean baseline characteristics were similar for TI and s.c. insulin (age 38.4, 38.5 years; disease time since diagnosis 16.5, 16.6 years; baseline HbA_{1c} 8.59%, 8.56%; BMI 26.12, 26.03 kg/m^2). Subjects treated with TI experienced fewer hypoglycemic events with regard to both incidence and frequency compared with subjects treated with other s.c. insulins. For incidence, fewer subjects reported hypoglycemia with TI: 75.9% vs. 81.0% for total hypoglycemia; 75.6% vs. 80.8% for mild/moderate hypoglycemia; and 24.3% vs. 27.5% for severe hypoglycemia, with the comparison p values substantially in favour of TI for total hypoglycemia and mild/moderate hypoglycemia. For frequency,

TI had a comparable (not statistically different) number of events, evaluated by event rate (number of events per 100 subject-months), with event rates for severe hypoglycemia lower for the TI group. When evaluated for those subjects with blood glucose values ≤2 mmol/l, TI also was comparable (not statistically different) to s.c. insulin treatment with a lower event rate.
Conclusion: TI, in combination with a basal insulin, consistently reduced the incidence of total and mild/moderate hypoglycemic events and had a lower frequency of severe hypoglycemic events under conditions of comparable glycemic control.

Hypoglycemia	Incidence (%)		Odds Ratio	Odds Ratio *p* Value	Event Rate (per 100 subject-months)		Event Rate *p* Value
	TI + basal insulin	Comparator + basal insulin			TI + basal insulin	Comparator + basal insulin	
Mild/moderate	75.6	80.8	0.743	0.0354	133.16	117.74	0.9097
Severe	24.3	27.5	0.826	0.1576	5.16	6.03	0.5901
Total	75.9	81.0	0.749	0.0413	138.60	124.06	0.9242

984

Better metabolic control, less hypoglycaemia and less weight gain with insulin detemir versus NPH insulin in intensive insulin therapy for patients with type 1 diabetes. A meta-analysis

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Background and aims: Detemir insulin characterizes more stable acting profile compared to NPH insulin due to binding with albumins and slow dissociation. In comparison with NPH insulin, basal-bolus treatment with insulin detemir might provide better glycemic control with lower risk of hypoglycaemia. The aim of this study was to compare the effect of treatment with detemir insulin vs. NPH insulin on metabolic control, hypoglycaemic episodes and body weight gain in patients with type 1 diabetes mellitus.
Materials and methods: This analysis is based on studies carried out longer than 12 weeks, comparing basal-bolus regimen therapies using detemir insulin vs NPH insulin in patients with type 1 diabetes mellitus. Two reviewers independently screened randomized controlled trials. Systematic overview included data from MEDLAINE, Cochrane Library and EMBASE until December 2008.
Results: This analysis included 7 studies. Meta-analysis of data of 3026 patients showed statistically significant reduction in HbA1c (WMD -0.10%, 95%CI -0.16 to -0.03, p=0.005) and fasting plasma glucose (WMD -1.11 mmol/L, 95%CI -1.60 to -0.52, p<0.001) in the detemir group compared with the NPH group. Meta-analysis of records obtained from 2667 subjects (5 studies) confirmed statistically significant reduction in all day hypoglycaemic episodes (RR 0.98, 95%CI 0.96 to 0.99, p=0.032) and nocturnal hypoglycaemia (RR 0.91, 95%CI 0.86 to 0.95, p<0.001) in patients using detemir insulin. Analysis of data of 3067 patients pointed out to 34% less frequent severe hypoglycemia (RR 0.66, 95%CI 0.54 to 0.81, p<0.001) and results of 5 studies (n=2306) showed smaller body mass gain (WMD -0.76 kg, 95%CI -0.99 to -0.53, p<0.001) in the detemir group.
Conclusion: In comparison with NPH insulin, basal-bolus treatment with insulin detemir resulted in a significantly better glycemic control expressed as reduction in glycated hemoglobin and fasting plasma glucose. The treatment with detemir insulin was superior to NPH insulin in reducing the risk of all day, nocturnal and severe hypoglycaemia, with added benefits of less weight gain.

985

Comparison of insulin detemir and NPH insulin in children and adolescents with type 1 diabetes mellitus aged 2-16 years: a 52-week randomised clinical trial

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Background and aims: This randomised, multinational, open-labelled, parallel-group trial compared 52 weeks of treatment with insulin detemir (IDet) and NPH insulin (NPH) in subjects aged 2-16 years with type 1 diabetes (T1DM). This trial was the longest and largest insulin trial ever performed in this age group and 82 (23.6%) of the individuals included were less than 6 years.
Materials and methods: The study investigated the efficacy and safety of IDet vs. NPH used in a basal-bolus multiple daily insulin injection regimen. Of the 347 randomised and exposed subjects (baseline characteristics: mean (SD) age: 9.9 (3.99) yrs, males: 51.9%, diabetes duration: 3.69 (2.58) yrs, HbA_{1c}: 8.4 (1.1)%, fasting plasma glucose (FPG): 8.52 (4.48) mmol/L), 177 received IDet and 170 received NPH, both administered once or twice daily in combination with mealtime insulin aspart (IAsp).
Results: After 52 weeks, non-inferiority of IDet vs. NPH was established for HbA_{1c} (IDet 8.75%, NPH 8.64%, mean difference 0.12% points, 95% CI (-0.12; 0.36%)). Change in mean FPG was -0.60 mmol/L with IDet vs. 0.02 mmol/L with NPH, (NS) and the within-subject variation in self-measured FPG was lower with IDet than with NPH (SD: 3.01 vs. 3.68 mmol/L, p <0.001). The estimated mean rate of 24-hour hypoglycaemia and nocturnal hypoglycaemia (between 22:00 and 07:00) was lower with IDet than with NPH, (respectively 55.4 vs. 72.5 events per exposure year, (rate ratio: 0.76, p <0.05) and 8.7 vs. 13.9 events per exposure year (rate ratio: 0.62, p <0.005)). The reduced rate of hypoglycaemia at night was observed even though pre-breakfast plasma glucose targets between 4.0 and 7.0 mmol/L was achieved by a slightly larger proportion of subjects on IDet compared to NPH (40% vs. 32%) at end of trial. Severe hypoglycaemic episodes were rare (3 with IDet and 12 with NPH) and none of these occurred at night with IDet. Apart from this, the adverse event profile was similar between IDet and NPH. SD scores for body weight (standardized by age and sex) decreased with IDet, but remained unchanged with NPH (change: -0.12 vs. 0.04, p <0.001). Thus, in contrast to NPH, IDet was not associated with inappropriate weight gain. At trial end, the mean daily basal insulin dose was comparable between IDet and NPH (0.60 vs. 0.58 U/kg) as was the dose of IAsp (0.48 vs. 0.45 U/kg).
Conclusion: In this unique trial, long-term treatment of children and adolescents aged 2-16 years with insulin detemir resulted in similar glycaemic control as NPH insulin, but with a significantly lower risk of overall and nocturnal hypoglycaemia and a reduction in weight SD scores.

986

A comparison of efficacy and safety of insulin aspart and human insulin in treatment of type 1 diabetes mellitus. The results of a systematic review

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Background and aims: There is an on-going debate concerning efficacy and safety of the rapid-acting insulin analogues in type 1 diabetes mellitus (T1DM). While a few systematic reviews have been published so far, most of them provide pooled results for two analogues (lyspro and aspart). Very limited data is available on each individual compound. Our aim was to perform a systematic review-based study to compare clinical outcomes of treatment with aspart (IAsp), a rapid-acting insulin analogue, and regular human insulin (RHI).
Materials and methods: The analysis was based on the results of clinical trials, randomized and their non-randomized continuations in T1DM. Relevant articles were identified by a systematic literature search in the electronic medical databases (MEDLINE, EMBASE, The Cochrane Library and others) up to February 2007. Two authors independently reviewed the articles.
Results: We identified 12 trials (3553 patients) that fulfilled the inclusion criteria. Results are presented as the weighted mean difference and 95%CI, if not

otherwise stated. No study provided data for mortality or morbidity. Pooled data for HbA_{1c} demonstrated a lower level in the IAsp than in the RHI group (-0.14; -0.20 to -0.07). The difference was greater in favour of IAsp in insulin pump therapy (-0.31; -0.55 to -0.08). In addition, meta-analysis revealed statistically significant differences in favour of IAsp for PPG after breakfast (-1.46; -1.80 to -1.11), lunch (-1.13; -1.88 to -0.38) and dinner, (-0.86; -1.16 to -0.55); but not for fasting plasma glucose (FPG) (0.12; -0.96 to 1.20). Data on treatment satisfaction and Quality of Life were presented only in two studies. We observed a benefit when comparing IAsp with RHI (0.33; 0.19 to 0.47) with respect to the part of the Diabetes Treatment Satisfaction Questionnaire scale evaluating treatment flexibility. The safety analysis showed significant reduction of nocturnal hypoglycemias in the IAsp group (relative risk 0.67; 95%CI 0.54 to 0.83).
Conclusion: In conclusion, analyses based on a systematic review showed that treatment with IAsp resulted in moderately better metabolic control, safety and treatment satisfaction in T1DM.
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987

Comparison between human insulin and insulin analogues treatment with regard to hypoglycaemia (HYPO score) and metabolic lability (Lability Index) in type 1 diabetes mellitus
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Background and aims: Intensive insulin therapy in order to achieve a strict glycemic control reduces the incidence of complications associated with type 1 diabetes mellitus but increases the risk of hypoglycemia. Therapy with insulin analogues has been associated with a lower risk of hypoglycemia, particularly nocturnal events, and reduced glycemic lability in comparison with traditional human insulin. However, methods for quantifying the global risk of hypoglycemia and metabolic lability are heterogeneous and poorly standarized. HYPO score and Lability Index (LI) are clinically validated quantitative measures associated with global risk of hypoglycemia and glycemic lability respectively. The aim was to evaluate, in type 1 diabetes mellitus patients treated with human insulin, whether treatment with insulin analogues was associated with a lower risk of hypoglycemia and glycemic lability (HYPO score and LI more favourable).
Material and methods: Our study was a 6 months prospective, randomized, open-labelled clinical trial. Consecutively attended patients in our unit were included if they had type 1a diabetes mellitus for at least 5 years, 18-60 years old, HbA_{1c} less than 10%, and were receiving treatment with three or more doses of human insulin (n=47). Patients were randomized in a 1:1 ratio to receive human insulin therapy (regular plus NPH; group 1, n= 21) or an insulin analogues regimen (aspart plus glargine; group 2, n=26). HYPO score, LI, and hypoglycemic episodes and their characteristics were assessed at baseline and at the end of follow-up. Age, gender, diabetes duration, BMI, HbA_{1c}, and insulin dose were also compared between groups at baseline and at the end of the study. A 72-hours continuous glucose monitoring was performed at the end of follow up in a subgroup of patients (n=12). Groups were compared with non-parametric tests. HYPO score and LI are expressed as median (25^{th} to 75^{th} interquartile range). Statistical significance was defined as $p<0.05$.
Results: At baseline both groups were comparable in terms of age (34.5 ± 5.6 vs 35.6 ± 7.8 years), diabetes duration (10.2 ± 4.1 vs 10.7 ± 7.3 years), BMI (26.8 ± 4.3 vs 26.4 ± 3.3 kg/m^2), HbA_{1c} (7.5 ± 0.9 vs 7.5 ± 0.7 %), and insulin dose (0.76 ± 0.16 vs 0.73 ± 0.17 IU/kg), groups 1 and 2 respectively. Initial HYPO score (223.5 (84.5-403.0) vs 149.0 (53.0-224.0)) and LI (89.8 (52.3-156.9) vs 77.4 (51.6-131.5) $mM/l^2/h.week^{-1}$) were also comparable. At the end of the study both groups continued to be comparable in terms of BMI, insulin dose, and HbA_{1c} (7.7 ± 0.9 vs 7.2 ± 1.0 %). However, HYPO score was significantly lower in group 2 at six months (260.0 (52.0-676.0) vs 71.5 (36.0-162.5), $p<0.05$) as well as nocturnal hypoglycemic events (1.4 ± 1.5 vs 0.4 ± 0.8 per 4 weeks, $p<0.05$) and episodes below 45 mg/dl (3.0 ± 2.9 vs 1.4 ± 1.3 per 4 weeks, $p<0.05$). LI was comparable at the end of the study in both groups. HYPO score was positively correlated with % time with glycemia below 70 mg/dl in continuous glucose monitoring (r=0,62, p <0,05).
Conclusion: treatment with insulin analogues in type 1 diabetes mellitus patients, in comparison with human insulin, was associated with a lower risk of hypoglycemia, particularly nocturnal and below 45 mg/dl events, despite comparable insulin dose and metabolic control. In contrast, metabolic lability was not different between groups.

PS 86 Diabetes education

988

Comparison between the "Diabetes Interactive Diary" telemedicine system and standard carbohydrate counting education: an open label, international, multicentre, randomised study
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Background and aims: Carbohydrates (CHO) counting is effective in promoting dietary freedom, quality of life, and glycaemic control, but its widespread use is limited by the complex education required. The Interactive Diary for Diabetes (DID) is an electronic diary/bolus calculator/ telemedicine system based on patient-specialist communication by mobile phone short messages. It enables patients to match insulin to CHO intake on a meal by meal basis. Aim of this study was to compare DID with standard CHO counting education in terms of metabolic and weight control, quality of life, and treatment satisfaction.
Materials and methods: Adults with T1DM were randomised to the DID educational program (Group A, N=67) or to CHO counting standard education (Group B, N=63). Patients were seen after 3 and 6 months. A subgroup of 60 patients was asked to fill in the SF-36 Health Survey and the WHO-Diabetes Treatment Satisfaction Questionnaire at each visit. Between-groups mean changes with respect to baseline values were evaluated using the Mann-Whitney U test.
Results: Of 130 patients enrolled (43% males; age 35.7±9.4 yr; T1DM duration 16.5±10.5 yr), 11 dropped out (9 in group A and 2 in group B). Time devoted to education was of 6 (2-15) hours in group A and 12 (2.5-25) hours in group B (p=0.07). After 6 months, HbA1c levels decreased from 8.2±0.8 to 7.8±0.8 in group A and from 8.4±0.7 to 7.9±1.1 in group B (p=0.68); FBG decreased from 183±86 to 163±67 mg/dl in group A, while it increased from 177±59 to 186±79 mg/dl in group B (p=0.13); body weight increased by 0.68±3.6 Kg in group A and 1.45±2.3 kg in group B (p=0.34). Mean daily doses of short-acting insulin were of 20.6±8.2 UI/die in group A and 20.1±7.8 UI/die in group B (p=0.92), while doses of long-acting insulin were lower in group A than in group B (17.4±7.4 UI/die and 21.4±10.0 UI/die, respectively; p=0.12). No severe hypoglycaemic episode occurred. DTSQ-scores increased significantly more in group A (from 26.7±4.4 to 30.3±4.5) than in group B (from 27.5±4.8 to 28.6±5.1) (p=0.04). Role physical, general health, vitality, and role emotional SF-36 scores improved significantly more in group A than in group B.
Conclusion: DID is at least as effective as traditional CHO counting education, allowing dietary freedom to a larger proportion of T1DM patients. DID is associated with lower weight gain, probably due to lower doses of long-acting insulin, it requires less time for education and does not increase the risk of severe hypoglycaemic episodes. DID significantly improved treatment satisfaction and several quality of life dimensions.
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989

Impact of the READ (Ramadan focused Education and Awareness in Diabetes) programme on HbA_{1c}, weight and hypoglycaemia
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Background and aims: 14 million Muslims are living in Europe and 1 million suffer with diabetes. During Ramadan, Muslims fast from dawn to dusk for

one lunar month. Type 2 Diabetes (T2D) patients who fast during Ramadan have a four-fold increase in hypoglycaemic events (HE) and gain weight. In 2007, we conducted a pilot study, which demonstrated that the READ (Ramadan focused Education and Awareness in Diabetes) programme reduced the risk of HE, prevented weight gain and empowered patients to change their lifestyle. In 2008, we extended the study, aiming to: 1)validate our results, 2)compare the impact of READ on HbA1c, weight, HE before and after Ramadan in a large cohort of Muslim patients with T2D, 3)implement treatment interventions to the education arm and compare their effects on HbA1c, weight, HE during Ramadan.

Materials and methods: 126 patients (Group A) took part in the READ programme about meal planning, physical activity, glucose monitoring, hypoglycaemia, medications. Group B did not attend (n=120). We retrospectively analysed mean weight, HbA1c, HE (blood glucose $\leq$3.5 mmol l^{-1} and/or symptoms) before and after Ramadan. Patients with HbA1c $\geq$ 8.5% who attended the programme were further assigned to two groups. In addition to Metformin 2g daily, group C received vildagliptin 50mg bd (n=26) and group D received gliclazide 80mg bd (n=28). HbA1c, weight, HE were compared before and after Ramadan. Patients on Human Mixtard30 bd insulin regime (n=52) were also divided into two groups before Ramadan. In group E, the evening insulin dose was changed to Humalog Mix50 insulin (n=26). Group F continued on Human Mixtard30 (n=26). We retrospectively analysed mean weight, HbA1c, HE before and after Ramadan.

Results: Group A had mean weight change of -1.52kg (p=0.07, 95%CI -3.41 to 0.37) and mean HbA1c change of -0.7% (p<0.001, 95%CI -0.83 to -0.57). Group B had mean weight change of +0.94kg (p<0.001, 95%CI -0.76 to 2.64) and mean HbA1c change of +0.1% (p<0.001, 95%CI 0 to 0.2). There was relative risk reduction in HE of 20% with education. Group C had mean HbA1c reduction of 1.3% (p<0.001) and mean weight gain of 0.34kg (p=0.08). Group D had mean HbA1c reduction of 1.3% (p<0.001) and mean weight gain of 0.8kg (p<0.001). During Ramadan there were 12 times more HE in the Gliclazide group (n=24) compared to the Vildagliptin group (n=2). Group E had mean weight gain of 0.81kg (p<0.001) and a mean HbA1c reduction of 0.49% (p<0.001). Group F had 0.33kg mean weight gain (p=0.08) after Ramadan and mean HbA1c increase of 0.33% (p<0.05). There was relative risk reduction of 27% in HE with Humalog Mix50.

Conclusion: The READ programme created an opportunity to study the outcomes of fasting in the UK among Muslims. It led to better metabolic control, through weight loss and significant decrease in Hba1c, whilst ensuring safer fasting by decreasing the risk of hypoglycaemia. Vildagliptin use is safer than gliclazide during the month of Ramadan. The Humalog Mix50 regime could be considered as a safer and more effective regime for T2D patients who fast during Ramadan. The benefit of patient education appears to be very relevant to T2D patients who fast during Ramadan. We encourage all healthcare providers in Europe to implement this novel education programme, which is the first to study outcomes of fasting in Europe.

990

International diabetes management practices study (IDMPS): outcomes comparison between educated and non educated people with type 2 diabetes

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Background and aims: Achievement of diabetes treatment goals greatly depends on the patient´s active and efficient participation in the control and treatment of the disease. Although education is a valuable tool to obtain such participation, many patients worldwide still lack appropriate diabetes education. This study shows significant improvement of treatment indicators in a population of educated persons with type 2 diabetes and values close to treatment goals according to EASD/ADA guidelines.

Materials and methods: The IDMPS is an international, multicenter, observational study performed in 27 countries within Africa, Asia, Eastern Europe, Middle East and Latin America. Data were collected from people with type 1 and 2 diabetes (≥18 years) seen in current medical practice in yearly cycles (2-week cross-sectional recruitment period followed by a 9-month longitudinal period for type 2 patients) for 5 years. IDMPS was performed in compliance with the Helsinki Declaration and Good Clinical Practice Standards. We currently report and compare results from 11384 people with type 2 diabetes (educated *vs.* non educated, 5692 in each group, paired by age, gender and diabetes duration) recruited during the second cross-sectional period (November and December 2006). Data were analyzed using the Wilcoxon and the chi^2 tests for continuous and categorical variables, respectively.

Results: Values of the different parameters measured in both groups were close to treatment goals proposed by EASD/ADA guidelines; however, the educated group had modestly but significantly (p< 0.001) higher figures of people with normal BMI (28.3 *vs.* 24.4%), diastolic BP <80 mm Hg (36.5 *vs.* 32.3%), HbA1c < 7.0% (38.1 *vs.* 35.8%), LDL-c < 100 mg/dL (39.5 *vs.* 33%) and triglyceride < 150 mg/dL (52.7 *vs.* 49%). The percentage of complications was low (<20%) in both groups but the educated group had significantly lower values of people with proteinuria (16.6 *vs.* 18%) and foot ulcer (2.4 *vs.* 3.5%).

Conclusion: These data represent one of the largest reported on education of people with type 2 diabetes and demonstrate that education can significantly improve treatment outcomes, even in persons with values close to treatment goals. Such improvements can help to prevent the development/progression of chronic complications and the consequent increase in treatment costs.

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991

Evaluating self-management support in type 1 diabetes: design and baseline data from the Irish Dose Adjustment for Normal Eating (DAFNE) Study

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Background and aims: Structured education programmes for individuals with Type 1 diabetes have become a recognised means of delivering the knowledge and skills necessary for optimal self-management of the condition. The DAFNE programme has been shown to improve biomedical (HbA1c and rates of severe hypoglycaemia) and psychosocial outcomes for up to 12 months following course delivery. The optimal way to support DAFNE graduates and maintain the benefits of the programme has not been established. We aimed to compare 2 different methods of follow-up of DAFNE graduates in a pragmatic clinical trial delivered in busy diabetes clinics across the island of Ireland.

Materials and methods: Six participating centres were cluster randomised to deliver either group follow-up or a return to traditional one-to-one clinic visits at 6, 12 and 18 months post DAFNE training. Generalisability (external validity) was maximised by recruiting study participants from existing DAFNE waiting lists in each centre, by using broad inclusion criteria (e.g., HbA1c values less than 13 percent with no lower limit) and by using existing clinic staff to deliver the training and follow-up. Internal validity and treatment fidelity were maximised by quality assuring the training of all DAFNE educators, by external peer review of the group follow-up sessions and by striving for full attendance at follow-up visits. Assays of HbA1c were undertaken in a central laboratory.

Results: The target sample size of 400 participants was reached in February 2009. Baseline analyses show that the study population comprises 55 percent females. The mean (±SD) age of the group is 38.1 (±11.7) years with duration of diabetes of 16.0 (±10.8) years and body mass index of 26.1 (±4.2). The mean HbA1c at baseline is 8.3 (±1.3) percent with no statistical heterogeneity across centres. A total of 89 out of 381 respondents (23%) reported one or more episodes of severe hypoglycaemia in the previous 12 months. Baseline psychosocial measures showed mean scores for Hospital Anxiety Depression Scale-Anxiety (HADS-A), HADS-Depression (HADS-D) and Problem Areas in Diabetes (PAID) of 5.0 (±3.5), 4.5 (±3.2) and 27.0 (±18.0) respectively. The percent of participants with values considered clinically significantly abnormal (greater than 8 for HADS-A and HADS-D and greater than 40 for PAID) was 17, 12 and 23 percent respectively.

Conclusion: This pragmatic clinical trial evaluating group follow-up after a structured education programme has been designed to have broad generalisability. The results should inform how best to manage the well educated patient with Type 1 diabetes in the real world of clinical practice.

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992

Patient Engagement And Coaching for Health *(PEACH):* baseline characteristics of patients with type 2 diabetes in cluster RCT of intensive treatment in general practice in Australia

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Background and aims: Significant gaps exist between actual clinical practice and evidence based treatment goals and
outcomes in type 2 diabetes (T2D) care in General Practice. Intensive treatment to reduce HbA1c and other risk factor for T2D complications can be hard to implement in the primary care setting. The PEACH study is a General Practice based, cluster RCT of practice nurse-led telephone coaching to improve health outcomes for patients with poorly controlled T2D in Melbourne, Australia. The practice nurse follows the COACHING program, a scripted format to motivate and empower patients in diabetes self management. Patients receive 8 telephone coaching sessions from the practice nurse over an 18-month period. Coaching focuses on having patients more actively engage with their General Practitioner to achieve HbA1c and other risk factor targets by intensifying pharmacologic treatment. This paper describes the demographics and clinical profiles of the participants at baseline, before randomisation into intervention and control groups.
Methods: T2D patients over 18 years with HbA1c>7.5% in the last 12 months without any complex debilitating coexisting medical condition were recruited from general practices. All patients were approached by mail and asked to fill in a brief personal survey irrespective of their willingness to participate. Those agreeing to participate completed baseline data on biochemical and lifestyle factors such as smoking, diet and physical activity with their practice nurses. Additional information such as diabetes self efficacy, depression, quality of life and level of social support were also obtained.
Results: 468 patients (42% female) from 59 general practices completed baseline assessments. Mean age was 62 years and 19% of participants were from non-English speaking background. Participants had a median duration of diabetes of 9 years with a median HbA1c of 7.8%. Over 90% of participants were overweight or obese, with average BMI of 33 kg/m^2 and over 70% had waist circumference above target. Blood pressure and total cholesterol means were 138/79mm/Hg and 4.5mmol/L, respectively. Proportion of people with normal ranges of albumin/creatinine ratio and eGFR were 73% and 82%, respectively. Nearly 40% of the sample reported mild to severe of depression level. Compared to non-participants, participants were more educated, more likely to have private health insurance, from higher socio-economic occupational groupings, had fewer macrovascular complications, and were more likely to be on combined insulin and oral medications. Participants reported slightly lower self efficacy in managing diabetes and slightly higher levels of perceived availability of support and support accessibility compared to community samples.
Conclusion: While participants are more educated and not as disadvantaged socio-economically as non-participants, this poorly controlled study population is at high risk of significant long term complications from diabetes. The high prevalence of depression and obesity are of concern and are likely to pose challenges to practice nurses undertaking coaching. The Practice Nurses will face additional challenges to coach these patients to improve their diabetes control in view of the natural progression of the disease.
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993

Attitudes and beliefs on self-care of diabetic foot among patients

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Background and aims: The risk of Diabetes Foot Syndrome has increased in patients with long diabetes duration. Preventive measures however can reduce lower limb amputations for diabetic foot disease. Tight glycemic control, patient education and preventive foot care behaviors can substantially reduce that risk. The aim of this study was to assess attitudes, beliefs and the level of preventive foot care among patients with diabetes in an outpatient foot clinic in Portugal.
Materials and methods: A retrospective descriptive analysis based on clinical data collected from all diabetic patients that attended for the 1st time our foot clinic, between January and December 2007. A cohort of 1.048 diabetic patients who went to our screening evaluation, were assessed by their preventive foot care attitudes and beliefs. A questionnaire including items such as foot hygiene, proper foot wear, identification and avoidance of risks, behavior in injury, routine foot surveillance and daily self feet inspection was used during the assessment. The level of awareness was categorized as having "No Awareness", "Some Awareness", "Acceptable Awareness" and "Good Awareness". Details of socio-demographic and clinical profiles were also available. Statistical analysis was performed through SPSS - Statistical Package for the Social for Science). Descriptive statistics are present and comparison between variables was performed using χ^2 test.
Results: We studied 1.048 patients, 46% were female and 54% male. Mean age of the group was 61 ± 14 years and duration of diabetes was 12 ± 9 years. 6,5% were type 1 diabetes, 88,1% were type 2 diabetes and 5,4% had other types of diabetes. The last Hb1A1c in patients with ≤ 6,5% was 19,37%. We found that 82,5% of patients had acceptable awareness of foot care behaviors. Comparative analysis between socio-demographic and clinical variables revealed statistic significant differences in gender (female 56,9% vs male 43,1%); $p < 0,05$. duration of disease for less than 9 years (Pearson Chi- Square value 77, df 42, p= 0.001), school degree to secondary school (Pearson Chi- Square value 2,3, df 3, p= 0,001) and type of diabetes for diabetes type 2 (Pearson Chi-Square value 21,8, df 5, p =0.001).
Conclusion: The evaluation in our sample showed an adequate awareness standard of preventive foot care behavior. Effective education is needed to increase awareness and preventive foot care attitudes (behavior) in the illiteracy community and, with longer duration of diabetes, especially in men and in type 1 diabetics.

994

Self-monitoring of blood glucose in patients with type 2 diabetes who are not using insulin: a 1-year randomised controlled trial

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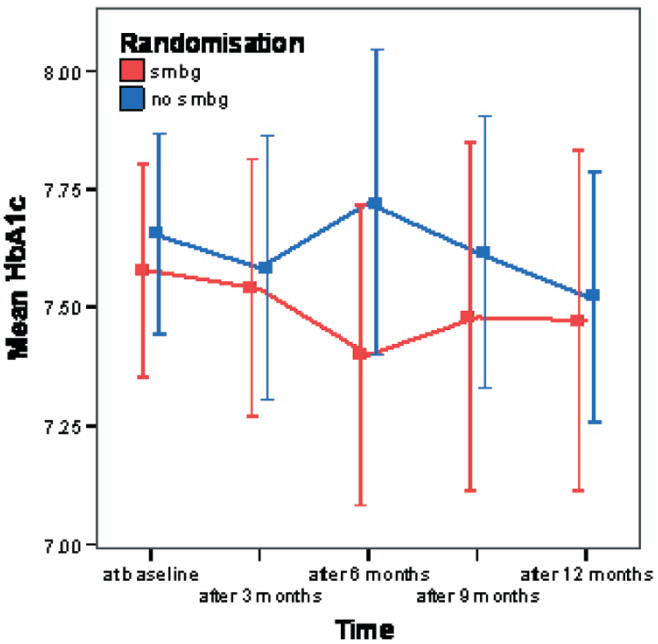
Background and aims: Self-monitoring of blood glucose (SMBG) improves glycemic control in patients using insulin. Whether this is also true for patients with type 2 diabetes mellitus (T2DM) not using insulin is questionable. Our aim was to investigate the effects of SMBG (postprandial values) in patients with T2DM not using insulin who are in persistent moderate glycemic control.
Materials and methods: Patients were eligible when between 18 and 70 years of age, with an HbA1c between 7 and 8.5% at the start of the study (at annual check-up) and at the previous annual check-up, using 1 or 2 different oral blood glucose lowering drugs without regimen change during the preceding 3 months, and without use of SMBG in the preceding 6 months. Forty patients were randomly assigned to receive either SMBG added to usual care or to continue with usual care during 1 year. SMBG was performed using the Accu-check Aviva blood glucose meter. Twice a week (including either Saturday or Sunday),a fasting glucose value and three postprandial glucose values were measured. All patients were instructed about acceptable glucose values (fasting: 4-8 mmol/L, postprandial: 4-10 mmol/L) and no specific (dietary) advice was given. HbA1c was measured every 3 months. Blood glucose lowering therapy was intensified when HbA1c was ≥ 8.5%. The primary efficacy parameter was change in HbA1c. An intention to treat analysis was performed.
Results: Patients were recruited from March 2006 until October 2007. Mean age was 59 years, median diabetes duration was 6.0 years, and mean BMI was 31 kg/m^2. Mean HbA1c at baseline was 7.58% vs. 7.66% in the intervention vs. control group, respectively. After 1 year HbA1c was on average 7.47% in the intervention group and 7.52% in the control group (see figure: Mean HbA1c values for smbg and no smbg groups with 95% CI). Change in HbA1c between groups was -0.05% (95%CI: -0.51, 0.41; p=0.507).
Conclusion: Although this study cannot fully exclude a possible clinical relevant change in HbA1c caused by SMBG in patients on oral therapy, it largely confirms the results of 2 previous trials with SMBG in T2DM (the DIGEM and ESMON trials). Our study adds data on patients with T2DM in persistent

moderate control, performing fasting and postprandial SMBG. We conclude that there is no evidence that SMBG should be considered of value in T2DM patients not using insulin.



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995

Successful optimizing of intensified insulin therapy using a new structured treatment and teaching programme for outpatients with diabetes mellitus type 1

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Background and aims: The only certified diabetes treatment and teaching programme (DTTP) for patients with diabetes type 1 in Germany was developed and evaluated by the group of Michael Berger. The programme aims for self management of diabetes and insulin dose adjustment. The evaluation of the DTTP is mainly based on studies on inpatients and one trial on outpatients from Austria. Primary objectives of the programme were starting insulin therapy and switching from conventional to intensified therapy. Today 99% of patients with type 1 diabetes in Germany are on intensified insulin therapy The current objective in the care of patients with diabetes type 1 is to optimize intensified insulin therapy. We adapted the original DTTP to improve metabolic control and reduce severe hypoglycaemia in patients with diabetes type 1 who are already on intensive insulin therapy. The evaluation was done in an outpatient setting in German diabetes practices.
Materials and methods: In an ambulant, multicentre, prospective trial, we recruited 85 patients with diabetes type 1 (age 44.1y, duration of diabetes 16.3y, BMI 26.6kg/m²). The DTTP consisted of 12 teaching sessions in a period of 6 weeks. Data were checked at baseline (V1), 3 weeks after DTTP (V2) and 12 months after DTTP (V3). 74 patients completed the DTTP and 62 patients could be examined after 12 months (V3: age 43y, duration of diabetes 13.7y, BMI 26.7kg/m²). Diabetes treatment satisfaction (DTSQs and DTSQc) and diabetes dependent quality of life (ADDQoL) were assessed with a questionnaire (C. Bradley); HbA_{1c} was DCCT adjusted.
Results: HbA_{1c} at baseline was 7.71%. In patients with baseline HbA_{1c} >7% (88%) HbA1c decreased by 0.36% (V1 8.17; V3 7.81%; p=0.004). The percentage of patients with HbA_{1c} ≤7% increased from 21.3% at V1 to 34.9% at V3 and the percentage of patients with HbA_{1c} above 10% decreased from 6.6% at V1 to 1.6% at V3. We did not observe a significant change in insulin dose (47.85 V1; 50.11 IE/d V3; p=0.380) or number of injections (4.89 V1; 4.77/d V3; p=0.349). The incidence of mild and severe hypoglycaemia decreased significantly: mild hypoglycaemia from 3.31 at V1 to 1.39/episodes/pat/wk at V3 (p=0.001) and severe hypoglycaemia (glucose i.v. or glucagon injection) from 0.16 at V1 to 0.03/episodes/pat/year at V3 (p=0.02). Ketoacidosis with hospital admission was rare (1 event/12 month at baseline; no event at V3). Diabetes treatment satisfaction increased by +10 of a maximal ±18 points. The negative influence of diabetes on quality of life decreased from -1.93 to -1.69 points (p=0.031).
Conclusion: In a group of moderately well-controlled patients with type 1 diabetes being already on intensified insulin therapy, metabolic control, treatment satisfaction and quality of life improved following participation in an ambulatory teaching and treatment programme.
Supported by: Roche Diagnostics Germany

996

Cost-efficacy of group care in the management of type 2 diabetes. Economic evaluation of the ROMEO (Rethink Organization to iMprove Education and Outcomes) data set

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Background and aims: ROMEO was a 4 years multicenter randomized controlled trial of Group versus individual care, which showed that the former helps achieving better metabolic control, quality of life and health behaviours in patients with type 2 diabetes. We have calculated the costs of hypoglycaemic, antihypertensive and lipid-lowering medication associated with the two approaches.
Patients and methods: the costs of drugs to the Italian National Health Service were calculated by summing the prices of all prescriptions to each patient for the duration of the trial. The data from 479 patients, 246 on Group Care and 233 controls from 4 participating centres were calculated
Results: On average, patients on Group Care cost 383.13 Euro/year and Controls 334.02 Euro/year. Over 4 years, patients on Group Care cost 81,5 Euro more than controls. Since at the end of study patients on Group Care had lower BMI (-1.09), HbA1c (-1.49%) and better quality of life (-16.8) p<0.0001 all Group Care cost Euro 74.78, 54.70 and 4.85 per point gained in body mass, metabolic control and quality of life respectively.
Conclusion: Group Care is a cost-effective alternative to traditional care for the management of type 2 diabetes.
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PS 87 Depression in diabetes

997

Impact of weight loss on anxiety and depression in obese patients with type 2 diabetes mellitus

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Background and aims: Obesity and type 2 diabetes mellitus (T2DM) are strongly associated with increased rates of depression. Modest weight loss (5-10%) in obese patients with T2DM has multiple metabolic benefits, improving glycaemic control, lipid profile and blood pressure. Effects of weight management on the psychological status of T2DM patients are less well documented. The aim of this study was to investigate the impact of weight loss on anxiety and depression in obese T2DM patients attending an outpatient specialist obesity clinic.
Materials and methods: Patients with T2DM, on oral hypoglycaemic agents, and body mass index (BMI) over 30 kg/m^2, followed in our obesity clinic, were invited to participate in the study. Weight loss interventions included lifestyle modification and pharmacotherapy with orlistat or sibutramine. Emotional disorders relating to anxiety and depression were assessed by the Hospital Anxiety and Depression Scale (HADS), a validated, self-report instrument consisting of 14 items (7 items for anxiety, HADS-A, and 7 items for depression, HADS-D). The two HADS subscales are independent measures. Anxiety cases on HADS-A and depression cases on HADS-D were defined by a score equal or greater than 8 on each subscale. Participants completed the HADS at baseline and at their annual follow-up visit.
Results: Complete data were available for 32 T2DM patients (40.6% male and 59.4% female, age range: 38-59). At baseline mean BMI was 48.26±7.90 kg/m^2 (mean±SD). Based on their baseline HADS scores (HADS-A mean score: 9.59±4.77 and HADS-D mean score: 8.31±4.47) 62.5% of the participants were classified as cases of anxiety and 53.1% as cases of depression. At 12 months mean body weight was significantly reduced by 5.80±5.23 kg ($p<0.01$) (BMI: 46.30±7.20 kg/m^2, $p<0.01$), representing a mean percent weight loss of 4.06±3.79% for the whole cohort. At 12 months 28.1% of the participants were classified as cases of anxiety and 31.3% as cases of depression with significantly decreased scores in both subscales (HADS-A mean score: 6.75±3.95 and HADS-D mean score: 5.75±3.76, $p<0.05$). Significant positive correlations were found between percent weight loss and reductions in both HADS-A and HADS-D scores ($p<0.05$).
Conclusion: These results suggest that weight loss achieved through a weight management programme in the setting of a specialist obesity clinic can improve the anxiety and depression status of obese patients with T2DM. Future studies are planned to examine the impact of weight management on psychological co-morbidities of T2DM in a larger cohort of patients and in the long-term.

998

Changes in eating behavior, depression and quality of life in patients with type 2 diabetes after a 3-months-intervention with low-fat vegetarian diet and conventional diabetic diet

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Background and aims: When compared to conventional diabetic diet, the clinical trials using vegetarian diets have shown greater effect on weight loss, greater reduction of LDL cholesterol, HbA1c and diabetes medication. The aim of our study was to determine eating behaviour, the degree of depression, and quality of life in patients with type 2 diabetes attending an intervention programme with different hypocaloric diets (low-fat vegetarian diet compared to conventional diabetic diet).
Materials and methods: Open, parallel randomized study. 70 individuals with type 2 diabetes were randomly assigned to a low-fat vegetarian diet (experimental group; EG) or conventional diabetic diet (control group; CG) with similar caloric restriction (-500 kcal/day). All patients were examined, using the The Three-Factor Eating Questionnaire (Stunkard and Messick), Beck's Depression Inventory (BDI), a questionnaire on Quality of life (OWL-QoL) and a questionnaire tracing negative symptoms caused by overweight and obesity (WRSM) at baseline (0) and after 12 weeks of diet intervention.
Results: *I. Both groups as a whole.* Patients in both groups lost weight ($p<0.01$). The average BDI score decreased significantly after the intervention ($p<0.05$). Patients in both groups noticed significantly lower feelings of hunger after the intervention ($p<0.01$). The quality of life improved in both groups ($p<0.01$). *II. Differences between groups.* Average weight loss was greater in the EG than in the CG (6.41±4.23 vs. 3.47±4.07 kg; $p<0.01$). There were no differences between groups in the average BDI score (8.3±5.5 vs. 8.6±8) at start, which did not reach the threshold of depressive symptoms. Nevertheless the average BDI score decreased significantly after the intervention ($p<0.05$). The interindividual variations are remarkable in both groups. The differences between both groups after the intervention are not statistically significant. In the disinhibition scale and hunger score, there are no statistically significant differences between the groups at start, but we can see a trend toward greater food disinhibition score in the EG (6.3 EG vs. 5.4 CG) and stronger feelings of hunger in the EG (4.9 EG vs. 3.4 CG) at start, whereas there is a trend toward greater decrease in feelings of hunger in the EG (1.88 EG vs. 0.83 CG) but there are no significant differences between groups in food disinhibition score after the intervention. The quality of life improved in both groups ($p<0.01$): the WRSM score decreased by 9.1 vs. 11.8 with no statistically significant differences between groups and the OWLQoL score decreased with no statistically significant differences between groups ($p<0.05$). There were no differences between groups in the restriction scale at start (8.7±3.8 vs. 8.4±4.8), whereas after the intervention the conscious control of food increased significantly in both groups, more in the CG (by 2.1 in the EG vs. 4.47 in the CG, $p<0.05$).
Conclusion: The results show a remarkable positive effect of the diet intervention on the quality of life, the BDI score and feelings of hunger. Patients from the CG expressed stronger feelings of restriction in food. In the EG patients lost more weight and expressed feelings of restriction in food and feelings of hunger less frequently.
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999

Screening performance of a short versus long version of the patient health questionnaire-depression in outpatients with diabetes

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Background and aims: Regular screening for depressive symptoms in diabetic outpatients might improve both diabetes and depression outcomes. This study was aimed at comparing performance of a two-item vs a nine-item version of the Patient Health Questionnaire (PHQ-9) defined as a proportion of positively screened patients with confirmed elevated depressive symptoms by means of a clinical interview.
Materials and methods: Two hundred sixty-three consecutively recruited diabetic patients attending their regular medical check-ups (90% with type 2 diabetes, aged 60±11 yrs., with diabetes duration of 10±8 yrs., 44% insulin-treated, with average HbA1C values of 7.4%±1.4 and BMI of 29.7 kg/m2 ±5.2) were screened for depression by using the PHQ-9 scale. Cut-off scores of 3 for the PHQ-2 and 10 for the PHQ-9 were used to discriminate between positive and negative screenings. Percentages of positive screenings on the two instrument's versions, and proportions of patients who were confirmed for elevated depressive symptoms by a clinical interview were compared.
Results: Twenty-two percent of the patients assessed for depressive symptoms were found to be above the cut-offs indicative of depression. The percengates of positive screening did not differ by using the PHQ-2 and PHQ-9 indicators (22% vs 22% p= 0.88). Patients with elevated depressive symptoms did not differ from those scoring below the cut-offs with respect to age (60±12 vs 60±11 yrs. p=0.86), diabetes duration (10±5 vs 10±7 yrs. p=0.94), type of diabetes ($\chi2$ =0.60 p=0.44), insulin therapy ($\chi2$=0.55 p=0.46), HbA1C values (7.5%±1.5 vs 7.3%±1.4) and BMI (30.2 kg/m2±5.4 vs 29.3 kg/m2±4.4 p=0.21). There were more female patients in the subgroup indicative of depression ($\chi2$=11.6 p=0.0007). Thirty percent of patients positively screened by PHQ-2 versus 37% of those positively screened by PHQ-9 were confirmed for elevated depressive symptoms using a clinical interview ($\chi2$ =0.04 p=0.85).
Conclusion: Finding elevated depressive symptoms in diabetic outpatients by using the PHQ-2 and PHQ-9 indicators was shown to be comparable with respect to both positive screenings per se and their proportion as confirmed by

a more comprehensive diagnostic procedure. PHQ-2 could be recommended for routine use in diabetological check-ups in order to detect diabetic patients at a risk of depression.
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1000

Investigation of depressive symptoms in diabetic patients with and without micro- and macro-angiopathic complications
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Background and aims: The prevalence of depression is twice as common in diabetic patients compared to the general population. Chronic complications of diabetes are thought to be a factor in the increased prevalence of depression. In the present study our goal was to assess depressive symptoms in a group of diabetic patients with macroangiopathy (MA), microangiopathy (MI) and in patients without complications.
Materials and methods: 271 diabetic patients were enrolled in our study. Participants were further divided into subgroups taking in account gender differences and diabetic complications. Patients with a positive medical history for myocardial infarction, stroke, or obliterative atherosclerosis of the lower limb were considered to have macroangiopathy. Individuals with a history of diabetic retinopathy, nephropathy or neuropathy were considered to have diabetic microangiopathy. Thus we gained a male and a female group without macroangiopathic diabetic complications (ACM, ACF) and a male and female group with diabetic macroangiopathic complications (MAM, MAF) respectively. Similarly for microangiopathic complications a male and female group with (MIM, MAF) and without the complications (ICM, ICF) has been established. There was no significant difference between the groups in age, BMI, HgA1C and TSH levels. Short version of the Beck's depression scale was used to evaluate depressive symptoms.
Results: Beck depression scores were higher in all female groups compared to males. Beck depression scores were significantly greater in both male groups with diabetic macro (ACM: 4,59±3,88 MAM: 6,44±3,96 $p<0,05$) and microangiopathic complications (ICM: 3,05±2,89 MIM: 5,29±3,77, $p<0,05$) compared to males without complications. This difference could not be detected in females regarding neither the macro (ACF Beck: 7,01±6,46 MAF Beck: 9,22±5,8) nor the microangiopathic complications(ICF Beck: 7,02±4,72 MIF Beck: 7,8±6,00).
Conclusion: Both MA and MI complications increase depressive symptoms of male diabetic patients. Depression scores of females are greater regardless of their diabetic complications.
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1001

Web-based cognitive behavioural therapy for diabetes patients with co-morbid depression: first findings
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Background and aims: Depression is a common co-morbidity in people with diabetes, negatively affecting well-being and glycaemic outcomes. Research on treatment of depression in diabetes is limited. We are the first to have developed a diabetes-specific version of the online cognitive behavioural self-help course "coping with depression", which is currently tested in an ongoing randomised trial (ISRCTN24874457). Socio-demographic and clinical characteristics of the patients who so far participated are being presented.
Materials and methods: The study is being advertised trough various media, including patient magazines and websites. Inclusion criteria are: 18 year or older, diagnosed with diabetes > 3 months ago, suffering from depressive symptoms, and sufficient language and computer skills. Exclusion criteria are: pregnancy, loss of significant other < 6 months ago, bi-polar disorder, using anti-psychotic medication, unstable antidepressant medication, co-morbid psychiatric disorder, history of suicide attempt, and current suicidal ideation. Patients sign up online for enrolment in the study and fill out questionnaires online. Self-reported information is gathered on depression (CES-D), diabetes-specific emotional distress (PAID), perceived health status, diabetes self-care behaviours, and diabetes outcomes (HbA1c, episodes of hypoglycaemia). If CES-D ≥ 16, patients are offered a telephone-administered diagnostic psychiatric interview (CIDI) for diagnosis of depression and/or anxiety disorders and then enrolled in the study.
Preliminary Results: Between the 24th of June and the 18th of March 2009, 293 participants signed up for our study. N=126 patients were excluded or dropped out, 20% due to not having elevated symptoms of depression (CES-D < 16). Mean age was 41 ± 11 years for T1DM and 56 ± 10 for T2DM (range 21 - 82); 61% was female; 91% of Dutch origin; 57% Type 2 diabetes, of which 45% on insulin treatment. Self-reported mean HbA1c was 7.5% ± 1.1 (range 4 - 13%); 25% did not know their HbA1c. Mean duration of diabetes 21 ± 12 years for T1DM and 8 ± 7 for T2DM. Mean CES-D was 29 ± 8 (ranging from 16 - 51), showing highly elevated symptoms of depression. Mean PAID score 40 ± 19, indicating high levels of diabetes-distress. The telephone administered CIDI was administered to 133 participants, where 59% suffered from Major Depressive Disorder (MDD), and 3% from Dysthymic disorder; 33% of the participants with depressive disorder were diagnosed with a co morbid anxiety disorder. Of the total group of participants, 12% was diagnosed with anxiety disorder only. Of all participants 40% reported a history of depression treatment.
Preliminary Conclusion: There has shown to be much interest in our online treatment of depression in diabetes patients, including a small group with no clear depressive symptomatology. This may point at a need for more education on the interplay between diabetes and depression. The majority of participants was diagnosed with depressive disorder (DSM-IV), warranting anti-depressant therapy. In contrast to our expectation, patients reported only moderately elevated HbA1c levels. Objective HbA1c values will be retrieved for comparison to validate this finding. High diabetes-related distress found in this group however does suggest serious coping problems and confirms the need to address diabetes-related emotional issues as part of the depression treatment.
Supported by: Dutch Diabetes Research Foundation

1002

Long term follow up of a cohort of patients with diabetes and their first foot ulcer-effect of depression on mortality
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Background and aims: Diabetic foot ulcers, infection and lower limb amputation have a severe negative effect on health related quality of life and may cause depression in individuals with diabetes. There have been no population based studies starting with patients presenting with their first foot ulcer and followed up over several years to assess how psychological and biological factors interact with each other to influence adverse outcomes. The aim of this study was to evaluate whether depression is associated with increased mortality in people with their first foot ulcer at 5 years.
Materials and methods: A retrospective follow up of a previously described cohort of patients, who presented with their first diabetic foot ulcer, were recruited from community and hospital foot clinics in south east London, UK. The original sample consisted of 253 patients prospectively followed up for 18 months. Post 18 months the same sample was retrospectively followed up for 5 years. At baseline, the Schedules for Clinical Assessment in Neuropsychiatry 2.1(SCAN 2.1) was used to define those who met DSM (Diagnostic and Statistical Manual of Mental Disorders)-IV criteria for depressive disorder. Logistic regression analysis controlled for potential covariates; age; sex; marital status; socio-economic status; smoking; A_{1C}; and University of Texas classification based- ulcer severity. The main outcome was mortality at 5 years.
Results: The prevalence of DSM-IV depressive disorder was 32.2% (n=82). There were 88 (34.8%) deaths at five years. Thirty seven patients out of the 82 (40.2%) who were dead at 5 years had clinically significant depression at baseline. In the adjusted logistic regression analysis DSM-IV depression was associated with mortality (odds ratio 2.54, 95% CI 1.31-4.93) compared with the group with no depression
Conclusion: More than a third of people with their first foot ulcer suffer from depression, and this is associated with an increased mortality at 5 years.

1003

Depression, coping strategies, metabolic control and compliance with diabetes care in diabetic patients

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Background and aims: Diabetic patients must cope with a wide range of challenges specific not only to the disease but also to other conditions in their lives, which may influence self-management of diabetes and metabolic control. In this study, we aimed to investigate the psychological and metabolic states of diabetic patients and the coping strategies they use.
Materials and methods: This descriptive, cross sectional study included 110 type 2 diabetic patients (62 female, 48 male) who were followed up in the Diabetes Outpatient Clinic of Baskent University. Demographic and clinical data were determined from questionnaires. Metabolic control was measured by glycosylated hemoglobin A1c (HbA1c) levels. The patients were asked to fill the Beck Depression Inventory (BDI) and Ways of Coping Questionnaire (WCQ). For statistical comparisons, Chi-square test, Independent sample T test and Pearson's correlations test were used.
Results: The mean age was 57.9&plusmn10.5 (range, 36-85). Mean HbA1c was 7.1&plusmn 1.7 % (range, 5.3-13.1). A good compliance with treatment and diet was reported among 93 patients (90 %) and 60 patients (57.6 %), respectively. Seventy two patients (65.5 %) had received patient education about diabetes care. Thirty four (30.9 %) diabetic patients were found to be depressed. There was a negative correlation between depression scores and compliance with diet (r = -0.2, p=0.01). Beck depression scores were significantly lower in patients receiving patient education about diabetes care (p&lt0.01) and having good compliance with treatment (p=0.03). The coping strategy most frequently used by the patients was problem solving-optimistic approach followed by fatalistic, escape, helplessness approaches in decreasing order of frequency. Patients who had Beck Depression Inventory scores 17 and over (moderate/severe depression) were more frequently using "fatalistic" and "helplessness" approaches as coping strategies. A positive correlation was found between female gender and depression scores (r =0.3, p=0.01), fatalistic approach (r =0.3, p&lt0.01) and helplessness approach (r =0.2, p&lt0.01). There was a positive correlation between depression scores and duration of diabetes (r=0.2, p=0.01). No correlation was found between HbA1c levels and type of diabetes treatment with depression scores and coping strategies. Also there was no correlation between new onset insulin therapy and depression scores.
Conclusion: Although the frequency of depression is consistent with that reported in the literature, it does not seem to effect glycemic control. Our data showed significant associations between depression scores, female gender, emotional-negative coping strategies and poor compliance with diet or treatment in our patients. This suggests that positive- problem solving coping strategies may be important factors for successful diabetes self-care and treatment. Additionally, we suggest supporting the patients by frequent monitoring, educating and problem solving counseling could be the first line interventions in diabetes care.
Supported by: Baskent University

PS 88 Psychological aspects of diabetes

1004

Health-related quality of life and macrovascular comorbidities among diabetes patients in the United States

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Background and aims: Diabetes mellitus has been defined as one of the global epidemics of chronic diseases by the World Health Organization. Patients with diabetes are at an increased risk of developing macrovascular disease. The purpose of this study was to examine the marginal impact of macrovascular comorbidities on health-related quality of life (HRQoL) of patients with diabetes in a United States nationally representative sample.
Materials and methods: Using the pooled Medical Expenditure Panel Survey (MEPS) 2001 and 2003 data, a nationally representative adult sample (age ≥18) was included in the study. The HRQoL outcomes included the SF-12 physical component summary (PCS) score, SF-12 mental component summary (MCS) score, EQ-5D preference-based index score, and visual analog scale (VAS). Ordinary least square regressions were used to identify the relationship between macrovascular disease conditions and PCS (and MCS) after controlling for age, sex, race, ethnicity, education, income, smoking status, health insurance, proxy response, and number of other comorbid categories. Due to the distributions of the EQ-5D preference-based index and VAS score, censored linear deviations estimator (CLAD) regressions were employed. All statistics were adjusted using the proper sampling weight from the MEPS.
Results: The average PCS, MCS, EQ-5D index, and VAS scores for patients with diabetes were 40.5, 48.5, 0.75, and 66.6, respectively for the sample. Compared to diabetes patients without macrovascular comorbidities (N=2,809), those with macrovascular comorbidities (N=654) had statistically significant lower PCS (-3.38, p<0.001), MCS (-1.43, p<0.05), EQ-5D index (-0.024, p<0.05), and VAS scores (-4.93, p<0.001). Patients with diabetes also had significantly lower HRQoL outcomes than those without diabetes (N=43,326) (p<0.001).
Conclusion: In summary, macrovascular comorbidities significantly decrease HRQoL in patients with diabetes.
Supported by: Merck and Company, Whitehouse Station, USA

1005

Coping styles influence diabetes-related outcomes in newly diagnosed type 1 diabetic patients

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Background and aims: Diabetes as a chronic condition influences many aspects of patients' life. Treatment of diabetes should not only concentrate on metabolic control, but also on psychological adaptation to the disease and good quality of life. Thus, it seems important to identify subgroups of patients requiring particular counseling and support. The aim of our study was to assess the influence of different ways of coping with stress on diabetes-related outcomes in newly diagnosed type 1 diabetic patients.
Materials and methods: A total of 152 adults with newly diagnosed type 1 diabetes were recruited for the study (98 men, 54 women, aged 16-35, mean age: 24.8 ± 4.8 years). All patients were admitted to hospital, educated and treated with intensive functional insulin therapy. Shortly after diagnosis each participant's coping styles were assessed with the use of Coping Inventory for Stressful Situations (CISS). Finally 108 patients (64 men, 44 women) were followed prospectively for 18.7 ± 7.2 months. At the end of the observation diabetes-related outcomes were evaluated including: HbA1c, acceptance of illness (Acceptance of Illness Scale) and quality of life (Quality of Life Questionnaire). Pearson's correlation coefficients were calculated. Statistical analysis was performed using Statistica 7.0 software.
Results: Task-oriented coping style at diagnosis correlated inversely with HbA1c value (r=-0.223, p=0.029). Emotion-oriented coping style correlated inversely with acceptance of illness (r=-0.334, p<0.0001) and quality of life (r=-0.283, p=0.003), but no association was found with HbA1c. Avoidance-oriented coping style did not correlate with any of the investigated variables.

Conclusion: The way of coping with stress may influence both objective (HbA1c) and subjective (acceptance of illness, quality of life) diabetes-related outcomes in newly diagnosed type 1 diabetic patients. Delineation of individual coping styles might be useful for identifying patients at risk for poor adaptation to disease.
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1006

Evaluation of personality with egogram in patients with diabetes
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Background and aims: Previous studies suggest that some psychosocial factors affect on blood glucose (BG) control in patient with diabetes mellitus. However, we have few useful methods which enable us predicting effectiveness of the treatment and the education on diabetic patients from psychological aspects. We examined ego state in diabetic patients with egogram, to clarify the influence of psychological factors such as ego state in BG control in diabetic patients.
Materials and methods: We evaluated ego state in 136 diabetic patients with the questionnaire, the Tokyo University Egogram version 2 (TEG-II). Ego state was assessed by calculating the scores of 5 components of ego, namely critical parent (CP), nurturing parent (NP), adult (A), free child (FC) and adapted child (AC). Of 136 diabetic patients, 71 patients had metabolic syndrome. Twenty-six patients had low compliance and resistance to any medical treatment despite of repeated administrations for education and treatment (RT group). Sixty-seven patients were elderly (more than 64 years of age) patients. We evaluated ego state especially in these patients groups. All results are expressed as means ± SEM. Statistical analysis was performed using Student's t test. P values less than 0.05 were considered significant.
Results: FC scores in RT group (n=26) were significantly ($p<0.05$) higher than those in other patients (n=110) (15.11 ± 0.60 vs. 13.70 ± 0.40) and AC scores in RT group were significantly ($p<0.05$) lower than other patients (5.54 ± 0.94 vs. 7.57 ± 0.43). (FC - AC) scores in RT group were significantly ($p<0.02$) higher than other patients (9.58 ± 1.35 vs. 6.14 ± 0.65). Other 3 scores of egogram did not have any significant differences between these 2 groups. Each egogram score was not significantly different between patients with metabolic syndrome (n=71) and patients without metabolic syndrome (n=65). FC scores and (FC-AC) scores of elderly patients (n=67) were significantly ($p<0.01$) higher (14.92 ± 0.44 vs. 13.06 ± 0.52, 8.93 ± 0.64 vs. 4.72 ± 0.93) and AC scores were significantly ($p<0.05$) lower(6.00 ± 0.46 vs. 8.33 ± 0.61)than younger patients (n=69). Thus, these results were similar to those observed in RT group. However, age was not significantly different between RT group and other patients (62. 46 ± 1.56 vs. 63.52 ± 0.49).
Conclusion: These results suggest that patients resistant to the education and the treatments have characteristic egogram pattern (high FC scores and low AC scores) and this seemed to be independent from aging. Such ego state can be one of factors which act to aggravate BG control in diabetic patients. Current data suggest that TEG-II give us useful information about personality of patients and it is helpful for us to make a program of education and treatment for each diabetic patient. We observed that elderly diabetic patients have similar characteristic egogram pattern. Further studies are expected to determine whether it is specific in diabetic patients or not.

1007

The youngest children with type 1 diabetes mellitus are affected with the same number of serious life events as children with diabetes recognized at later age
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Background and aims: Stress is one of factors seen as contributing to diabetes development. Psychological stress due to a link to hormonal levels and nervous signals increases insulin resistance and affects immune system. It may contribute to the induction of type 1 diabetes mellitus (T1DM) related autoimmunity. In the last years the highest increase in the development of T1DM has been noted in children at the age 0-4 years old. Clinical observation and recent studies show the high incidence of stressful events before diabetes diagnosis. The aim of this study was to assess incidence rate of serious life events prior to diabetes diagnosis and examine if there is a difference in occurrence of such events between families of children with T1DM recognized at the age < 5 years and > 5 years.
Materials and methods: There were included 347 participants: 123 parents of children (67 girls, 56 boys) with T1DM onset at the age < 5 years with the mean age 3,1 SD 1,4 ys (range 0-5) and 224 parents of children (108 girls, 116 boys) with T1DM onset at the age > 5 years with the mean age 9,9 SD 2,8 ys (range 6-17). Parents completed a questionnaire during a routine visit in the outpatient clinic. The questionnaire, especially constructed for the purpose of this study, included questions about experience of serious life events which can cause chronic stress (as seen in Posttraumatic Stress Disorder).
Results: The number of serious life events totaled to 243 (in 60% of families) In both groups serious life events appeared more often within 2 year period before T1DM recognition: in 47% vs 13% of families (RR 0,3 95%CI 0,3-0,4 $p<0,0001$). There was no difference in the number of families of children with T1DM recognized at the age < 5 years and > 5 years affected with serious life events 49% vs. 45% respectively. In <2 years before T1DM onset in both groups there were mostly 1 or 2 serious life events per family, without difference between groups. In both groups parents divorce was the most common serious life event which occurs in every 6th family. There was no statistical difference between groups in the number of serious life events except grandparents death (RR 0,9 95%CI 0,8-1, $p=0,004$).
Conclusion: 60% of families with children with T1DM have been affected by serious life events. The most serious life events occurred in the families within 2 year period before T1DM recognition. Children with T1DM onset at the age < 5 years were affected with the same number of serious life events as children with diabetes recognized at later age. Coping strategies for stress and attachment patterns should be carefully looked into in families of children with type 1 diabetes.

1008

Cognitive performance and chronic cerebral ischaemia in diabetes mellitus type 2
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Background and aims: Underlying mechanisms for decreased cognitive functions in patients with type 2 diabetes (DMT2) are unclear. Cerebral ischaemia is a risk factor for cognitive disorders and dementia. White matter lesions reflect cerebral small vessel disease and may account for cognitive impairments in diabetic patients.The aim of the present study was to compare cognitive functions and brain magnetic resonans imaging (MRI) between type 2 diabetic patients and non diabetic control subjects and assess the relationship between early cognitive impairment and brain MRI characteristics in DMT2.
Materials and methods: Our study included 126 (42 men and 84 women) patients with DMT2 (age 50-75 years) and 20 (3 men and 17 women) control patients without diabetes and hypertension. MRI scan (T1,T2,Flair) was performed to define white matter lesions(WMLs)as a marker of cerebral small vessel disease.Glycated hemoglobin (HbA1c),body mass index,systolic and diastolic blood pressure, biochemistry assay, albuminuria, neuropsychological tests: Frontal Assesment Battery (FAB), Mini Mental State Examination (MMSE),Clock Drawing Test (CDT),"10 words"test were measured.
Results: WMLs were found in 122 DMT2 cases (97%) and in 7 control cases (35%),$p=0,004$. Patients with DMT2 performed worse results in all neuropsychological tests, $p<0,05$. Early cognitive disfunction was found in 55%patients-mild cognitive disorder (MCD). Structural brain white matter abnormalities were interfacing with: arterial hypertension duration ($r=0,278$ $p=0,002$), intencity of carotid artery atherosclerosis ($r=0,323$ $p=0,02$),chronic inflammation ($r=0,211$ $p=0,024$),albuminuria ($r=0,334$ $p<0,001$), fibrinogen($r=0,351$ $p=0,001$), homocystein ($r=0,254$ $p=0,018$) and HbA1c ($r=0,201$ $p=0,001$). Periventricular lesions (PVLs) were inversely related with MMSE ($r=0,279$ $p<0,001$). Deep white matter lesions (DWMLs) were inversely related with MMSE ($r=0,239$,$p<0,001$), test "10 words" ($r=0,21$,$p=0,025$) and with CDT ($r=0,219$,$p=0,032$).
Conclusion: Early cognitive dysfunctions were associated with WMLs on MRI-scan in patients with DMT2 and reflected the cerebral microangiopathy. Presens of MCD may be a clinical condition of chronic cerebral ischaemia.
Supported by: National research program

1009

Patient reported outcomes in adults with type 2 diabetes using mealtime insulin monomer human (rDNA origin) inhalation powder or metformin+secretagogue or both

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Background and aims: This study investigated whether Mealtime Insulin Monomer Human (rDNA origin) Inhalation Powder alone or in addition to Metformin+Secretagogue was associated with improved patient reported outcomes (PRO) over Metformin+Secretagogue alone.
Materials and methods: In this 24-week randomized, multicenter study adults with type 2 diabetes used Mealtime Insulin Monomer Human (rDNA origin) Inhalation Powder (MIMHIP; n=177), Metformin+Secretagogue (MS; n=162) or both (I+MS; n=169). At 12 weeks subjects not adequately controlled with MIMHIP or MS were transferred to I+MS. PRO included: the SF-36, assessing health-related quality of life (HRQOL), and the Insulin Treatment Questionnaire (assessing diabetes worries and treatment satisfaction). Intent to treat (ITT) analysis used mixed effect models to estimate differences in mean group changes in PRO from baseline to week 12 (adjusted for baseline scores); t-tests assessed within-group change from baseline to 12 weeks in all groups and from 12 to 24 weeks in patients adding MIMHIP to MS.
Results: At Week 12 SF-36 mental health composite scores (MCS) increased (p=.0010) in MIMHIP but not in MS or I+MS; the difference in change between MIMHIP and MS was not significant in ITT analysis (p=.0612), but was in the per protocol population (p=.0233). MIMHIP improved more than MS in Role-Emotional scores (p=.0044). At Week 12 SF-36 physical health composite scores (PCS) increased in MIMHIP and I+MS and declined in MS; although none of these changes were significant, the difference in change between I+MS and MS was significant (p=.0084). I+MS showed greater improvement than MS for Bodily Pain (p=.0148), Physical Functioning (p=.0020), and Role Physical scores (p=.0172); MIMHIP showed greater improvement than MS for Role-Physical scores (p=.0124). There were no changes in diabetes worries from baseline to week 12, and no differences in change. There was an increase in treatment satisfaction in MIMHIP (p=.0003) and I+MS (p<.0001); these improvements were greater than MS (MIMHIP p=.0116; I+MS p=.0009). There was no change in MCS, PCS or diabetes worries between weeks 12 and 24 among subjects who added MIMHIP to MS (n=79), but treatment satisfaction increased (p=.0007).
Conclusion: HRQOL and treatment satisfaction improved more among those taking Mealtime Insulin Monomer Human (rDNA origin) Inhalation Powder (with or without oral medications) than those taking oral medications only. Those who added Mealtime Insulin Monomer Human (rDNA origin) Inhalation Powder to oral medications during the trial experienced increased treatment satisfaction.
Supported by: MannKind Corporation

1010

The importance of parent and friends diabetes social support for adolescents with type 1 diabetes of different age and gender

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Background and aims: This study aimed to determine the instrumentality of diabetes social support in adolescents with type 1 diabetes mellitus.
Materials and methods: 437 adolescents (ages 11 to 19 years) with type 1 diabetes participated in the present study. Multiple linear regression analysis was conducted to investigate moderating effects of emotional problems between diabetes related stress and support from parents and friends. Sample was categorized in four subgroups using a 2x2 design to test the effect of age and gender on importance of support from parents and friends.
Results: Results showed that Emotional Problems moderated the relation between Diabetes Stress and Parent Support ($\beta = 0.125$; $p < .05$), whereas they did not moderate the relation between Diabetes Stress and Friends' Support. The results confirmed our hypothesis that the effect of Parent Support changes direction in the presence of emotional problems which may represent autonomy seeking. To investigate the potentially changing effects of Diabetes Social Support across gender and age, we developed a hierarchy of autonomy seeking based on the assumption of change in importance of support received from parents to friends across age. We assumed that younger girls are most prone to evoke parental support followed by younger boys and older girls who evoke less support from parents but more support from friends as compared to younger girls and finally older boys, who are expected to evoke least support from parents but most support from friends as compared to the other three groups. We also assumed that younger adolescents and girls would get more support from parents and older adolescents and boys get more support from friends. As expected, for younger girls Emotional Problems appeared to moderate the relationship between Parent Support and Diabetes Stress ($\beta = 0.24$, $p < .05$, and $\Delta R^2 = .04$), whereas for older boys Emotional Problems appeared to moderate the relationship between Friends Support and Diabetes Stress ($\beta = 0.27$, $p < .05$, and $\Delta R^2 = .06$). Analysis for specific Parent Support factors showed that Emotional Problems moderated the relationship between Diabetes Stress and two aspects of Parents support: Guidance and Supervision ($\beta = 0.24$, $p < .05$, and $\Delta R^2 = .04$), and Encouragement for Self-care and Exercise ($\beta = 0.25$, $p < .05$, and $\Delta R^2 = .04$) only for younger girls. Analysis for specific Friends Support factors showed a moderation by Emotional Problems of the relation between Diabetes Stress and Friends' Support: Guidance and Encouragement ($\beta = 0.32$, $p < .01$, and $\Delta R^2 = .09$), and Nourishment ($\beta = 0.33$, $p < .01$, and $\Delta R^2 = .10$) only for older boys, whereas for remaining three factors (i.e., Help in Critical Situations, Empathy, and Exercise) emotional problems appeared to have no moderating effects.
Conclusion: Our results are helpful in resolving two critical issues; first they explain, how a positive factor of adjustment (i.e., Diabetes Social Support) may have negative consequences, and second, they enlighten the role of Parent and Friends Support at different ages for girls and boys.

1011

Effects of gender, age, duration of diabetes on dietary self-care in adolescents with type 1 diabetes: a self-determination theory perspective

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Background and aims: Evidence suggests that age, diabetes duration, and gender are important non-modifiable risk factors associated with non-adherence, with older adolescent girls having more difficulties following their dietary plan than adolescent boys. However, little is known about the mechanisms underlying these findings. Based on Self-Determination Theory (SDT), we hypothesized that for adolescent girls perceived support from parents or health care practitioners does not adequately map onto their needs for emotional support. Alternatively, because of social pressures, older girls may feel less competent (self-efficacious) or motivated to follow a well-balanced diet. The aims of the study were (1) to examine whether age, duration of diabetes and gender, and their interactions, were related to dietary self-care in adolescents with type 1 diabetes; and (2) to test a theoretical model in which these non-modifiable factors influence perceptions of support from parents and health care practitioners, and, in turn, predict dietary self-care through motivational resources (i.e., perceived competence and autonomy).
Materials and methods: 289 adolescents with type 1 diabetes (54% male), recruited from the outpatient clinics from two diabetes centres in Quebec City, Canada, completed questionnaires assessing perceptions of self-efficacy (competence), dietary motivation, support fostering autonomy from parents and health care professionals, and dietary self-care. Duration of diabetes and BMI were also assessed.
Results: Structural Equation Modelling analyses revealed that longer diabetes duration and female gender were indicative of poorer dietary care. This effect was mediated by contextual and motivational factors as posited by SDT. Poorer perceived autonomy support from health care practitioners was predominant in girls with greater diabetes duration. Perceptions of autonomy and competence were associated with greater autonomy support and, subsequently, better self-care. The model was well supported by the data, $\chi^2\ (121) = 132.194;\ p=.23;$ NNFI = 1.00; CFI = 1.00; RMSEA = .02 [.00; .03]; χ^2/df ratio = 1.60.
Conclusion: The study shows that young patients who experience greater feelings of autonomy and competence toward dietary self-care, also display better dietary self-care. The study further indicates that, even if supporting actions of health care practitioners do not differ upon patient gender, girls who have diabetes for longer may perceive and evaluate these support actions quite differently. Correspondingly, health care practitioners may want to adapt the way they support adolescent girls with diabetes to attain more autonomy in dealing with their dietary self-care activities.
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1012

A holistic programme of cardiac rehabilitation is able to improve anxiety and depression in diabetic men with coronary heart disease

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Background and aims: Anxiety and depression are very common for cardiac patients and have effects on the development of coronary heart disease (CHD). Moreover in patients with CHD, anxiety and depression influence the outcomes including an increased incidence of ischemic events and higher mortality. The aim of this prospective study was to evaluate the effects of a holistic ambulatory cardiac rehabilitation (CR) program on anxiety and depression in diabetic' men with CHD.
Materials and methods: 275 diabetic' men with CHD (D) and 430 non diabetic' men with CHD (ND) used as control group, with identical age (57.2 +-9 / 54 +-55.5 +-11 years respectively) were included in the study. All patients performed a 6-week multidisciplinary holistic CR program including exercise training, psychological screening and support as well as education concerning lifestyle. Reasons of admission were: coronary heart disease artery bypass graft surgery (45%), angioplasty percutaneous coronary interventions and stent (41%), angor pectoris or myocardial infarction (14%). Level of anxiety and depression were measured by the psychologist using the Hospital Anxiety and Depression (HAD) scale, before and after the cardiac rehabilitation program.
Results: At baseline, prevalence of anxiety [HAD≥ 11] was similar in D and ND (31% vs 32%). The prevalence of depression [HAD≥ 8] was higher in D compared with ND (31% vs 24%, p<0.05)At the end of the program of CR, the entire population studied achieved significant improvements characterised by a statistically significant fall in anxiety/depression (HAD scale). The prevalence of anxiety [HAD≥ 11] was similar in D and ND (11% both). The prevalence of depression [HAD≥ 8] was higher but statistically insignificant in D compared with ND (10 vs 6%, p<0.08).
Conclusion: Our study confirms that anxiety and depression are frequent in men with CHD. The prevalence of depression is higher in diabetics compared to non diabetics. A holistic CR program including psychological screening and support is able to reduce significantly the level of anxiety and depression in both diabetics and non diabetics' men. Our results emphasize the need to a systematic screening for depression particularly in diabetics and the positive impact of the CR which should be relevant to improve prognosis, even if it is needed to be confirm.

1013

Development of the diabetes treatment satisfaction questionnaire (DTSQ) for teenagers and parents: the DTSQ-Teen and the DTSQ-Parent

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Background and aims: To report psychometric development of the DTSQ-Teen and -Parent, designed by Woodcock et al (2007) from the adult DTSQ using qualitative interviews with 14 teenagers with diabetes and 32 parents of children with diabetes. To compare the views of teenagers and their parents.
Materials and methods: The 12-item Diabetes Treatment Satisfaction Questionnaire for Teens (DTSQ-Teen) and the 13-item questionnaire for Parents (DTSQ-Parent) were completed by 38 adolescent offspring (aged 12-17 years) and 91 parents participating in a study by Pacaud et al (who provided baseline data from their study for questionnaire development). Non-parametric tests were used when Kolmogorov-Smirnoff test indicated data not normally distributed. All probabilities are 2-tailed.
Results: All 13 items on the DTSQ-Parent, except *perceived frequency (p. freq) of hyper- and hypoglycaemia*, had loadings from between 0.509 and 0.746 on a forced one-factor solution of a principal components analysis. Cronbach's alpha=0.787. Ten items retained for the new Treatment Satisfaction-Parent subscale (TS-Parent) were: *satisfied, ease, flexibility, day treatment, liked activities, family life, understanding, low discomfort, medical support, continue treatment*; alpha=0.801. With the 12-item DTSQ-Teen (items as in the DTSQ-Parent, except *family life* which teens do not view as affected by diabetes), factor loadings for all items except *p. freq of hypoglycaemia* and *low discomfort* were between 0.497 and 0.870 on a forced one-factor solution. When the *p. freq hyper-* and *hypoglycaemia* and *control* items were removed for comparability with the TS-Parent, alpha improved from 0.769 to 0.832, and to 0.856 when *low discomfort* was removed. A 2-item subscale, reflecting Perceived Diabetes Control (PDC), emerged from unforced factor analyses extracting factors with eigenvalues >1. Loadings were 0.820 and 0.722 (Parent) and 0.809 and 0.864 (Teen) for *p. freq of hyperglycaemia* and the *control* item. Alphas=0.567 (PDC-Parent) and 0.768 (PDC-Teen). Comparison of parent and teen ratings on DTSQ items, the PDC and the TS subscales indicated moderate agreement: Bivariate correlations between parent and teen ratings were significant for 6 of 12 items (p<0.05, r ranging from 0.38 to 0.67), and for each total subscale score (PDC Spearman's r=0.63, p<0.01; TS r=0.36, p<0.05). Differences between parent and teen ratings on the PDC scale were significant: mean % parent=55.3, teen=62.2 (Wilcoxon matched-pairs signed-ranks p<0.025). On individual items, parents over-estimated discomfort compared to their offspring (means on 0-6 low discomfort scale: parent=4.18, teen=4.99 (Wilcoxon p<0.01) and assessed diabetes as less well controlled (means on 0-6 scale: parent=3.93, teen=4.38, Wilcoxon p<0.05).
Conclusion: The DTSQ-Teen and DTSQ-Parent instruments include subscales to measure diabetes treatment satisfaction which have clear structures and excellent internal consistency reliability in addition to content validity previously demonstrated. *P.frequency of hypoglycaemia* is treated as a single item. Analyses confirmed the importance of examining the teens' reports, since these did not always relate closely to the parents' reports, differing significantly from them on PDC scale, and on two individual items. Both the TS and PDC measures are likely to be useful outcome measures in clinical trials of diabetes treatments for children and teenagers.
Funding for psychometric analyses from Health Psychology Research Ltd, Orchard Building, RHUL

1014

Personality traits in association with type 2 diabetes or pre-diabetes in Swedish middle-aged men and women

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Background and aims: Personality traits such as hostility have been recognized as risk factors for CHD. Whether hostility/antagonism or other personality traits may influence the risk of type 2 diabetes is not extensively studied. The aim was to examine the association between personality traits and abnormal glucose regulation (type 2 diabetes or pre-diabetes).
Materials and methods: This cross-sectional study comprised 2152 men and 3143 women aged 43-66 years, in Stockholm Diabetes Prevention Program. All participants had been classified with normal glucose tolerance 8-10 years earlier and about 50% had a family history of diabetes (FHD). None had known type 2 diabetes. OGTT identified 316 and 213 women with type 2 diabetes or pre-diabetes (impaired fasting glucose, IFG; impaired glucose tolerance, IGT; and IFG+IGT). Personality traits were measured by questionnaire with five subscales evaluating facets of the Big Five personality factors, i.e. antagonism (low agreeableness), impulsivity (low conscientiousness), hedonic capacity (high extraversion), negative affectivity (high neuroticism) and alexithymia (low openess). Each subscale comprised four items followed by a four-point Likert response format ranging from 1) "does not apply at all" to 4) "applies completely". The sum of each subscale was divided by the number of items included, and divided into three groups labelled "low" (<1 SD), "middle" (±1 SD) and "high" (>1 SD) according to the gender specific mean distributions. The middle group was considered unexposed to the particular personality sub-trait. OR's and 95% CI's were estimated in logistic regression for associations between personality sub-traits and abnormal glucose regulation, adjusted for age, BMI, FHD, smoking, physical activity, socio-economic position and psychological distress.
Results: For men reporting low values on the antagonism scale the risk of having abnormal glucose regulation was reduced with 70% compared to men that scored in the middle group, age-adjusted OR 0.3 (CI 0.2-0.6), which was not altered when controlled for the other potential confounders. For high antagonism in men there was no association. In women, neither high nor low values on the antagonism scale were associated to pre-diabetes or type 2 diabetes. For the hedonic capacity scale, men and women with high scores showed a 50% and 40% decreased risk of having abnormal glucose regulation, age-adjusted OR's 0.5 (0.3-0.9) and 0.6 (0.4-1.0) respectively, which was not

changed when controlled for the other potential confounders. For the group reporting low hedonic capacity there were no associations, although in women an increased risk was observed, age-adjusted OR 1.7 (1.1-2.6) that was no longer significant when controlled for the other potential confounders, OR 1.4 (0.9-2.4). The results for the alexithymia, impulsivity and negative affectivity scales illustrated no significant associations in either men or women.
Conclusion: In this study, a reduced risk of abnormal glucose regulation was observed in men with low antagonism, and in men and women with high hedonic capacity. For impulsivity, negative affectivity and alexithymia there were no significant associations in either men or women.
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PS 89 Diabetes in the hospital

1015

Stress hyperglycaemia in a coronary intensive care unit
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Background and aims: Newly diagnosed hyperglycemia (NDH) and stress hyperglycemia (SH) among hospitalized patients during acute illness is reported as a non-physiological condition. Affected cases seem to have higher mortality rates and worse functional outcomes than the ones with known diabetes or normal glucose. The aim of this study is to determine the rate of NDH and SH among cases admitted to coronary ICU with coronary disease and to inquire the relationship of SH with disease severity and functional outcomes such as longevity of ICU stay.
Materials and methods: Patients with acute coronary syndrome (ACS) and myocardial infarction (AMI) admitted to our emergency room were recruited consecutively. Admission plasma glucose (APG) and fasting plasma glucose (FPG); the first morning after admission, measurements were obtained and each participant was subjected to capillary glucose measurement (CGM) every six hours within the first day. Patients were separated into 4 groups: GROUP 1: Normoglycemic cases: No known diabetes, APG < 200 mg/dl and FPG < 126mg/dl and all of the CGM < 200 mg/dl. GROUP 2: Newly diagnosed hyperglycemic cases: No known diabetes, APG > 200mg/dl and/or FPG > 126mg/dl and/or any of the CGM > 200 mg/dl. Group 2a: Unrecognized diabetes, hbA1c: >6.0% Group 2b: Stress hyperglycemia <6.0%. GROUP 3: Known diabetes. Age, gender, co-morbidities on admission, adverse outcomes in hospital, duration of stay in ICU, deaths, drugs, and glucose levels were all recorded. Throughout the hospital stay of Group 2 and Group 3, CGMs were performed and treated when necessary (target glucose<180mg/dl). Acute Physiology and Chronic Health Evaluation II (APACHE-II); a severity of disease classification system was used for each case. Duration of ICU stay was categorized into two groups; ≤ 3 days and > 3 days. A logistic regression analyses was performed using ICU stay as dependent variable and age, groups, co-morbidity, additional problem in ICU, APACHE-II score and CGM were used as independent risk factors.
Results: A total of 494 patients; 321 (65%) men, 173 (35%) women with 372 (75.3%) ACS and 122 (24.7%) AMI was included. There were 255 (51.6%) cases in Group 1, 82 (16.6%) in Group 2; 37 (7.5%) cases in Group 2a, 45 in Group 2b (9.1%) and 157 (31.8%) patients in Group 3. Group 2 had more cases with MI (n=35, 42.7%) (p=0.0001). On admission Group 3 had more patients with atherosclerotic co-morbidity (n=138, 87.9%) (p=0.001). The mean APACHE-II score were 11.9±2.9 in Group 1, 12.5±2.8 in Group 2a, 14.1±4.2 in Group 2b and 13.1±2.9 in Group 3 (p=0.0001). The mean duration of ICU stay of the groups were 2.27 days (median:2, min:1-max: 9), 2.95 days (median:3, min:1-max:6,), 4.56 days (median:3, min:1-max:25), and 3.03 days (median:3.0, min:1-max:16), respectively (p=0.0001). Group 2b also exhibited longer ICU stay than Group 1 (p=0.000) and Group 3 (p=0.03). Two (4.4%) cases in Group 2b and 8 (5.1%) in Group 3 died in hospital (p=0.608). In multiple logistic regression analysis, SH was shown to be an independent risk factor for the duration of ICU stay (OR:2.8, 95% CI:1.3-6.2).
Conclusion: In the present study, in accordance with literature, 16.6% of the cases had the diagnosis of NDH and 9.1% were found to be SH. The poor clinical pictures and functional outcomes of the SH cases pointed that these patients had to be considered more carefully.

1016

In hospitalized patients with diabetes mellitus screening for hereditary hemochromatosis by using transferrin saturation is cost effective
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Background and aims: Homozygosity for C282Y mutation in hemochromatosis gene (*HFE*) is the major contributor to hereditary hemochromatosis (HH). In caucasians, the prevalence of this homozygote mutation is 0.1 to 0.5 %. Due to incomplete penetrance, population-based screening in healthy subjects is not appropriate. However, in patients with diabetes the risk for HH is 5-fold higher. Therefore, screening in hospitalized patients with diabetes

may be relevant. To address this topic, in consecutive patients with all types of diabetes admitted to our institution, we determined the iron indices and assessed cost effectiveness of laboratory screening.
Materials and methods: In a prospective manner, for three consecutive months, phenotypic screening in 527 hospitalized diabetic patients was performed by obtaining the iron indices. Genotyping of *HFE* gene (C282Y mutation) was performed in patients with a transferrin saturation > 45 %. We assessed the diagnostic efficiency and the cost effectiveness of two strategies. The estimated total costs were based on costs for laboratory tests (reagents and manpower) for iron (2.23 €), transferrin (2.76 €) and ferritin (11.62 €) in serum and for genotyping of the *HFE* gene (180,70 €). (Strategy A) Preselection by elevated ferritin (> 400 ng/ml), followed by obtaining transferrin saturation (iron in serum [µg/dl] / transferrin [mg/dl] x 70.9), and genotyping if transferrin saturation was elevated (> 45 %); (Strategy B) Determination of transferrin saturation in all patients, followed by genotyping if transferrin saturation was elevated.
Results: With strategy A, we identified 94 patients with elevated ferritin, 9 of them had elevated transferrin saturation, and two of the latter were homozygous for the C282Y mutation. Costs were 15.60 € per patient, and 4,109.55 € per identified case with HH. The positive predictive value for an elevated ferritin was 2.1 %. With strategy B, we identified 27 patients with elevated transferrin saturation, two of them were homozygous for C282Y mutation. The costs were 14.25 € per patient, and 3,754.32 € per identified case with HH. Positive predictive value for an elevated transferrin saturation was 7.4 %.
Conclusion: Due to relevant clinical consequences of non-treatment of HH and the costs for screening as determined by our analysis, we suggest that phenotypic (laboratory) screening is appropriate in patients with diabetes mellitus. Primary screening by using an elevated transferrin saturation is more efficient and cost effective.

1017

Initiation of glucose lowering therapy among hyperglycaemic patients without prior diabetes hospitalized for acute myocardial infarction

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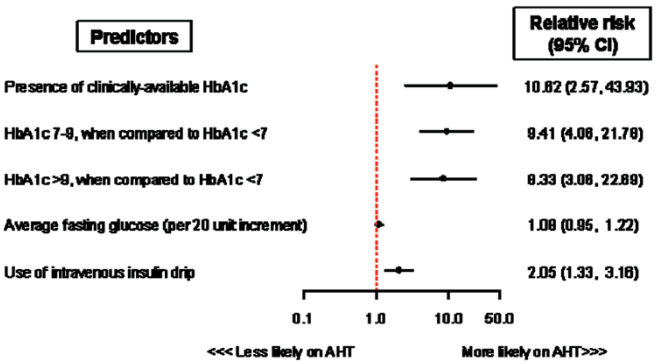
Background and aims: Hyperglycemia is common in acute myocardial infarction (AMI) and predicts adverse outcomes, particularly in patients (pts) without known diabetes (DM). AMI hospitalization therefore represents an opportunity for DM recognition and initiation of antihyperglycemic therapy (AHT). However, the incidence, predictors, and the relationship between AHT and post-discharge glycemic control are unknown.
Materials and methods: TRIUMPH is a prospective registry of AMI pts from 26 US centers; we analyzed AHT initiation among hyperglycemic pts (initial glucose ≥140 mg/dL) without known DM. AHT was defined as initiation of a new oral antihyperglycemic agent, exenatide, or insulin at discharge. Baseline HbA_{1c} was measured during hospitalization; follow up HbA_{1c} was obtained at 6 months in pts who consented. Independent predictors of AHT initiation were identified using hierarchical Poisson regression. Among pts with baseline and 6-month HbA_{1c}, the association between AHT and post-discharge glucose control was evaluated, adjusting for baseline HbA_{1c} and hospital site.
Results: Between 2005-08, TRIUMPH enrolled 3469 pts of whom 601 (17%) had hyperglycemia and no known DM. HbA_{1c} was assessed in 332 (55%) of these pts; the level was <7% in 271 (82%), 7-9% in 34 (10%), and >9% in 27 (8%) pts. Overall, 56 hyperglycemic pts (9.3%) were initiated on AHT; a greater proportion of these pts had HbA_{1c} assessment (95% vs 51%, p<0.001). Pts with AHT also had higher initial glucose (248 vs 174 mg/dL, p<0.001), fasting glucose (161 vs 114 mg/dL, p<0.001), HbA_{1c} (8.8% vs 6.4%, p<0.001), and higher rate of intensive in-hospital insulin (23% vs 2%, p<0.001). Independent predictors of AHT initiation are listed in the Figure. In 107 pts with complete baseline and 6-month HbA_{1c}, AHT was associated with significant improvement in glucose control at 6 months (crude ΔHbA_{1c} -2.5 ± 2.1% vs 0.0 ± 0.5%, p<0.001; adjusted 6-month HbA_{1c} 0.5% lower in pts with vs without AHT, p=0.04).
Conclusion: Both HbA_{1c} assessment and higher HbA_{1c} levels are key predictors of in-hospital AHT initiation after AMI in hyperglycemic patients without known diabetes. When HbA_{1c} assessment is performed, about one in five hyperglycemic patients without known diabetes have evidence of suboptimal or poor long-term glucose control. Initiation of AHT in these patients, while uncommon, is associated with significant improvement in post-discharge glucose control.



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1018

Prediction of glucose concentrations following cardio-thoracic surgery using continuous glucose monitoring

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Background and aims: The maintenance of near normoglycaemia is of fundamental importance for critically ill patients. Continuous glucose concentration measurements could facilitate this by enabling glucose excursions to be detected at a stage early enough to allow adverse events to be avoided through corrective action. The aim of the present investigation was to examine the predictive power of continuous glucose data using simple linear extrapolation of the trend signal in critically ill patients.
Materials and methods: 20 patients (69±7 years, BMI 28.2±4.9 kg/m, APACHE II score 10.1±3.2) with a blood glucose level >120 mg/dl (6.7 mmol/l) upon admission to the ICU were included in the trial. Glucose was continuously monitored using microdialysis coupled with a glucose sensor. In parallel, throughout the study period (up to 48 hours) whole blood samples were collected at hourly intervals via an arterial line. The prediction horizon was determined by extrapolation of the actual linear trend and assessed in two ways: 1) Analytical assessment: the degree of divergence between the predicted glycaemia and corresponding reference values was calculated using the Root mean squared error (RMSE). 2) Clinical assessment: the predictive power of continuous sensor glucose readings with respect to the reference glucose values was clinically evaluated by Insulin Titration Error Grid (ITEG) analysis.
Results: A total of 538 hours of continuous glucose measurement data were analysed. These correlated very well with the conventional blood glucose reference measurements (mean of residuals: 5.4 mg/dl; relative system error: 4.03%; mean absolute relative difference (MARD): 1.27 %). This correlation was mirrored by a high degree of clinical reliability: when analysed using the ITEG, 98.66% of the data were located in the acceptable treatment zone. A first estimate of a reasonable prediction horizon (PH) - 17.6 ± 4 min - was derived as the mean time interval between successive peak maxima and trough minima. A prediction horizon of 37 min was associated with an increase in the root mean squared error of 10 mg/dl. In clinical terms, a strong increase in the number of data points in the unacceptable violation zone was observed when the prediction horizon exceeded approximately 20 min.
Conclusion: Our data provide evidence that simple linear extrapolation of glucose trend information obtained by continuous glucose monitoring can be used to predict the course of glycaemia in critically ill patients for up to 20 to 30 minutes. This "glimpse into the future" can be used to proactively prevent the occurrence of adverse events.
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1019

Risk factors for hypoglycaemia in patients with diabetes during continuous intravenous insulin infusion in hospital setting

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Background and aims: Continuous intravenous insulin infusion (CIVII) is often used in hospital setting in the treatment of poorly controlled diabetes. Hypoglycemia, especially with rapid onset and of severe intensity, is the most common adverse event of this mode of diabetes treatment. The aim of this prospective study was to identify risk factors of hypoglycemia during CIVII.
Materials and methods: The study group was 200 patients with type 1 and type 2 diabetes treated with CIVII at the university hospital department (mean age 55±16 years, BMI 29.3±6.4 kg/m2, diabetes duration 10±9 years, HbA1c 10.1±2.5%). CIVII consisted of basal insulin infusion and three 90-min insulin boluses per day administered at the meal time. The boluses consisted of three time periods (20+20+50 min),each with different rate of insulin infusion, which was adjusted individually to the meal absorption and postprandial glucose. In normal conditions capillary blood glucose was measured every 90-120 min throughout the day and night. Hypoglycemia was diagnosed if capillary blood glucose was <60 mg/dl or when CIVII was stopped due to the symptoms of hypoglycemia.
Results: Mean duration of CIVII was 3.8±1.5 days. 135 episodes of hypoglycemia were noted in 48 (24%) patients. Duration of diabetes in the patients with hypoglycemia was significantly longer than in the patients in whom hypoglycemia did not develop (12.4±10.8 vs 9.2±8.2 years; p<0.05), the former had also lower body weight (74.2±15.6 vs 83.9±19.8 kg; p<0.01; BMI 27.0±5.9 vs 30.0±6.4 kg/m2; p<0.01) than patients without hypoglycemia. The strongest risk factor for hypoglycemia was having type 1 diabetes (hypoglycemia occurred in 48% patients with type 1 and 20% with type 2 diabetes), particularly of long duration (14.3±12.4 vs 6.8±7.6 years in type 1 diabetes without hypoglycemia; p<0.01). Degree of metabolic control, presence of late diabetes complications, kidney, gastrointestinal tract, neurological or cardiovascular diseases or complications as well as insulin dose per kilogram of body weight did not contribute to the risk of hypoglycemia.
Conclusion: Patients with long-standing type 1 diabetes are at particularly high risk of hypoglycemia occurrence during CIVII, regardless of present complications or significant co-morbidities. CIVII should be used in this group of patients with utmost caution.

1020

Glucose variability predicts mortality in the ICU

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Background and aims: Mounting evidence suggests a role for glucose variability in predicting ICU mortality. We investigated the predictive value of glucose variability for ICU death across several ranges of mean glucose.
Materials and methods: Two measures of variability, Mean Absolute Glucose change per hour (MAG) and standard deviation (SD), were calculated as measures of glucose variability from patients admitted to an 18-bed medical/surgical ICU from 2004 to 2007. Readmissions, patients with a withholding care policy and patients with only one glucose value measured during admittance were excluded. Patients were treated with a computerized insulin algorithm (target glucose range 4-7 mmol/l).
Results: Mean age was 65 ± 13 years, 34% was female and 6.3% of patients died in the ICU. MAG was a stronger predictor for ICU death than SD; crude Odds Ratio (OR) (highest vs. reference quartile) for ICU death 7.7 (95% CI 5.2-11.6) and 5.8 (4.0-8.4), respectively. In multivariate logistic regression, OR's for death in the ICU were calculated for quartiles of mean glucose, with quartiles of glucose variability as covariate, correcting for age, sex, diabetes mellitus and mean sequential organ failure scale (SOFA) during admittance. The highest OR for ICU death was found in patients with the highest MAG in the upper glucose quartile: OR 15.1 (95% CI 4.8-47.2, p<0.001). The OR's for ICU death were equally low in the lowest MAG quartile, independently of the mean glucose.
Conclusion: High glucose variability is a strong predictor of ICU death, even more in combination with increased mean glucose levels. Low glucose variability seems protective, even when mean glucose levels remain elevated despite glucose lowering therapy.

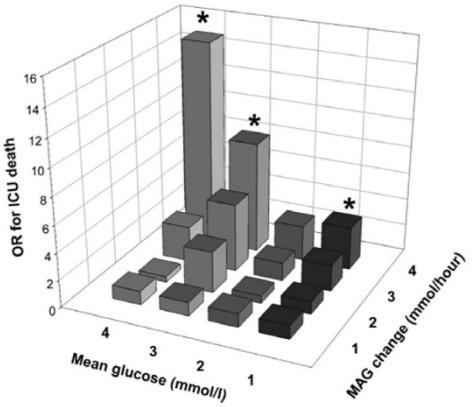

Figure 2: OR for ICU death, adjusted for age, sex, DM and mean SOFA score, *P<0.05 as compared to the lowest MAG quartile.

Quartile	Range mean glucose (mmol/l)	Range MAG (mmol/hour)
1	<6.93	<0.39
2	6.93-7.60	0.39-0.60
3	7.61-8.91	0.61-0.87
4	>8.91	>0.87

1021

Screening of cardiometabolic risk factors in patients with severe enduring mental illness: results and potential

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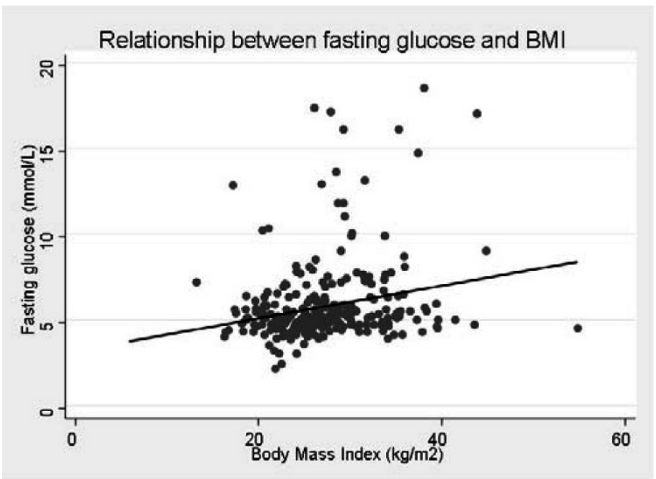
Background and aims: People with severe enduring mental illness (SMI) are reported to be twice as likely to die from cardiovascular disease (CVD) than those in the general population with the excess mortality even higher in younger individuals. In a meta-analysis of 18 international studies, 60% of the excess mortality of schizophrenia was attributable to physical illness, with CVD being the major contributor.
Materials and methods: We report the implementation of a primary care programme of screening and intervention for cardiometabolic risk factors in one region of the UK. This involves incentivation of General Practioners by the UK Department of Health through the Quality and Outcomes Framework (QOF). Data search was performed using a database generated through the EMIS software provider.
Results: 453 patients (55.8% male 44.2% female) on the SMI Register in Cheshire, UK were screened for dysglycaemia (screening rate 57.3 %) and dyslipidaemia (screening rate 36.2%). There were no differences in BMI by gender, but a greater proportion of women (25% vs 20%) were obese (BMI ≥ 30 kg/m2). Fasting glucose was in the impaired fasting glycaemia range (6.1-6.9mM) in 6.5% of those screened and at or above the threshold for type



2 diabetes (7.0mM) in 17.3% of the group. Fasting serum cholesterol was high at >5mmol/L in 62.8% of those screened for whom the mean cholesterol was 6.2±0.8 mmol/L). Despite high rates of dysglycaemia and dyslipidaemia, systolic blood pressure was high at >140mmHg in only 13% of those examined. Multivariate linear regression analyses revealed a direct relationship between fasting glucose levels and BMI (Spearman's rho = 0.22, p<0.001) independent of age, sex, systolic blood pressure and fasting cholesterol and triglycerides.
Conclusion: Screening in SMI patients for cardiometabolic risk in a Primary Care setting is feasible. However screening rates need to be improved. The relatively low prevalence of hypertension is likely a consequence of the alpha 1 receptor blocking properties of many neuroleptics. There is scope for cardiometabolic risk reduction with targeted intervention in this group, with potentially significant associated reduction in CVD mortality. Any programme must include active lifestyle measures to encourage weight reduction in addition to pharmacotherapy.

PS 90 Diagnostic tools

1022

Urinary myoinositol index: a new and better marker for postprandial hyperglycaemia

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Background and aims: A growing body of evidence suggests that reducing postmeal plasma glucose (PG) excursions as well as achieving HbA_{1C} goals is important for the prevention of the cardiovascular disease. Therefore, we examined the relationship between postprandial hyperglycemia and HbA_{1C}, plasma 1,5-anhydroglucitol (1,5-AG) or urinary mioinositol index (UMI) in patients with type 2 diabetes mellitus (T2DM).
Materials and methods: Fifty-eight T2DM patients (18 males and 40 females) were enrolled in this study. The patients with renal or hepatic disease, using insulin or α-glucosidase inhibitors, and with HbA_{1C} above 8.0% were excluded. Test-meal tolerance test was done after informed consent was obtained. Test-meal consists of carbohydrates 51.4% (57.6g), protein 15.3% and lipid 33.3% with total energy of 460kcal. Plasma 1,5-AG was measured by a fully enzymatic method, and UMI by an improved enzymatic cycling method.
Results: Their age, BMI and waist circumference were 67.6 ± 7.9 year-old, 24.9 ± 3.8 kg/m^2, and 90.2 ± 10.4 cm, respectively. Fasting, 1- and 2-hour postmeal PG were 130 ± 23 (7.2 ± 1.3), 178 ± 46 (9.9 ± 2.6) and 149 ± 49 mg/dl (8.3 ± 2.6 mmol/l), respectively. HbA_{1C}, 1,5-AG and 2-hour UMI (three PG markers) were 6.3 ± 0.6 %, 11.9 ± 5.7 μg/ml and 52.0 ± 35.9 mg/gCr, respectively. The correlation coefficient (*r*) between 1-h PG and three PG markers were 0.558, 0.256, 0.496, and that between 2-h PG and the three PG markers were 0.605, 0.306, 0.606, respectively.
Conclusion: Myoinositol is one form of inositol; inositol is a circular D-glucose isomer. Myoinositol concentrations in blood or in urine are reported to be higher in patients with diabetes or chronic renal failure than in healthy individuals. It's concentration in biological samples has been measured by various methods, but it has been difficult to measure accurately. We used the above-mentioned, easy-to-use and highly sensitive method. We calculated a urinary mioinositol/creatinine ratio as UMI at 2-h after meal load. When UMI was measured hourly from fasting until 3 hours at the time of 75g-OGTT for patients with normal glucose tolerance (GT), impaired GT and diabetes, its peak was with 2-h urine in any case though UMI admitted rising according to the level of the GT. The advantages of UMI are to collect samples noninvasively, and to respond rapidly to changes in PG. In this study, 1-h PG was higher than 2-h, but these three PG markers were better related to 2-h PG than to 1-h. Then, the *r* between 2-h PG and UMI was higher than that between 2-h PG and 1,5-AG or HbA_{1C}. Recently, HbA_{1C}, glycoalbumin, 1,5-AG and PG are the mainstream as markers for glycemic control. In these markers, 1,5-AG has been proposed as a marker for postmeal hyperglycemia. However, in this study the *r* between postmeal PG and 1,5-AG was lower than that between postmeal PG and 2-h UMI. Although HbA_{1C} is also a good marker for 1- or 2-h PG, it neither accurately reflects transient elevations of glucose within a few days, nor responds quickly to changes in PG. In conclusion, UMI seems to be a better marker for monitoring postprandial hyperglycemia in comparison with plasma 1,5-AG or HbA_{1C}.

1023

Assessing postprandial hyperglycaemia and glucose variability. The ADAG study

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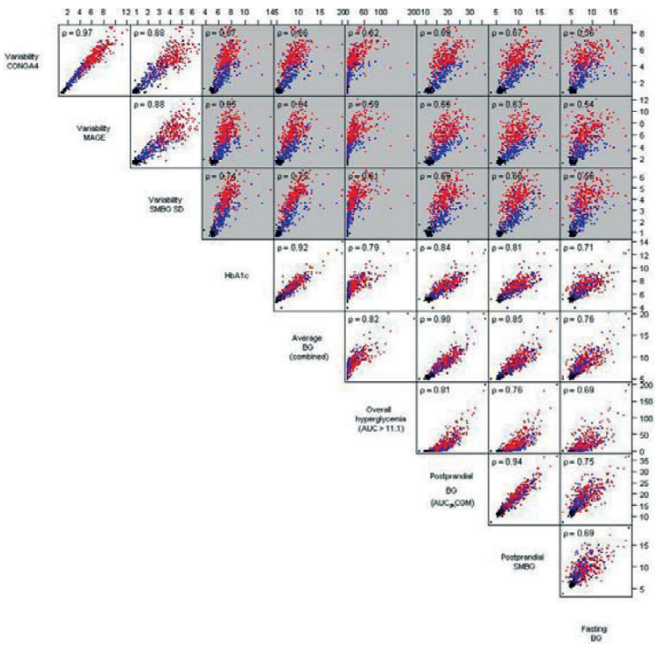
Background: Glucose variability and postprandial hyperglycaemia have been implicated in risk of cardiovascular disease in patients with diabetes. However, little information exists on the relationship between the different indices of glycaemia.
Materials and methods: 507 participants (268 with type 1 diabetes, 159 with type 2 diabetes, 80 without diabetes) from the ADAG (A1c Derived Average

Glucose) study underwent intensive glucose monitoring during 16 weeks. For each participant, approximately 2,700 glucose values were obtained using continuous glucose monitoring (CGM) and seven-point self-monitored capillary blood glucose (SMBG) on different days. HbA1c was measured. We derived common indices of postprandial glycaemia, over-all hyperglycaemia and glucose variability and studied their association. A combined CGM/SMBG average blood glucose (BG) was calculated. Intraday glucose variability indices were calculated from CGM: the Mean Amplitude of Glycaemic Excursions (MAGE) and the Continuous Overlapping Net Glycaemic Action (CONGA). The standard deviation (SD) was calculated from CGM and SMBG data

Results: CONGA calculated with 1, 2, 4, and 6 hour windows showed strong intercorrelation. The $CONGA_4$ and CGM SD are both strongly related to MAGE with correlation coefficients of 0.97. The SMBG variability index (SD) relates with the CGM indices MAGE, CGM SD, and the $CONGA_4$ with correlation coefficients of 0.88, 0.90, and 0.88, respectively. Selected correlations are shown in the figure. For postprandial indices, the area under the glucose curve (AUC) calculated from CGM 2 hours after a meal is related to the SMBG postprandial measurements with a correlation coefficient of 0.94. FBG only correlates moderately (correlation coefficient between 0.69 - 0.76) with indices of hyperglycaemia, average or postprandial BG levels.

Conclusion: As expected, the different indices of glycaemic variability, average and post-prandial glycaemia correlate within each category and moderately between categories. In particular, the variability indices do not correlate strongly with indices of fasting, postprandial or elevated BG. This suggests that the variability indices convey different information from the other indices of hyperglycaemia, average or postprandial BG levels (shaded area of figure). FBG only has a moderate correlation with other indices of glycaemia, indicating that it is not a clear indicator of general glycaemia. Within the categories, SMBG derived indices are strongly correlated with those derived from CGM, indicating that much information regarding postprandial BG levels and glucose variability is captured by 7-point profile SMBG.



Pairwise scatter diagrams illustrating selected Correlations of Glycaemic indices with Pearson correlation coefficients (ρ) for each pair.

Supported by: EFSD, ADA, Abbott Diabetes Care, Merck & Company, Bayer Healthcare, Lifescan, GlaxoSmithKline, Medtronic, sanofi-aventis, Hemocue

1024

Analytical and clinical evaluation of Lucica GA-L plasma glycated albumin assay in subjects with and without diabetes

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Background and aims: The measurement of glycated proteins, including HbA1c and plasma glycated albumin (PGA), is a key indicator of glycaemic status in diabetic patients. PGA specifically provides a useful measure of short-medium glycaemic control and may also provide alternative information on the risk of the long-term hyperglycaemic-related complications of diabetes.The objective of this study was to undertake both an analytical and clinical evaluation of a new robust enzymatic assay for PGA in a healthy non-diabetic cohort (controls) and in a cohort with diabetes.

Materials and methods: PGA was measured using a Lucica GA-L Glycated Albumin assay (Asahi Kasei Pharma Corporation) on a Roche Modular-P platform. Control samples were obtained from our Irish Construction Worker cohort and diabetes samples from our Case Control cohort. The baseline characteristics of the two cohorts are outlined in Table 1.Data are expressed as Mean (SEM) or *Median (IQR)

Results: The analytical performance of the assay was very satisfactory with (1) intra-assay precision between 0.9-1.2% and (2) interassay precision between 0.8-1.8%, across a range of concentrations. The reference range for PGA, established using healthy non-diabetic Irish control subjects (n=87), was 8.9% - 14.6%. PGA levels were significantly elevated in diabetic subjects [median (IQR) 17.0% (14.1% - 19.8%)] compared with non-diabetic control subjects [median (IQR) 11.0% (10% - 12%)] (P<0.005). In addition, a significant correlation was found between PGA and fasting plasma glucose in the whole cohort (r=0.58, p<0.05), while PGA was significantly correlated with HbA1c (r=.70, p=<0.005) in diabetics only.

Conclusion: The Lucica GA-L enzymatic assay is a robust and reproducible analytical method for routine measurement of PGA. Furthermore in a cross sectional evaluation it clearly differentiated diabetic and non diabetic subjects, indicating its clinical value as a marker of glycaemic status.

Table 1

	Control (n=184)	Diabetes (n=158)	p value
Age (Years)	40.0 (0.1)	61.3 (1.06)	<0.005
Male/Female	98/86	101/57	---
HbA1c (%)	5.4 (0.05)	7.6 (0.14)	<0.005
*Glycated Albumin (%)	11.0 (10.0-12.0)	17.0 (14.1-19.8)	<0.005

1025

The validity of the CPR index (CPI) in selecting treatment in a patient with type 2 diabetes mellitus

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Background: Evaluation of residual pancreatic beta-cell function is important in choosing appropriate treatment for diabetic patients, especially deciding whether insulin treatment is required for controlling type2 diabetes. The homeostasis model assessment-β(HOMA-β) and urinary C- peptide collected during 24hours (U-CPR), have been so far widely validated clinical and epidemiological tools for estimating beta-cell function. Recently, we proposed that CPR index (CPI), which is derived from following equation [=fasting serum C-peptide (F-CPR)/ fasting plasma glucose (FPG) x 100], was a useful marker of insulin secretion. In this study, we examined whether CPI is correlated with HOMA-β, F-CPR in a general population. Then, we further examined the validity of the CPI by comparing these indices in deciding which treatment is suitable for patients with type 2 diabetes mellitus.

Group1; Subjects were 658 adult subjects (average age±SD 64.9±10.1 years, range: 31-88 years old: 290 men, 368 women) who had received medical examination in 2008 at Fuchu town, Toyama Prefecture, central Japan. Group2; Subjects were 160 (average age±SD 62.9±12.1 years, range: 28-86 years old:114 men, 56 women) inpatients with type 2 diabetes mellitus admitted to the University of Toyama hospital for controlling glycemic control. In group1: we analyzed relationship between HOMA-βand F-CPR or CPI by simple cor-

relations. In group2: we calculated the CPI in 160 inpatients measuring FPG and F-CPR on the second or third hospital day. They were divided into three groups according to the therapy they received at 3 months after discharge: insulin treatment; n=86 (Insulin group), oral medication; n=50 (O group), and diet therapy; n=24 (D group). We determined CPI cutoff levels between Insulin group and O+D group by histogram and compared the power of CPI, F-CPR, U-CPR and HOMA-β to predict requirement of insulin therapy by receiver operating characteristic (ROC) analysis.
Results: In group1, both F-CPR and CPI were significantly and positively correlated with HOMA-β. However, the correlation coefficient with HOMA-β was significantly greater for CPI (r=0.76) than for F-CPR (r=0.59) (p<0.05). CPI, F-CPR, U-CPR and HOMA-β in Insulin group were significantly lower than those of O+D group: CPI(I: 1.05±0.59 vs O+D: 1.91±0.99, p<0.01),HOMA-β(I: 20.89±21.06 vs O+D: 53.67±35.22, p<0.05),F-CPR(I: 1.84±0.93 vs O+D: 2.37±0.87 , p<0.01),U-CPR(I: 42.24±30.71vs O+D: 57.87±30.72, p<0.01). ROC analysis has shown that the area under the curve (AUC) of CPI was significantly larger than that of F-CPR and U-CPR (p<0.05). The AUC of CPI and HOMA-β were comparable. Histogram of CPI and HOMA-β has shown that insulin treatment is required to control diabetes in patients with the CPI≤1.00 or HOMA-β≤13.69, but not in those with CPI≥1.86 or HOMA-β≥44.55.
Conclusion: CPI and HOMA-β were significantly correlated with each other. ROC analysis revealed that those two indices are useful for a clinical decision of whether insulin was required for controlling Japanese type 2 diabetic patients. When we decide an appropriate therapy in patients with type2 diabetes mellitus, the CPI may be useful because it is an index calculated simply.

1026

Evaluation of exocrine pancreatic function in patients with type 1 and type 2 diabetes

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Background and aims: Although the impairment of pancreatic exocrine function in patients (pts) with non-pancreatic diabetes (DM) is documented, its incidence and consequences are not clearly known. The aim of our study was to assess the exocrine pancreatic function in pts with type 1 or 2 DM.
Materials and methods: The stool elastase activity of 146 pts with non-pancreatic DM was measured. Cardiovascular reflex tests (CRT), peripheral sensory function (applying Neurometer; Neurotron Inc. Baltimore, MD), retinopathy, HbA1c, BMI, microalbumin excretion (MAU) were measured and the quality of life (QoL) was assessed by an EORTC QLQ-C30 questionnaire.
Results: 28% of all pts had an affected exocrine pancreatic function. Two groups (n=21 and n=10) were selected from pts based on decreased or normal elastase (mean elastase of pts: 141±11 vs 417±37 μg/g, p<0.00001). The duration, age, BMI, HbA1c, MAU and retinopathy frequency did not differ significantly between the groups: (duration: 16.2±2.7 vs 16.3±3.6 years, p=0.98; age: 58.7±2.6 vs 60.3±4.3 years, p=0.74; BMI: 27.4±1.98 vs 30.5±1.27, kg/m^2 p=0.08; HbA1c: 8.25±0.3 vs 8.98±0.2 % p=0.17; MAU: 20 vs 16 % p=0.98, retinopathy: 50 vs 48 %; p=0.93 decreased vs normal elastase group). A CRT (30/15 ratio) was lower in pts with impaired elastase (0.98±0.01 vs 1.03±0.02, p=0.04), but differences in the overall autonomic neuropathy (AN) scores were not significant (AN score: 4.35±0.75 vs 3.9±0.38, p=0.50). The threshold of perception of both limbs showed a non-significant tendency to increase in pts with low elastase (n. medianus 2000 Hz: 3.07±0.6 vs 2.49±0.55 mA, p=0.64, 250 Hz: 2.26±0.6 vs 0.96±0.2 mA p=0.16, 5 Hz: 1.68±0.63 vs 0.66±0.2 mA p=0.42, n peroneus: 2000 Hz: 5.10±0.76 vs 3.61±0.87 mA p=0.24, 250 Hz: 3.91±0.81 vs 2.1±0.78 mA p=0.26, 5 Hz: 2.82±0.8 vs 1.85±0.75 mA p=0.42). Qol scores were not significantly different between the groups.
Conclusion: Decreased stool pancreatic elastase activity can be found in almost one third of diabetic patients, and it might be related to a neuronal dysfunction without affecting further complications. Prospective trials with a higher number of patients and the investigation of the effect of enzyme substitution are needed to clarify the clinical role of exocrine pancreas function in non-pancreatic diabetes

1027

A new strategy for the sensitive and simultaneous analysis of human insulin and insulin analogues using LC/MS^2 in combination with a tailored sample clean-up procedure

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Background and aims: In recent years, a number of insulin analogues with altered pharmacokinetic (PK) properties have been designed to improve glucose control in diabetes. Currently, the measurement of insulin in body fluids for the purpose of PK profiling is still performed by immunoassays. These methods however suffer from certain limitations, including cross-reactivity with structurally related peptides, a lack of assays specific for individual insulin analogues and the inability to assay multiple insulins simultaneously. An LC/MS approach able to simultaneously quantify multiple insulins in one sample without the risk of cross reactivity would clearly represent a useful alternative to immunoassays.
Materials and methods: B chains were obtained from human insulin, insulin aspart, insulin glulisine, insulin lispro, insulin glargine and porcine insulin by reduction using TCEP. LC-MS^2 analysis was performed using a LC-Packings Ultimate 3000 HPLC system in the capillary mode. Insulin B chains were eluted with shallow water: acetonitrile gradients with 0.1% formic acid at a temperature of 50°C (column: ACE 4, 150 x 0.3 mm, 3 microm particle size) and detected with a TSQ Quantum Ultra AM mass spectrometer using positive ESI in SRM mode.
Results: The new LC/MS^2 method allowed simultaneous analysis of human insulin and several insulin analogues (insulin lispro, insulin aspart, insulin glulisine, insulin glargine, bovine or porcine insulin) without cross reactivity. A sophisticated sample clean-up procedure involving protein precipitation (PP), solid phase extraction (SPE) and ultrafiltration (UF) enabled the efficient removal of interfering proteins from serum samples and thereby compatibility with LC/MS analysis. The procedure showed excellent sensitivity: 1 fmol (= 1x10^{-15} mol) on column - corresponding to insulin levels of around 4 pM in 250 µl serum - could be detected with signal/noise ratios of >1000 in serum samples.
Conclusion: A sensitive LC/MS^2 method in combination with a tailored sample clean-up procedure enables the sensitive, simultaneous specific detection of human insulin and insulin analogues in serum samples down to physiological basal levels (~ 36 pM) of human insulin in serum.
Supported by: the Austrian Federal Ministry of Transport, Innovation and Technology (BMVIT)

PS 91 Health care delivery

1028

Diabetes care protocol: effects on patient-important outcomes. A cluster randomised non-inferiority trial in primary care

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Background and aims: The Diabetes Care Protocol (DCP) combines task delegation, intensification of diabetes treatment and feedback. It reduces cardiovascular risk in type 2 diabetes (DM2) patients. This study evaluates the effects of DCP on perceived health status, treatment satisfaction and psychosocial self-efficacy.
Materials and methods: A Cluster randomized, non-inferiority trial, by self-administered questionnaires in fifty-five primary care practices across the Netherlands: 29 practices DCP, 26 usual care. DM2 patients treated by their primary care physician were included. Main outcome was the one-year between group difference in Diabetes Health Profile (DHP-18) total score. Secondary outcomes: DHP-18 subscales, general HRQoL (SF-36, Euroqol 5D/ EQ-VAS), treatment satisfaction (DTSQ-status) and psychosocial self-efficacy (DES-SF). Per protocol (PP) and intention-to-treat (ITT) analyses were performed: non-inferiority margin -Δ2%. 1692 patients participated in the usual care group (UCG), 1699 in the DCP group (DCPG). At baseline 2333 questionnaires were returned, 1437 yearly thereafter, 1060 patients (517 UCG; 543 DCPG) completed both DHP questionnaires.
Results: Compared to usual care, in the DCPG the DHP-18 total score decreased 0.88 more in PP (CI: -1.94 - 0,12) and 0.439 more in ITT (CI: -1.01 - 0.08); demonstrating non-inferiority. SF-36 "Health change" increased 3.51 in PP (CI: 1.23 - 5.82) and 1.91 in ITT (CI: 0.62 - 3.23), demonstrating superiority. Other differences in perceived Health status, treatment satisfaction or psychosocial self-efficacy were inconclusive, inconsistent or non-inferior.
Conclusion: Despite intensification of advices and therapy, DCP is non-inferior to usual care regarding perceived diabetes specific healt status and improves the perceived change in general health.
Supported by: Pfizer BV

1029

Impaired fasting glucose and impaired glucose tolerance: follow up rates in the community

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Background and aims: Both impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) convey an increased risk of developing type 2 diabetes (T2DM) in the future. Current guidelines advocate that patients with IFG, IGT or both are to be screened at least annually with either a oral glucose tolerance test (OGTT) or fasting plasma glucose.(FPG). We examined a group of patients with OGTT-diagnosed IFG and IGT to determine the proportion of patients who were subsequently retested based on these recommendations.
Materials and methods: All patients undergoing OGTT from Primary Care in our region between Oct and Dec 2006 were included in the analysis. Patients who were deceased over this period and patients tested for gestational diabetes were excluded from the analysis. Approval was obtained from the Trust Audit Department prior to this undertaking. Data for this study was obtained from the laboratory serving the whole population. The criterion for diagnosis of IFG and IGT were based on the WHO.
Results: There were a total of 683 patients included in the analysis. 199 patients were diagnosed with T2DM and 246 patients had either IGT or IFG. Of the 246 patients with IFG/IGT, (median (IQR) age 67 (54 to 74) years, 127F, 119M), 196 patients had repeat glucose testing in the following 26 months after a mean (SD) of 13(5.5) months. 59% (115/196) were screened using a repeat FPG and 41% (81/196) with an OGTT. Using FPG alone, 52 (45%) patients were noted to have persistent IFG and 11 (9.6%) patients had a glucose level of 7 mmol/L or higher. Repeat OGTT showed 15 (18.5%) patients with persistent IGT, 30 (37%) with IFG and 19 (23.5%) patients with both. 13 (16%) had a glucose level of 7 mmol/L or higher. Crucially, this meant 19% (47/246) of patients (median (IQR) age 60(41 to 69) years, 24F, 23M) did not have a repeat test at all over the 26 months from the initial diagnosis
Conclusion: Patients with IFG and IGT are at greater risk of developing future cardiovascular disease and T2DM It is also known that lifestyle intervention can reduce the risk of progression to T2DM. This data has shown that the follow-up of patients with IFG and IGT in primary care is not systematic, with approximately 1 in 5 patients with IFG and/or IGT not being followed up at all after 26 months of being diagnosed. There are challenges in moving more diabetes care from secondary to primary care environments. This study has shown that one of these challenges is to it develop a consistent means of recalling patients with abnormal OGTTs order to establish (and try to prevent) progression from IFG/IGT to T2DM.

1030

Clinical decision support delivered through EMRs improved glucose and blood pressure control in adults with diabetes

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Background and aims: Medical groups have invested billions of dollars in outpatient Electronic Medical Records (EMR), but few studies demonstrate a positive impact of EMR-based clinical decision support on clinically important patient outcomes. We conducted this randomized trial to assess the impact of an EMR-based diabetes clinical decision support system on control of glycated hemoglobin (A1c), blood pressure (BP) and LDL-Cholesterol (LDL) in adults with diabetes.
Materials and methods: The study was conducted from October 2006 to May 2007 in Minnesota. Eleven clinics with 41 consenting primary care physicians (PCP) and these physicians' 2556 diabetes patients were randomized either to receive or not to receive an EMR-based advanced pharmacologic and clinical decision support system designed to improve A1c, BP, and LDL in diabetes patients not at recommended clinical goals at the time of an office visit.
Results: Intervention group PCPs used the EMR-based decision support system at over 62.6% of all diabetes patient visits, and intensified drug therapy at 61.9%, 43.6% and 18.8% of visits with uncontrolled A1c, BP, and lipids, respectively. Intervention group diabetes patients had significantly better A1c (p=.014) and SBP (p=.035) but not LDL (P=.63) relative to patients of PCPs randomized to the control arm of the study based on general linear mixed models with a repeated time measurement to control for clustering. Additional analytic models that adjusted for patient age, gender, and comorbidity showed similar results. 94% of PCPs expressed satisfaction with the intervention, and moderate rates of use persisted for over a year after withdrawal of feedback and incentives to encourage use.
Conclusion: An EMR-based advanced pharmacologic and clinical decision support system significantly improved glucose control and BP control in adults with type 2 diabetes who were not at recommended clinical goals.
Supported by: NIDDK, NIH, DHHS

1031

Management of diabetes in migrants - does ethnical background matter?

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Background: Migrants constitute a growing part of the Austrian and European population. There seem to be barriers to the use of health services, access to appropriate treatment and knowledge transfer regarding diabetes due to cultural and religious particularities. We aimed to identify differences between diabetic subjects (TDM2) with different countries of origin regarding knowledge, treatment, as well as self-capacitance to take a responsibility for the diabetes management.
Methods: TDM2 -patients (first generation migrants from Serbo-Croatia (SC, n=52) and Turkey (T, n=54)) living in Austria, aged between between 35 and 60 years were randomly selected. Information was collected by structured interviews in their first language and anthropometry and glycemic parameters were recorded.
Results: Nearly all patients were overweight (T: 96%, SC 85%). Overweight T misclassified themselves regarding the weight category more often than SC

(22% vs.6%, p<0.05). Migrants, independent from the country of origin, were often diagnosed when already presenting with diabetes- related problems (T 79%, SC 77%). Most migrants were treated with oral antidiabetics (OAD)(T 63%, SC 60%) or a combination of OAD and insulin (T 21%, SC 15%). While knowledge regarding the medication was reported to be unproblematic (T 93%, SC 90%), one third of T did not know how to correct hypoglycemia (T 30%, SC 15%, p<0.05) and two thirds were not used to examine their feet for lesions (T 70%, SC 15%, p<0.0001). For both patient groups, physicians are the primary source of information (T 85%, SC 100%), nurses are nearly never experienced as a possible source of support (T 4%, SC 4%). The chance of gathering additional information about diabetes through internet or diabetes associations was completely unused. T report less often recommendation of lifestyle changes (diet: T 41%, SC 81% p<0.001; exercise: T 24%, SC 56% p<0.001). Implementation of dietary changes was difficult for nearly every second patient (T 56%, SC 44%). Regarding physical activity, more T reported difficulties with change (T 44%, SC 27%, p<0.05). Knowledge about causal factors was little in most patients. But there were differences between the groups (table 1). Noticeably, nearly every SC believed in a relationship between diabetes and stress.

Conclusion: We conclude that screening examinations for diabetes are not used by migrants independent of country of origin. All patients exhibit deficits in diabetes knowledge and its transfer into daily practise. Regarding knowledge and self-capacitance the country of origin matters and should be borne in mind when treating migrants with diabetes as well as when planning migrant oriented health promoting initiatives.

Table 1: Knowledge about causal factors for diabetes (% patients agree) #p<0.05 between T and SC

	Genetics	Age	Body weight	Nutrition	Excercise	Stress	God's will
T	33#	7#	37	35#	22	50#	13
SC	46	37	48	56	29	81	8

1032

Insulin dose titration system in diabetic patients using a short messaging service automatically produced by a knowledge matrix

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Background and aims: We designed the system in type 2 diabetic patients treated with long acting insulin to produce an automatic adjustment of insulin dose based on real time glucose level data and to provide to the patients the needed insulin dose by using a short message service (SMS) and apply to the clinical practice.

Materials and methods: We developed the system to produce an automatic adjustment of insulin dose based on the mean fasting blood glucose (FBG) level for consecutive 3 days. Moreover we created a web site program (https://uc.healthkorea.net/UDang/dclinic/index.aspx) that was used to formulate messages of insulin dosage through an automated algorithm. By connecting specially designed glucometer to the cellular phone, the measured glucose data is automatically sent to patient's personal data sheet on the web site, then our system automatically composed messages that were then sent to the patient. According to the mean FBG level, patients were asked to increase in glargine dose by 6 U (≥ 180 mg/dL), 4 U (140~179 mg/dL), 2 U (120~139 mg/dL), respectively. If patients showed descended FBG levels for consecutive 3 days, the dose was adjusted based on the last measured day. If one FBG level is higher than 60 mg/dL compared with other levels measured for 2 days, the mean FBG level of other 2 days was used for dose adjustment. 100 type 2 diabetic patients suboptimally controlled on their previous antidiabetic treatment were included. Each participant was assigned to either the intervention or control group, each with 50 patients using adaptive randomization. We applied this system for 12 weeks in the intervention group. In the control group, a conventional titration schedule was used, seeking a target FBG of < 120 mg/dl. If the mean FBG level for consecutive 3 days of ≥ 120 mg/dL, patients were asked to increase in glargine dose by 2 U. In both group, 2 U decreases were allowed if any time glucose level of < 80 mg/dL, insulin dose was decreased by 4 U if glucose level of < 60 mg/dL. Then, we compared A1C and FBG after 12 week trial in both groups. The protocol was approved by the Ethics Committee of the Hallym University. Informed consent was obtained from each participant. Comparison of both groups was based on the independent t-test or Chi-square test, appropriately.

Results: 47 patients in the intervention group (47.8 ± 9.6 yrs, M = 24) and 45 patients in the control group (49.0 ± 10.7 yrs, M = 22) completed the experimental protocol over a 12 week period. A greater ($P = 0.023$) baseline to end point reduction in A1C was observed in the intervention group (9.8 ± 1.3 to 7.4 ± 0.7%) than in the control group (9.8 ± 1.2 to 7.8 ± 0.8%). The FBG after 4 weeks was lower ($P = 0.013$) in the intervention group (121.8 ± 35.9 mg/dL) than in the control group (141.4 ± 38.1 mg/dL), however, the reduction of FBG after 12 wks in the intervention group (200.1 ± 53.7 to 113.1 ± 15.3 mg/dL) was similar with the control group (196.3 ± 48.9 to 117.4 ± 26.5 mg/dL). The incidence of symptomatic, asymptomatic, nocturnal hypoglycemia was similar in both intervention and control groups. There was a small increase in body weight from baseline to end point with both intervention (2.4 ± 3.0 kg) and control group (2.2 ± 2.8 kg).

Conclusion: This study demonstrated that SMS based on our specialized internet supported system is an effective and safe approach to long acting insulin dose adjustment in patient with type 2 diabetes.

We thank "UBCare Company" for its large contribution to the development of our specialized system

1033

A nationwide diabetes campaign: Portuguese pharmacies identify uncontrolled diabetic patients

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Background and aims: The National Association of Pharmacies (ANF) developed a model and tools for a national pharmacy-based intervention campaign targeted to adult patients on diabetes therapy, in collaboration with 2 medical societies in the field of diabetes disease. Tools provided included a Pharmacists' Intervention Protocol on Diabetes©, with capillary blood glucose recommended values, and a spreadsheet to document care provided. The Campaign was launched in November 2007 and was preceded by evening sessions for pharmacists held in the 3 major cities.

Aim: To assess capillary blood glucose (BG), out of reference values, in diabetic patients during the nationwide campaign.

Materials and methods: Between 12 and 17 November 2007, all diabetic patients aged 18 and higher on antidiabetic drugs were included in the Campaign. Pharmacists' intervention were focused on adherence to therapy, self surveillance counselling and capillary BG assessment.

Results: 1 763 pharmacies participated in the Campaign, out of which 723 have sent data to ANF (41.0%). 7 719 adult diabetic patients were assessed, an average of 13 patients per pharmacy. 57.2% were female and average age was 57. A high percentage of patients (91.2%) did not smoke and 36.2% had BMI over 30 kg/m^2. The majority (87.3%) of patients were on oral antidiabetic drugs (ADO), 7.6% were only on insulin and 5.1% were on combined insulin and ADO therapy. Pharmacists performed 11 102 measurements in the 5 days of the Campaign. Average postprandial BG values were 189.5 mg/dL and average fasting BG values were 144.8 mg/dL. Patients with high BMI tend to have higher fasting BG values (p<0.001). The % of patients with postprandial BG > 180 mg/dL or fasting BG > 130 mg/dL were 47%. Compared with the older patients (≥ 65 years old), younger patients have higher probability of having capillary BG within the recommended values (18-44 years old: OR=2.502, IC95%:[1.928-3.247]; 45-64 years old: OR=1.142, IC95%:[1.023-1.275]). Compared with those only on insulin therapy, the patients on ADO had higher probability of being within the recommended BG values (OR=1.297, IC95%:[1.061-1.587]) and the patients on combined ADO and insulin therapy had lower probability of being within the recommended BG values (OR=0.563, IC95%:[0.413-0.768]). A referral to the prescriber was reported for 23.9% of patients, out of which 72.7% had BG above the recommended values.

Conclusion: These results suggest pharmacists may have an important role in identifying diabetic patients with BG values out of the recommended targets, as well as, in reinforcing adherence to therapy and self surveillance counselling.

1034

Organizational characteristics influence quality of diabetes care. The QUASAR (quality assessment score and cardiovascular outcomes in Italian diabetes patients) study

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Background and aims: While process and outcome measures are usually considered for quality of care profiling, the way diabetes care is organized and delivered can undoubtedly play an important role in achieving the desired results. We evaluated how structural and organizational characteristics influence process and intermediate outcome indicators in T2DM, taking patient case-mix into consideration.
Materials and methods: Seventy-eight clinics described their structure and organization and were classified in 3 levels of organizational complexity (high, intermediate, low). Each clinic collected data on 100 random patients, including frequency of HbA1c, blood pressure (BP), lipid profile, and renal function monitoring in the last 12 months and frequency of patients reaching HbA1c<=7%, BP<=130/85 mmHg, LDL-cholesterol<=100 mg/dl, and albumin/ creatinine ratio <=30 mg/g. Multilevel models were applied to identify correlates of the selected indicators.
Results: Overall, 6702 patients were enrolled. Structures with high complexity ensured the highest average number of visits per patient/year. The likelihood of monitoring HbA1c increased by 3 times (OR=3.06; 95%CI 1.14-8.24) for every additional unit in the average number of per-patient visits performed by the centre, while the likelihood of monitoring BP increased by over 4 times (OR=4.35; 95%CI 3.57-5.26). The chance of reaching levels of HbA1c<=7% increased by 62% (OR=1.62; 95%CI 1.01-2.61) for each additional unit in the number of per-patient visits. A lower level of complexity of organization was associated with a lower likelihood of achieving the BP target. Process indicators were not independently associated with outcomes.
Conclusion: Our study suggests that, in addition to process and intermediate outcome indicators, measures able to capture the complexity of structural/organizational aspects should be incorporated in any initiative to evaluate the quality of diabetes care. Diabetes clinics that provide better quality of care are those with higher level of organization and able to ensure an adequate frequency of encounters with patients.
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1035

Screening for deprivation using EPICES score: a tool to detect patients at high risk of diabetic complications

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Background and aims: Several studies reported that deprivation was associated with complications in diabetic patients. We hypothesized that screening for deprivation could lead to a more intense action to try and prevent complications. We analyzed the interest of a deprivation score, EPICES (Evaluation of Precarity and Inequalities in Centres d'Examens de Santé), validated in the French general population.
Materials and methods: The study was developed in a single hospital diabetes center from November 2006 to July 2007. Diabetic patients were split in two groups, either deprived (EPICES score > 30.17), or not. Diabetes control, estimated by HbA1c, and prevalence of chronic complications were compared between the two groups, as well as the quality of life, estimated by SF-36 questionnaire.
Results: Of the initial 102 patients, 97 subjects, among which 18 type 1 and 79 type 2 diabetics ,were included in the trial. Interestingly, HbA1c was not correlated to EPICES score. Yet nephropathy was associated with deprivation ($p = 0.049$) and possibly neuropathy ($p = 0.06$; NS). No other complication seemed to be linked to deprivation. Deprived diabetic patients presented with more co morbidities : excess weight ($p = 0.001$), lower HDL-cholesterol ($p = 0.04$). Eventually, quality of life, in all aspects of daily life, was much lower in deprived diabetic patients.
Conclusion: Our results demonstrated that even in an area of moderated deprivation intensity (mixed rural and urban population in Central France), a high EPICES score, an individual index of deprivation, is associated with nephropathy, higher cardiovascular risk factors and lower quality of life in diabetic patients. EPICES score is therefore an easy to calculate useful tool to detect diabetic patients needing specific focus on management of their disease.

1036

Better %HbA_{1c} control among type 2 diabetes patients is associated with higher patient-reported satisfaction with treatment: a meta-analysis of trial results

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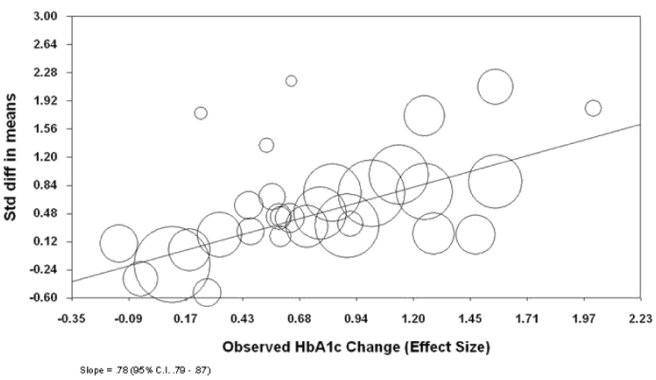
Background and aims: We tested the hypothesis that control of HbA1c associated with current treatments for Type 2 diabetes mellitus (DM) increase patients' satisfaction with treatments. Data were obtained from an empirically-based systematic literature review of 280 randomized controlled trials examining medications used to treat Type 2 DM published after 1998. After restricting the articles to adult samples and removing duplicates, we identified 230 articles for consideration. Fourteen of these studies provided quantitative assessment of patient self-reported satisfaction measures associated with treatment-related changes in %HbA1c.
Materials and methods: Fourteen randomized clinical studies where included that involved Type 2 DM patients (n = 2880) that included both patient-reported treatment satisfaction surveys and treatment-related changes in %HbA1c from baseline. %HbA1 changes were standardized using Hedge's g effect size statistics with positive values indicating improvement from baseline. After standardization, we then performed a fixed effects meta-regression on changes in treatment satisfaction; assessed almost exclusively using the Diabetes Treatment Satisifaction Questionnaire (11/14 articles). Both of these meta-analytic methods were applied to: 1) all scales measuring any aspect of treatment satisfaction, and 2) scales which measured overall satisfaction or product preference.
Results: Our fixed effects meta-analysis revealed a statistically significant improvement in treatment satisfaction with a improvements %HbA1c from baseline ($p < 0.0001$). The effect size in the meta-analysis resulted in a positive mean difference with a point estimate and 95% CI of 0.51 (95% CI: 0.47 to 0.55). When we pooled studies in a meta-regression, the slope of the association between %HbA1c change and all treatment satisfaction scales was 0.50 (95% CI: 0.44 to 0.56), $p < 0.0001$. In a meta-regression on overall satisfaction or product preference versus %HbA1c change, the slope of the association was 0.78 (95% CI: 0.70 to 0.87), $p < 0.0001$ (see Table).
Conclusion: Our meta-analyses on randomized controlled trials showed a significant, positive improvement in patients' self-reported treatment satisfaction scores when associated with a positive baseline change in %HbA1c. We can conclude that, for every percentage point improvement in %HbA1c over time, Type 2 DM patient satisfaction improves by 10-20%.



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PS 92 Health economics

1037

Obesity increases healthcare resource use in patients with type 2 diabetes: a UK database analysis

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Background and aims: Little information is available on resource use and diabetic medication prescribing by BMI category in type 2 diabetic (T2DM) patients in general practice in the United Kingdom. This study was designed to investigate these factors from the time of insulin initiation.
Materials and methods: A cohort of patients with diagnostic codes for T2DM was selected from the General Practice Research Database (GPRD) for the period January 1st 2002 - March 31st 2008. No patient had been prescribed insulin in the 6 months prior to the first insulin prescription (index date). Additionally patients had to have ≥ 2 further prescriptions for insulin in the 12 months post index date as well as ≥ 2 BMI and ≥2 HbA1c recordings (one baseline, one post index). Patients also had to have a minimum of 12 months data post the index date. The cohort was stratified into BMI categories at the time of insulin initiation: normal (18.5-24.9 Kg/m^2); overweight (25.0-29.9 Kg/m^2); obese (30.0-34.9 Kg/m^2); clinically obese (35.0-39.9 Kg/m^2); morbidly obese (≥40 Kg/m^2). Prescribed insulin quantities were used to estimate daily insulin requirements at 6, 12, 18 and 24 months. Oral diabetic drug prescribing, GP visits and hospital referrals were calculated and stratified by patients' BMI category in the first year after initiation and in year 2. Rates were calculated with 95% confidence intervals and presented per 100 patient years of observation.
Results: 3783 patients fulfilled the inclusion criteria (normal weight, n=672; overweight, n=1259; obese, n=1070; clinically obese, n=480; morbidly obese, n=302). Mean daily insulin requirement appeared relatively stable between 6-24 months post initiation in normal weight patients (41 [SD 30] rising to 45 [SD 36]units/day) but appeared to increase in all other groups e.g. clinically obese 67 [SD 50] rising to 78 [SD 60] units/day). The rate of oral diabetic therapy prescribed was statistically signficantly higher with increasing BMI, ranging from 699 scripts per 100 patient-years (95% CI 680,720) in normal weight patients to 1066 scripts (95%CI 1038,1095) in clinically obese. GP visit rates for diabetes increased signficantly by BMI from 40.0 per 100 patient-years (95%CI 36.4, 43.8) in normal weight up to 55.7 (95%CI 51.3,60.1) in the clinically obese. Secondary care non-diabetes referrals increased signficantly with BMI from 70.7 per 100 patient-years (95%CI 67.2,74.0) in normal category to 85.2 (95%CI 81.7, 88.1) in clinically obese.
Conclusion: The results from this study indicate that patients who are obese have higher resource use including oral diabetic agents and higher daily insulin requirements as well as higher rates of GP and secondary care non-diabetes referrals.
Supported by: Eli Lilly & Co Ltd.

1038

Cost and resource use following insulin initiation in Europe: 24-month follow up data from the INSTIGATE study

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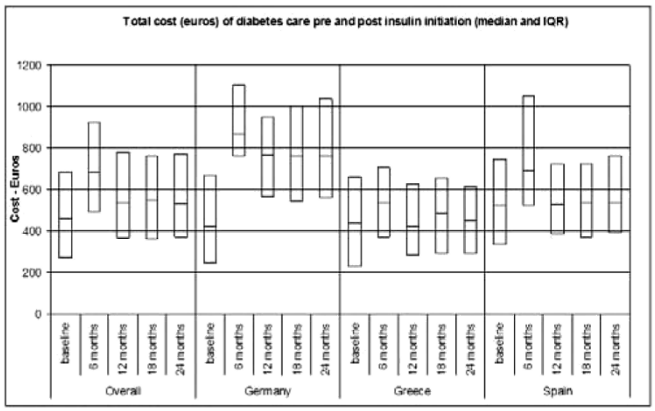
Background and aims: INSTIGATE is a prospective observational study in Europe, investigating patients with type 2 diabetes (T2DM) who initiate insulin during usual care. Follow up data to 24 months (m) were collected in Germany (De), Greece (Gr) and Spain (Es). The primary objective of the study was to assess the direct costs of care for T2DM following insulin initiation.
Materials and methods: Direct costs of diabetes care were calculated by assigning local unit costs (2006) to data collected at each visit on individual resource use (diabetes medication; blood glucose monitoring (BGM); and for the period since the last visit, contact with health care professionals (HCPs) and diabetes related hospitalisations). Reported costs and resource use are for the 6m prior to insulin initiation (baseline) and the 6m between study visits.
Results: Of 726 patients entering the study (De 256; Gr 263; Es 207) in these 3 countries, 564 (De 155; Gr 237; Es 172) had at least a 12m visit and 498 (De 119; Gr 227; Es 152) were followed to 24m. In all three countries median and mean total direct cost of diabetes care increased in the first 6m following insulin initiation then fell and remained reasonably stable over the following 18m (Figure 1). In Gr and Es median costs fell to around the baseline level. In De median costs remained higher than prior to insulin initiation. The main contributors to total costs differed between countries. In De patients were prescribed more intensive insulin regimens (at 6m 79% of patients (119/151) were on prandial based regimens) with mean 3.4 injections per day. In contrast in Gr and Es at 6m; 78% (185/237) and 88% (152/172) respectively were prescribed either basal only or premix only and the mean daily injections were Gr 1.9 and Es 1.4 although OAD usage was higher than in De. There were few changes in insulin regimens over the first 24m. BGM was a major component of total cost. Prior to insulin initiation patients monitored approx. once per day (mean strips/week De 8; Gr 7; Es 7). Following insulin initiation this increased in De to 3 times daily, but changed little in the other countries. In the 6m following initiation mean strips per week were De 21, Gr 8, Es 8; and 18 to 24m following initiation De 21, Gr 8, Es 7. Mean total contact (number of visits & phone calls) with HCPs in the 6m prior to initiation were De 12, Gr 8, Es 10; rising in the 6m following initiation to De 15, Gr 10, Es 12; and falling thereafter, at 18 to 24m following initiation total contacts were De 6, Gr 4, Es 7. There were few hospitalisations in any country
Conclusion: There are considerable additional direct costs associated with starting insulin therapy, particularly during the first 6m, but the cost burden reduces over time. The main contributing factors for cost were BGM and insulin.

Figure 1 Course of total cost of diabetes care (Euros)



Abbreviations: IQR=Interquartile range.

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1039

Swedish patients with type 2 diabetes show willingness-to-pay for health improvements including weight loss, reduction in hypoglycaemia and glycaemic control

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Background and aims: Patient preference for treatments and interventions do not always concur with the physicians' choice or scientific evidence base. This study aimed to investigate the most important aspects of diabetes medication, as measured by the patients' willingness-to-pay (WTP), an indication of patient preference.
Materials and methods: The WTP survey used a discrete choice experiment methodology to evaluate the convenience and clinical effects normally associated with type 2 diabetes treatments. Patients in Sweden were recruited using an existing nationwide e-mail panel administered by GfK Health Care. Patients were included if they were adults (≥18 years) with type 2 diabetes and currently receiving antidiabetic medication(s). Data were collected electronically between September 2008 and November 2008, and results were analysed using a standard statistical model designed for choice games (con-

ditional logit). The WTP was examined for 6 characteristics: weight (gain or loss), HbA_{1c} levels, hypoglycaemic events, nausea, injections (with or irrespective of meals) and blood glucose monitoring.
Results: In total, 461 of 537 (86%) patients with type 2 diabetes completed the internet questionnaire and were eligible for inclusion. The survey sample matched the National Diabetes Register (NDR) in Sweden for all key parameters such as age, duration of diabetes, HbA_{1c} level and BMI, except gender distribution (291 males; 170 females). Patients placed the highest value on the avoidance of weight gain; they needed to receive as much as 795 Swedish kronor (SEK) per month in order to accept a 3kg weight gain. They would also pay as much as 528 SEK per month to lose 3kg. Women were more concerned about gaining weight than men (p=0.0074) and obese patients (BMI>30 kg/m^2) had a higher WTP to lose 6kg compared with non-obese patients (BMI≤30 kg/m^2; p<0.0001). Patients wanting to reduce the number of hypoglycaemic events from two per month to none were willing to pay 419 SEK per month. Patients who had never experienced a hypoglycaemic event had a significantly higher WTP (300 SEK per month) to avoid them completely than patients already experiencing hypoglycaemic events (p=0.0004). Patients valued a 1% reduction in HbA_{1c} level at 414 SEK per month. Patients would require 140 SEK per month in order to accept a 25% risk of medication-related nausea. Participants preferred taking tablets to injections and required a compensation of 376 SEK to accept one injection. Patients already taking tablets did not have a higher WTP to avoid injections than those already having injections. Injections irrespective of meals were preferred to injections with meals (WTP: 140 SEK per month).
Conclusion: Patients were willing to pay a considerable amount of money each month for the health benefits associated with improved diabetes treatment. In particular, patients wanted to avoid gaining weight above other benefits.
Notes: Exchange rates: 100 SEK = 9.15 EUR or 12.41 US$ (26 March 2009)
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1040

Patient willingness-to-pay for liraglutide and exenatide in Sweden based on head-to-head clinical trial results

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Background and aims: Liraglutide is a once-daily human glucagon-like peptide-1 (GLP-1) analogue. As part of the Phase III trial programme the LEAD-6 (Liraglutide Effect and Action in Diabetes 6) trial compared liraglutide 1.8 mg once-daily with exenatide 10 μg twice-daily as add-on to oral antidiabetic drug therapy. Here we assessed patients' willingness-to-pay (WTP) for liraglutide versus exenatide by applying clinical data from LEAD-6 to results generated by a willingness-to-pay (WTP) survey. The WTP survey investigated patients' preferences and WTP for the most important aspects of diabetes medication.
Materials and methods: The LEAD-6 trial was a 26-week, randomised, open-label, parallel group study in patients with type 2 diabetes who were either taking liraglutide 1.8 mg once-daily irrespective of meals (n=233) or exenatide 10 μg twice-daily at the two main meals (n=231). The WTP survey involved 461 participants with type 2 diabetes from Sweden. The electronic WTP survey used a discrete choice experiment methodology to evaluate the convenience and clinical effects of treatments associated with type 2 diabetes. The values from the WTP study were applied to the efficacy and safety data from the two treatment groups in LEAD-6.
Results: The main results from LEAD-6 were differences in change in HbA_{1c} levels (liraglutide -1.12%; exenatide -0.79%, p<0.0001), change in BMI (liraglutide -1.145 kg/m^2; exenatide -1.015 kg/m^2, NS), minor hypoglycaemia rate/month (liraglutide 0.161; exenatide 0.217, p=0.0131), change in SBP (liraglutide -2.51 mmHg; exenatide -2.00 mmHg, NS), and difference in the frequency of nausea (equivalent to a 4.9 % and 12.1% of subjects in liraglutide and exenatide groups respectively with nausea for the 26 weeks trial period) (p not available). Analysing the data with the results from the WTP survey revealed that patients were willing to pay an extra 17 Swedish kronor (SEK) per day in the first year of treatment for liraglutide compared with exenatide. The largest component of this WTP amount was the once-daily dosing of liraglutide; patients were willing to pay an extra 10 SEK per day to reduce drug administration to once per day irrespective of meals (liraglutide) from twice a day in relation to meals (exenatide). They would also pay an extra 5 SEK per day to reduce their HbA_{1c} levels to a greater extent with liraglutide compared with exenatide. Patients were willing to pay 1 SEK per day for the relative reductions in SBP and BMI with liraglutide compared with exenatide, and were willing to pay an extra 0.7 SEK per day in the first year to take liraglutide to reduce their incidence of nausea. Despite a significant difference in minor hypoglycaemic event rates between liraglutide and exenatide, patients were not willing to pay more than 0.2 SEK per day extra to receive liraglutide on this basis.
Conclusion: Patients were willing to pay 17 SEK per day to use liraglutide rather than exenatide. Willingness-to-pay can be calculated for any combination of investigated effects and can be used to make comparisons between treatments from a patient perspective.
Notes: Exchange rates: 100 SEK = 9.15 EUR or 12.41 US$ (26 March 2009)
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1041

Cost-effectiveness of motivational enhancement therapy with and without cognitive behaviour therapy for type 1 diabetes

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Background and aims: Diabetes' high prevalence, chronic nature and association with complications incur substantial costs for services, patients and wider society, necessitating cost-effective approaches to treatment and long-term management. We examined the cost-effectiveness of two manualised psychological treatments for adults with Type 1 diabetes (T1DM) as part of a multi-centre randomised controlled trial in the absence of such evidence.
Materials and methods: A representative sample of adults with T1DM and persistent suboptimal glycaemic control (HbA1c >8.2%) were recruited from 8 UK hospital diabetes clinics and randomised to motivational enhancement therapy (MET), MET + cognitive behaviour therapy (MET+CBT) or usual diabetes care. We measured costs and health (HbA1c improvement) and quality of life (quality-adjusted life years; QALYs) outcomes over one year. Health care and societal costs (2005/06 prices) were estimated from resource use data collected by interview at 6 and 12 months. Outcomes and costs were linked/compared between groups using incremental cost-effectiveness ratios (ICERs) and cost-effectiveness acceptability curves (CEACs).
Results: 344 participants were randomised to MET (n=117), MET+CBT (n=106) and usual care (n=121); 62%, 69% and 58% of each group respectively had requisite cost and outcome data for the economic evaluation. Only MET+CBT produced a statistically significant HbA1c improvement (+0.45 compared to usual care; 95% CI: 0.10 to 0.78) and neither intervention significantly increased QALYs. Mean costs of the psychological interventions were £195 for MET and £660 for MET+CBT. Both interventions led to significantly higher mean total health care costs compared to usual care alone: +£535 (95% CI: 171 to 857) in MET group and +£790 (95% CI: 5.7 to 1072) in the MET+CBT group. Societal costs were also higher but not statistically significant. ICERs for additional HbA1c point improvements ranged from £514 (MET+CBT vs. MET) to £3,821 (MET vs. usual care) from the health care perspective. At thresholds of £5,000 or higher for an additional HbA1c point improvement, the probability of MET+CBT being cost-effective compared to MET or usual care exceeded 0.7; probabilities for MET versus usual care were lower. Due to minimal QALY differences between groups, cost-effectiveness conclusions based on these were unfavourable for both interventions, although better for MET than MET+CBT: the minimum ICER was £48,636 (MET vs. usual care, health care perspective) and probabilities of cost-effectiveness peaked at 0.39 (MET vs. usual care, societal perspective) for thresholds up to £45,000 per QALY gain.
Conclusion: Additional costs of the two psychological interventions were not offset by savings elsewhere within one year. MET+CBT had high chances of cost-effectiveness based on HbA1c outcomes; conclusions were broadly more favourable in relation to this outcome than QALYs. Treatment provision decisions should thus account for the relative importance of these two outcomes.
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1042

Assessment of healthcare resource utilization among type 2 diabetic patients with pre-existing macrovascular comorbidities in Europe: a matched cohort study

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Background and aims: T2DM is a chronic metabolic disorder characterized by hyperglycemia and associated with significant morbidity. This may be particularly so among patients with pre-existing macrovascular conditions (MVC). This study was undertaken to examine differences in healthcare resource utilization between T2DM patients with and without pre-existing MVC in Europe.
Materials and methods: This is a matched cohort study based on the Real-Life Effectiveness and Care Patterns of Diabetes Management (RECAP-DM) study, a multi-center, observational study with retrospective medical chart reviews of T2DM patients in Spain, France, UK, Norway, Finland, Germany, and Poland. Included patients were aged ≥30 year at time of diagnosis of T2DM and added a SU or a TZD to failing metformin monotherapy (index date), and had pre-existing (i.e., with onset date prior to index date) MVC. A control cohort with T2DM but without pre-existing MVC was identified using 1:1 propensity score matching. Logit models were used to identify the relationship between pre-existing MVC and the likelihood of emergency room admission, receiving medical/surgical procedures, and hospitalization during the study period. Negative binomial models were used to predict the number of office visits and length of hospital stay per year attributable to the pre-existing MVC.
Results: Of the 453 patients with pre-existing MVC (cases) and eligible for inclusion, 64% were male, mean (SD) age and time from T2DM diagnosis was 64.5 (9.1) and 6.2 (5.3) years, respectively. HbA_{1c} prior to the index date was 8.0 (1.2). Relative to controls, patients with pre-existing MVC were significantly more likely to report emergency department admissions (Odds Ratio 3.1; 95%CI 1.8-5.2), receiving medical/surgical procedures (OR 2.72; 95%CI 1.7-4.3), and hospitalizations (OR 2.6; 95%CI 1.7-4.0) after controlling for other predictors. Similarly, pre-existing MVC was associated with 2.9 additional office visits per year ($p<0.001$) and 0.41 days of hospital stay per year ($p=0.004$).
Conclusion: This study found that T2DM patients with pre-existing MVC were more likely to use various types of healthcare resources.
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1043

Pharmacoeconomic analysis for the future treatment of diabetes mellitus after gestational diabetes

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Background and aims: To outline the risk of developing Diabetes Mellitus (DM) during the first year after giving birth for women with previous Gestational Diabetes Mellitus (GDM), as well as to estimate the social efficiency value of the applied prophylactic method.
Materials and methods: The study has been performed among 50 women, with GDM for one year after delivery. During that period a prophylactic program has been applied for DM prevention. The social efficiency of the applied prophylactic method is presented using the "decision tree" model. All indirect costs for future DM treatment are presented, as well as calculations are given for the disability adjusted life years (DALY).
Results: DM has been observed at 13 (or 26%) out of 50 women with previous GDM in the first year after birth delivery. The total cost per women for the applied preventive programme have been calculated at 12.1€. The total annual expenses for future treatment and control of a women with type 2 DM and good metabolic control is 98.9€, for satisfying metabolic control - 122.1€ and for poor metabolic control - 241.8€. The total annual expenses for future treatment and control of one women with type 1 DM and good metabolic control is 241.8€, for satisfying metabolic control - 303.7€, and for poor metabolic control. The social cost of late diagnosed or complicated type 2 DM is 10,994.80 €. The cost for the complicated type 1 DM treatment is 20,979.38 € at 5% discount. The rate of DALY also varies according to the rate of diabetic complications. The calculated DALY for women with DM at stage of disability are: - 10.1 year for women with DM with no complications, 12.1 years for women with DM and a mild rate of complications, 13.6 years for women with DM and with moderate complications and 15.1 years for women with DM and a severe rate of complications.
Conclusion: Future DM treatment costs depend entirely on the extent of metabolic compensation, probability for later complications and the method of treatment. However, the prophylactic screening the women with previous GDM can considerably save these costs.

1044

Health care expenditures following initiation of insulin glargine or exenatide in type 2 diabetic patients on oral agents in US

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Background and aims: Increasing health care expenditures are a significant challenge when deciding on an effective course of treatment for type 2 diabetes.
Materials and methods: A managed care database of 47 health plans was analyzed between May 2005 and September 2007 to evaluate health care expenditures after initiation of insulin glargine (GLAR; n= 15,695) or exenatide (EX; n= 21,516) in T2DM patients treated with only oral antidiabetic agents (OAD) during the 6 months (baseline) prior to initiation.
Results: Baseline characteristics for GLAR patients vs EX patients, respectively, were mean age, 54 vs 53 years; female, 43% vs 54%; glycated hemoglobin A1C, 9.5% vs 7.9%; hypoglycemia rate on OADS, 4.8% vs 2.7%; use of endocrinologist care, 16% vs 31%; retinopathy, 9.8% vs 7.8%; nephropathy, 5.4% vs 2%; neuropathy, 9% vs 8.3%; myocardial infarction, 3.5% vs 1.1%; and any history of pancreatitis, 1.5% vs 0.3%. Baseline hospitalization rate was 22% vs 5% in GLAR vs EX with 7.5 vs 3.5 hospital days and 22% vs 12% emergency service use, respectively, reflecting possibly a sicker population in the GLAR group. Baseline health plan expenditures for GLAR vs EX, respectively, were as follows: for antidiabetic medications, $626 vs $673; for all medications, $1,805 vs $1,986; for diabetes-related non-pharmacy inpatient and outpatient medical services, $3,206 vs $1,022; and for total medical services, $9,044 vs $2,956. During the year post initiation, 5% in the GLAR group started EX, and 11% in the EX group started insulin. The 1-year adjusted health plan total expenditures for all medical and pharmacy utilization (glucose monitoring supplies not available), were $25,596 vs $26,566 ($\Delta$=$970, $P<0.001$) for GLAR vs EX, respectively (after adjustment for baseline age, sex, total health care expenditures, hospitalization, and Charlson comorbidity). After accounting for concomitant medications, the total expenditures were $24,852 for GLAR vs $27,219 for EX ($\Delta$=$2,368, $P<0.001$). Pair-wise comparisons for GLAR vs EX showed lower 1-year expenditures for antidiabetic drugs (Δ=$419, $P<0.001$), total medications (Δ=$246, $P<0.001$), diabetes-related medical (Δ=$730, $P<0.001$) and total medical (Δ=$207, $P=0.486$).
Conclusion: This retrospective analysis showed lower health care expenditures 1 year following initiation of GLAR compared with initiation of EX. However, differences in patient populations may have affected the results despite adjustment. Our findings require further confirmation with well-designed prospective real-world studies.
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1045

Cost-effectiveness analysis of medical intervention in patients with early detection of diabetic nephropathy in a tertiary care hospital in Bangladesh

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Background and aims: The economic burden resulting from diabetic nephropathy (DN) consumes a major portion of resources allocated for healthcare services. Cost-effectiveness of various interventions on DN & its complications has relatively been well explored in developed countries, but these are almost absent in developing countries. The present study was undertaken to assess the cost-effectiveness of medical intervention in patients with DN.

Materials and methods: Two hundred patients with DN, with at least 1 year of follow-up, were purposively selected from Out Patient Department of BIRDEM (tertiary diabetes care hospital), Bangladesh. Of them 100 were late in detection of DN and 100 were detected early. The degree & extent of complications like cardiopathy, peripheral neuropathy, retinopathy & vasculopathy, treatment outcome, clinical effectiveness of interventions and direct, indirect & incremental cost of complications were calculated. Comparison was made between the groups. Cost included drugs, hospitalizations, diagnostics & visits.
Results: A total of 200 patients were considered for an average of 365 days, amounting to 651 person-years of observation in total. The mean±SD serum creatinine of the groups (LDN & EDN respectively) was 4.90±1.17 & 0.89±0.03 mg/dL, fasting serum glucose was 9.36±0.40 mmol/l & 4.78±0.38 mmol/l, total cholesterol was 206.50±42.60 & 104.20±35.50 mg/dl, HbA_{1c} was 9.80±0.50% & 5.70±0.38%, TG was 163.76±99.46 & 155.67±94.84 mg/dl. About 19% patients in LDN & 36% in EDN were free of diabetic complications other than DN. In LDN, 20% had one complication, 29% had two & 32% had more than two complications. On the other hand, in EDN the corresponding values were 48%, 10% & 6% respectively. The most frequent complication was cardiopathy, which affected 33% patients in LDN & 27% in EDN, followed by peripheral neuropathy 21% & 18%, retinopathy 17% & 13%, and vasculopathy 10 % & 6% respectively. The average annual cost of care was US$ 27954 (direct US$ 16983 & indirect US$ 10971), with an average US$ 140 per patient. Among the average annual cost LDN consumed US$ 19837 (US$ 198 per patient) & EDN US$ 8117 (US$ 81 per patient). US$ 13473 (48%) of costs was attributable to drugs for both groups of which US$ 10817 (80%) was for LDN & US$ 2656 (20%) for EDN, US$ 8739 (31%) to hospitalizations of which US$ 5211 (60%) for LDN & 3528 (40%) for EDN. In case of diagnostics & visits the corresponding values were US$ 2136 (60%) & 1419 (40%) and US$ 1673 (76%) & 514 (24%) for LDN & EDN respectively. The annual medical costs increased with the increased number of complications from US$ 1320 to 2296 & to 3989 in LDN with one, two & more than two complications (other than DN) which is increasing at a rapid rate and US$ 917 to 1556 & to 2372 in EDN respectively, increasing at a diminishing marginal rate. The regression equation showed that medical cost is significantly related to complications tested in both univariate (P<0.0001) & multiple linear regression analyses (R^2=0.52; F=82.3, P<0.0001).
Conclusion: Proper management with regular screening substantially reduces the expenditure related to care of patients with diabetic nephropathy & related complications even in a developing country. Strategies aimed at preventing diabetic nephropathy & early detection of the onset of nephropathy complication will reduce medical costs in a substantial way.

PS 93 Nephropathy - experimental

1046

Defensive role of mitochondrial fusion against podocyte injury in diabetic nephropathy

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Background and aims: Podocytes are highly specialized cells covering the exterior glomerular basement membrane surface of the glomerular capillary, and constitute the final barrier to prevent albumin excretion from the kidney. Podocyte injury is one of the main events that lead to diabetic nephropathy. Podocytes have large amount of mitochondria, but pathogenic role of podocyte mitochondria in diabetic nephropathy is unknown. We unexpectedly found that podocyte mitochondria in animal models of diabetic nephropathy are elongated. We thus investigated the pathogenic role of this mitochondrial pathology (mitochondrial fusion) in diabetic nephropathy.
Materials and methods: Podocyte mitochondrial morphology was examined in the kidneys of two animal models of diabetic nephropathy; Otsuka Long-Evans Tokushima Fatty (OLETF) rats, obese type 2 diabetic animals, and streptozotocin-induced diabetic rats that underwent unilateral nephrectomy. We also examined the effects of combination of various risk factors of diabetic nephropathy i.e., high glucose, high free fatty acid and angiotensin (GFA), on cell apoptosis and mitochondrial morphology in cultured podocytes.
Results: OLETF rats exhibited pronounced albuminuria from 20 weeks of age ($P < 0.001$ vs age-matched control LETO rats). Focal and segmental sclerotic lesions and an increase in desmin expression, a marker of podocyte injury, were noted in the kidney of OLETF rats at 44 weeks of age. Electron microscopy showed extensive effacement with distorted interdigitation of foot processes in podocytes of OLETF rats. In addition, elongation of mitochondria was seen in podocytes of OLETF rats, starting from 12 weeks of age and peaking at 32 weeks of age. This elongation (fusion) of mitochondria was not found in mesangial cells or endothelial cells. At 44 weeks, discontinuation and obliteration of mitochondrial cristae were also noted, and mitochondrial number was markedly lower in OLETF rats than in LETO rats ($P < 0.05$). Consistent with the results in OLETF rats, mitochondrial elongation was also found in the podocytes of type 1 diabetic rats that underwent unilateral nephrectomy. GFA induced significant cellular apoptosis in cultured podocytes ($P < 0.001$ vs untreated cells). Podocytes cultured with GFA displayed morphologic features of fusion and also an increase in the expression of fusion-associated protein, OPA1. OPA1 expression in podocytes was preceded by an increase in the mitochondrial reactive oxygen species (ROS) production as measured by mitoSOX. In addition, downregulation of OPA by siRNA significantly increased cellular apoptosis and mitochondrial ROS production ($P < 0.05$ vs control siRNA-transfected cells), indicating that mitochondrial fusion is an adaptive process to prevent podocyte apoptosis in states of high ROS.
Conclusion: Podocyte mitochondrial fusion is a defensive mechanism that protects the cells from injury in diabetic nephropathy. Podocyte mitochondrial fusion machinery can become a previously unrecognized target to control diabetic nephropathy.
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1047

FT011, a novel synthetic anti-fibrotic drug attenuates the progression of experimental diabetic nephropathy

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Background and aims: Pathological fibrosis is a key feature of diabetic nephropathy (DN) that correlates closely with renal dysfunction and that can be partially attenuated with agents that block the renin-angiotensin system. However, the effects of a more direct approach that specifically targets fibrosis has not been studied in detail, mostly due to a lack of suitable agents. Accordingly, we synthesized a novel anti-fibrotic drug based on a cinnamoyl core structure, FT011 (Fibrotech Therapeutics Pty Ltd, Melbourne, Australia), to

test the hypothesis that preventing pathological fibrosis will attenuate renal injury in advanced experimental model of DN.
The aims of the present study were to determine the effects of FT011 on TGF-beta stimulated matrix synthesis on cultured mesangial cells, and to test the efficacy of FT011 *in vivo* on the structural and functional manifestation of experimental DN.
Materials and methods: Mesangial cells (1097 clone) were studied *in vitro* to determine the effects of FT011 on TGF-β induced collagen synthesis by measuring ^{3}H-proline incorporation. Forty, six-week old female, heterozygous (mRen-2)27 rats, were assigned to receive either 55 mg/kg of STZ or citrate buffer alone (non-diabetic) by tail vein injection following an overnight fast. Control and diabetic groups were then each randomised into 2 groups (n=10), receiving either treatment with FT011 (100mg/kg bid gavage) or without treatment for 16 weeks.
Results: In response to TGF-beta stimulation, mesangial cells demonstrated a dose-dependent inhibition of proline incorporation in response to FT011. Without affecting blood pressure or hyperglycaemia, diabetic Ren-2 rats treated with FT011 had less albuminuria (10.10 ± 1.11 *vs* 1.97 ± 1.46, geometric mean ± tolerance factor mg/day, $P < 0.01$), tubulointerstitial fibrosis (2.28±0.46 *vs* 0.64±0.07, %/area, $P < 0.01$), glomerulosclerosis (1.05±0.05 *vs* 0.63±0.04, glomerulosclerotic index $P < 0.05$), macrophage infiltration (62±16 *vs* 26±5, number/area $P< 0.05$) and glomerular endothelial cell loss (1.04±0.5 *vs* 4.1±0.9, %/glomerular cross section, $P < 0.05$) when compared with untreated diabetic rats. Non-diabetic rats were unaffected by FT011 and no adverse effects of FT011 were observed in either diabetic or non-diabetic groups.
Conclusion: FT011 is a novel anti-fibrotic drug that attenuates the functional and structural manifestations of experimental model diabetic nephropathy, providing a novel therapeutic strategy for the treatment of progressive kidney disease.
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1048

Antioxidant tempol reduces albuminuria by decreasing PARP-induced podocyte apoptosis in diabetic hypertensive rats
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Background and aims: It has recently been demonstrated that in diabetic hypertensive rats a superoxide desmutase mimetic (SOD), tempol, reduces albuminuria by restoring the redox imbalance. Increased formation of intracellular reactive oxygen species (ROS) leading to activation of poly(ADP-ribose) polymerase (PARP)-1 and podocyte loss by apoptosis contributes to albuminuria in diabetes mellitus (DM). In the present study we investigate the hypothesis that in diabetic hypertensive rats tempol reduces albuminuria by inhibition of PARP-induced podocyte apoptosis.
Materials and methods: DM was induced in spontaneously hypertensive rats (SHR) by streptozotocin at 4 weeks of age. Blood glucose levels of ≥ 270 mg/L were considered diabetic. The diabetic rats were randomly assigned to receive or not tempol intraperitoneally at a dose of 250 mg/kg. After 20 days, rats were euthanized and kidneys were collected. Glomeruli isolation was performed by differential sieving technique. Apoptosis of renal cells was evaluated by TUNEL staining and expression of WT-1, a marker of podocyte, was estimated by immunohistochemistry. Nephrin expression was assessed by Western blot analysis and immunofluorescence. PARP-1 expression, a nuclear enzyme activated by DNA strand breaks due to oxidative stress, was assessed by Western blot analysis.
Results: Plasma glucose levels were higher in diabetic rats and it was not affected by tempol treatment (169±24 *vs.* 482±52 *vs.* 444±276 mg/dl, respectively, p<0.0001). Systolic blood pressure was unaltered by diabetes or tempol treatment, (control=159±11, untreated diabetic=156±7, tempol diabetic=152±9 mmHg). Albuminuria was significantly higher in diabetic rats (680 (180 - 1815) μg/24h), compared to the control rats (229 (109 - 540), p=0.012) and it was significantly reduced by tempol treatment (366 (109 - 1342), p=0.052). DM increased the number of glomerular apoptotic cells compared to the control group (p=0.0013), and it was markedly reduced by tempol (p=0.0057). DM leads to podocyte loss as accessed by immunohistochemistry for WT-1 (p=0.0143) that was reduced in tempol treated rats (p=0.0369). Similarly, the incubation of isolated glomeruli with high glucose concentration (mimicking DM) or with H_2O_2 (ROS) leads to higher number of apoptotic cells compared with respective controls. Number of apoptotic cells was significantly reduced when isolated glomeruli were incubated with tempol. The expression of nephrin was decreased in the glomeruli of diabetic SHR and restored by tempol treatment. DM increases the expression of PARP-1 in isolated glomeruli from 2490±1801 to 7337±1128 arbitrary units (p=0.0379), and it was markedly reduced (p=0.0262) by tempol treatment to 1977±3242.
Conclusion: In diabetic hypertensive rats treatment with antioxidant tempol reduces albuminuria by prevention of PARP-induced podocyte apoptosis.
Supported by: FAPESP, CAPES and CNPq

1049

Upregulation of chemokines induced by AGEs was inhibited by Chinese herbal medicine YuQuiQing
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Background and aims: Previous work shown that advanced glycosylation end products (AGEs) increased the expression of chemokines in human renal mesangial cells (HRMCs). The aim of this study was to further investigate the effect of a Chinese traditional drug YuKuiQing on the upregulation of monocyte chemoattractant protein-1 (MCP-1) and fractalkine (FKN) induced by AGEs.
Materials and methods: HRMC cultured in the serum-free medium was incubated with AGE-BSA in the presence or absence of neutralizing anti-MCP-1 and anti-FKN antibody (Ab). HRMC incubated with AGE-BSA was cultured in the medium containing rat serum or special YuKuiQing serum which prepared by using Chinese herbal medicine serum pharmacological approach. The capacity of monocytes transmigration to HRMC was detected with a transwell system. The expression of MCP-1 and FKN mRNA in HRMC were analyzed by RT-PCR, and the content of MCP-1 and FKN in the supernatant of HRMC was measured by ELISA.
Results: 1. The number of monocytes transmigration to HRMC treated with AGE-BSA was significantly higher than control group (17.8±2.04 vs 0.7±0.84, $P<0.01$). But that of anti-MCP-1 Ab (8.6±1.14, $P<0.01$) and anti-FKN Ab (2.2±1.10, $P<0.01$) group was lower than AGE-BSA group. 2. The number of monocytes transmigration to HRMC treated with AGE-BSA in the presence of different concentration of rat serum (0.313%, 0.625%, 1.25%) was increased dose dependently (19.6±0.89, 23.6±1.14, 27.2±0.84, $P<0.05$). But those of YuKuiQing serum groups (17.0±1.00, 14.8±0.84, 12.0±1.22, $P<0.01$) were decreased compare to similar concentration of control serum. 3. The mRNA expression (FKN: 1.03±0.05 vs 0.22±0.05, MCP-1:0.74±0.05 vs 0.26±0.03, $P<0.01$) and the supernatant content (FKN: 289.49±9.77 vs 20.15±8.84 pg·L^{-1}, MCP-1: 33.47±3.14 vs 16.19±0.66 pg·L^{-1}, $P<0.01$) of MCP-1 and FKN in the HRMC treated with AGE-BSA was significantly higher compared to control group. 4. The mRNA expression (FKN: 0.950±0.051 vs 1.106±0.045, 0.885±0.041 vs 1.148±0.044, 0.833±0.040 vs 1.199±0.044,$P<0.01$. MCP-1: 0.724±0.043 vs 0.800±0.060, 0.663±0.031 vs 0.836±0.055, 0.604±0.028 vs 0.856±0.053,$P<0.01$) and the supernatant content (FKN: 280.19±9.31 vs 310.87±10.70, 268.41±9.77 vs 332.57±9.31, 251.98±9.80 vs 343.73±6.33 pg·L^{-1}, $P<0.01$. MCP-1: 27.49±2.16 vs 33.69±3.24, 26.77±2.13 vs 34.03±3.31, 26.06±1.65 vs 34.72±3.01 pg·L^{-1}, $P<0.01$) of MCP-1 and FKN in the HRMC treated with AGE-BSA in the presence of different concentration of YuKuiQing serum was lower than control serum.
Conclusion: 1. AGE-BSA enhance the capacity of MCP-1 and FKN to induce monocytes transmigration to HRMC, which are inhibited by anti-MCP-1 and anti-FKN antibody partially. These findings suggest that MCP-1 and FKN might mediate the role of AGEs in the development of diabetic nephropathy. 2. YuKuiQing markedly reduce the upregulation of MCP-1 and FKN induced by AGE-BSA, and attenuate the capacity of MCP-1 and FKN to induce monocytes transmigration to HRMC, These results indicate that YuKuiQing might play its role of reno-protection via inhibition of MCP-1 and FKN.
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1050

Metabolomic profile delineates potential defect in renal transporter function in the proximal tubule of monkeys with diabetic nephropathy

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Background and aims: Non-invasive biomarkers that predict future disease development will enhance efforts to identify the earliest indications of type 2 diabetes mellitus (T2DM). The urinary metabolomes of well-characterized rhesus macaques that were normal, prediabetic, or overtly spontaneously and naturally diabetic, and had been held under consistent, healthy dietary and environmental conditions, were examined to discover biochemical indicators of T2DM disease pathogenesis and progression.
Materials and methods: The rhesus monkeys selected for this study were under longitudinal study and were fully phenotyped for glucose and lipid metabolism, insulin sensitivity, beta cell function, and renal function prior to collection of clean urine samples. High-resolution ultra-performance liquid chromatography and the accurate mass determination of time-of-flight mass spectrometry were used to analyze spot urine samples from normal (n = 10), pre-T2DM (n = 5), and T2DM (n = 11) male monkeys. Models generated using the random forests machine learning algorithm revealed candidate biomarkers from approximately 8500 ions. Biomarkers were identified based on accurate masses and confirmed by tandem mass spectrometry of authentic compounds.
Results: Random forests models had a misclassification error less than 5%. Urinary compounds significantly increased in T2DMs compared with normals included glycine betaine (9-fold above normal), citric acid (2-fold above), kynurenic acid (1.8-fold above), glucose (67-fold above), and pipecolic acid (2.5-fold above). Compared with the conventional definition of T2DM, the biomarker-based definitions were also useful in defining the three groups.
Conclusion: Elevation of these urinary biomarkers suggest defective renal transporter function in the proximal tubule. This non-invasive urinary metabolomics approach is likely to be valuable in developing a more detailed understanding of complex diseases such as T2DM.
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1051

Kidney injury molecule 1 (KIM1) and neutrophil gelatinase-associated lipocalin (NGAL) as markers of tubular kidney injury in type 1 diabetic patients

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Background and aims: Urinary(u) KIM1 and u-NGAL have been suggested as markers of tubulointerstitial damage in renal disease, mostly acute renal failure. In kidney injury, KIM1 is present on the proximal tubule apical membrane. Inflammation increases NGAL production in tubular cells. Our aim was to evaluate u-KIM1 and u-NGAL in type 1 diabetic patients with different levels of albuminuria and in controls. Subsequently we studied the effect of renoprotective treatment with the ACE inhibitor lisinopril on the level of u-NGAL.
Materials and methods: Cross sectional study of adult Caucasian type 1 diabetic patients from a tertiary referral centre. The patients were divided into three groups: normoalbuminuria (u-albumin <30mg/ 24h), microalbuminuria (u-albumin 30-300mg/24h) and macroalbuminuria (>300 mg/24h) based on three 24h urine collections. We measured u-KIM1 (ELISA, Roche®) and u-NGAL (ELISA, Bioporto®) in morning spot urine adjusted for creatinine concentration. We included 55 patients with normoalbuminuria, 42 with microalbuminuria and 44 with macroalbuminuria. Control group: 53 healthy controls. The groups were matched by gender and duration of diabetes. In addition, in 56 T1D patients with diabetic nephropathy a double blind, placebo controlled randomized crossover trial was performed. After 2 months washout period (baseline), patients were treated with lisinopril 20, 40, and 60 mg once daily, each dose for 2 months. U-NGAL was measured in 24h urine samples after each treatment period.
Results: U-KIM1 [(geometric mean (95%CI), pg/ml]: Normoalb 3.42 (2.62-4.49), microalb 4.06 (3.15-5.23), macroalb 4.17 (3.04-5.71), control 1.50 (1.11-2.03). u-KIM1 was higher in all diabetic groups than in controls (p<0.001). No difference in u-KIM 1 between diabetic groups. No association were found between u-NGAL and age, sex, eGFR, cholesterol, BMI or blood pressure. U-NGAL [Geom.mean (95% CI)]: normoalb 147 (98-221) (ng/mmol creatinine), microalb 225 (159-318), macroalb 257 (173-380), controls 74 (52-104). U-NGAL was higher in macroalb than normoalb and controls (p<0.05). Microalb was not different from macroalb or normoalb. U-NGAL was higher in normoalb vs. controls (p<0.01). U-NGAL was elevated in women and increased with age. No association with cholesterol, BMI or blood pressure. U-NGAL was correlated with u-albumin/creatinine ratio (R^2=0.18, p<0.01) and with eGFR in the macroalb group (R^2=0.15, p<0.01).
U-NGAL did not change significantly during lisinopril treatment: u-NGAL (geometric mean (SD): baseline 8.9(2.5), 20 mg 7.7(2.5), 40 mg 7.9(2.3) 60 mg 7.9(5.4) (N.S)
Conclusion: U-NGAL and u-KIM1 are elevated in type 1 diabetes, with or without albuminuria, indicating tubular damage. U-NGAL, but not u-KIM1, increases significantly with increasing albuminuria suggesting that u-NGAL is a better marker of diabetic nephropathy than u-KIM1.
Short-term treatment with lisinopril does not affect u-NGAL significantly, suggesting that lisinopril does not affect the renal damage reflected by u-NGAL.

1052

Bezafibrate suppresses the expression of TGF-β and type IV collagen mRNA in human mesangial cells loaded with remnant lipoproteins

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Background and aims: Diabetic nephropathy is one of the main causes of death in diabetic patients. Hyperglycemia and hypertension are risk factors for diabetic nephropathy. Recently it has been proposed that hypertriglyceridemia contributes to diabetic nephropathy. In JDCS (Japan Diabetes Complications Study) and UKPDS (UK Prospective Diabetes Study) 74, hypertriglyceridemia was detected as one of the risk factors for diabetic nephropathy. In FIELD (Fenofibrate Intervention Event Lowering in Diabetes) study, fenofibrate therapy reduced plasma level of triglyceride (TG), and prevented and improved diabetic nephropathy in type 2 diabetic patients. Increased remnant lipoproteins underlie hypertriglyceridemia. We first found that increased remnant lipoproteins contribute to diabetic nephropathy. It is proposed that remnant lipoproteins are taken up by mesangial cells, and then cause renal damage. Diabetic nephropathy is characterized by the accumulation of extracellular matrix protein (type IV collagen) in the glomerular mesangium and expansion of the mesangial matrix, resulting in glomerulosclerosis. TGF-β stimulates type IV collagen protein synthesis. Fibrate, which is a ligand for PPARα, reduces plasma levels of TG and remnant lipoproteins and possibly may protect and improve nephropathy thorough PPARα.In the present study we examined the effect of bezafibrate on TGF-β and type IV collagen expression in human mesangial cells (HMCs) loaded with remnant lipoproteins in the medium.
Materials and methods: Remnant lipoproteins were isolated from plasma of a patient with type 2 diabetes and type III hyperlipoproteinemia (apo E2/2 genotype) by ultracentrifugal method. HMCs loaded with remnant lipoproteis were incubated for up to 24 h with bezafibrate at the concentration of 0, $3x10^{-6}$, $3x10^{-5}$ and $3x10^{-4}$ mol/L. To evaluate the expression of PPARα mRNA, TGF-β mRNA and type IV collagen m-RNA in mesangial cells, RT-PCR procedure was performed. All data were analyzed using the expression of the gene encoding GAPDH as a reference.
Results: Remnant lipoproteins significantly (p<0.001) stimulated the expression of TGF-β mRNA (1.38 vs baseline 0.57) and type IV collagen mRNA (0.92 vs baseline 0.72) in HMCs. Bezafibrate significantly (p<0.001) stimulated the expression of PPARα mRNA (1.33 vs baseline 0.20) in HMCs loaded with remnant lipoproteins. Bezafibrate significantly (p<0.001 or p<0.01) suppressed the expression of TGF-β mRNA in HMCs loaded with remnant lipoproteins in a dose-dependent manner (0.82 at $3x10^{-6}$, 0.67 at $3x10^{-5}$ and 0.65 at $3x10^{-4}$ mol/L vs. 1.38 at 0 mol/L). Bezafibrate significantly (p<0.001 or p<0.05) suppressed the expression of type IV collagen mRNA in HMCs loaded with remnant lipoproteins in a dose-dependent manner (0.74 at $3x10^{-6}$, 0.68 at $3x10^{-5}$ and 0.69 at $3x10^{-4}$ mol/L vs. 0.92 at 0 mol/L).

Conclusion: Remnant lipoproteins stimulated the expression of TGF-β and type IV collagen in HMCs. It is concluded that remnant lipoproteins play an important role in the development and progression of diabetic nephropathy through increased TGF-β and type IV collagen. In addition, bezafibrate suppressed the expression of TGF-β and type IV collagen in HMCs loaded with remnant lipoproteins. It is suggested that fibrate is effective to protect and improve diabetic nephropathy in early diabetes as shown in FIELD study.
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1053

The orphan nuclear receptor small heterodimer partner attenuates renal fibrosis in obstructive nephropathy

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Background and aims: The accumulation of extracellular matrix proteins is the key feature of chronic fibrotic kidney disease including diabetic nephropathy. Accumulating evidence suggests that transforming growth factor-β (TGF-β) and plasminogen activator inhibitor type 1 (PAI-1) act as central components in the development of renal fibrosis by stimulating matrix protein generation and inhibiting matrix protein removal. Previously, we demonstrated that small heterodimer partner (SHP) represses PAI-1 expression in the liver by inhibiting the TGF-β signaling pathway. Therefore, we examined whether SHP prevents renal fibrosis in the unilateral ureteral obstruction (UUO) model and elucidated its mechanism in cultured renal cells.
Materials and methods: The effects of adenovirus mediated overexpression of SHP on PAI-1 expression in Rat mesangial cell (RMC)s and renal tubular epithelial cells (NRK-52E cells) were measured by Northern blot analysis. Transient transfection study with reporter constructs of PAI-1 promoter was performed to measure the effect of SHP on PAI-1 promoter activity. The effects of SHP on Smad 3-DNA binding activity were examined by electrophoretic mobility shift assay. The effects of SHP on renal interstitial fibrosis in vivo were assessed by UUO-induced renal fibrosis model.
Results: UUO markedly increased the expression of PAI-1, type I collagen, and fibronectin but decreased SHP gene expression. Moreover, in kidneys of SHP-/- mice, the expression levels of PAI-1, type I collagen, fibronectin and α-smooth muscle actin (α-SMA) were increased compared with those in kidneys of wild-type mice. In addition, loss of SHP accelerated renal fibrosis after UUO. Adenovirus-mediated overexpression of SHP in cultured RMCs and NRK-52E cells inhibited TGF-β-stimulated PAI-1, type I collagen, and fibronectin expression. SHP inhibited TGF-β- and Smad3-stimulated PAI-1 promoter activities and TGF-β-stimulated Smad3 binding to its response consensus element on the PAI-1 promoter. Moreover, up-regulation of SHP expression in the kidney by adenovirus expressing SHP inhibited the expression of UUO-induced PAI-1, type I collagen, fibronectin, and α-SMA.
Conclusion: This study shows that SHP prevents renal fibrosis in obstructive nephropathy and raises the possibility that SHP can be a target for the prevention of renal fibrosis.

1054

Preliminary study of bone marrow mesenchymal stem cells on diabetic nephropathy

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Background and aims: Mesenchymal stem cells (MSCs) have been widely studied in protecting against acute renal failure in animals, but studies on the role of MSC in models of chronic kidney disease are largely lacking. Diabetic nephropathy is a very common complication of diabetes mellitus. Current therapies can only delay its progress. In the present study, we aim to discover whether MSC transplantation could treat or attenuate diabetic nephropathy in rats.
Materials and methods: Sprague-Dawley (SD) rats were randomized to five groups: control group (C), diabetic nephropathy control group(DN), ciclosporin A (CsA) group, MSC group, and MSC+CsA group. Bone marrow stem cells were cultured, identified and labeled by 5-bromo-2'-deoxyuridine (BrdU) in vitro. Then they were transplanted to rats with diabetic nephropathy via introcardiac infusion(2×10^6MSCs/200μl). Ciclosporin A was administered daily after transplantation at a concentration of 5mg/kg body weight in CsA group and MSC+CsA group. 1, 2, 4, 8 week after transplantation, rat blood glucose, body weight (BW), kidney weight (KW), urine protein, urine micro albumin, endogenous creatinine clearance rate (Ccr) and profile of kidney hypertrophy (KW/BW) were tested. Renal morphology and labeled cells were examined in the kidney.
Results: 1. The cultured bone marrow stem cells expressed mesenchymal cell phenotype, and could be multidifferentiated to osteogenic and adipogenic cells.
2. Labeled stem cells could be detected in the kidney of nephropathic rats, but they did not propagate after engrafting in kidney.
3. Blood glucose in MSC+CsA group was significantly lower than that in MSC group, CsA group and DN group, but higher than control group at day 3 and 7 after transplantation; blood glucose in MSC group had a transient decrease at week 2, but increased to pretreatment level at week 8 for two groups.
4. At week 4, urine protein in MSC+CsA group was lower compared to MSC group, CsA group and DN group, but higher than control group. The 24h urine micro albumin in MSC+CsA group became significantly lower than other diabetic groups at week 1 and remained low in the first 4 weeks post transplantation; in MSC group urine micro albumin decreased at week 4, but rebounded to the pretreatment level at week 8. At week 1 and 2 urine micro albumin in MSC+CsA group was lower than that in MSC group. At all time points the Ccr in MSC+CsA group and MSC group were higher than control group, though without significant difference, while they were much higher in CsA group and DN group at week 4 compared to control group. KW/BW in MSC+CsA group was significantly lower than that in CsA group and DN group, though still higher than control group at week 2. The morphology of glomerulus was ameliorated after bone marrow stem cell transplantation, while the morphology of renal tubles was improved by CsA.
Conclusion: The cultured bone marrow stem cells express mesenchymal cell phenotype with multilineage potential. Bone marrow stem cells can track to the kidney of diabetic nephropathic rats after cardiac injection. Mesenchymal stem cells can ameliorate diabetic nephropathy temporarily, but long term effects are limited.

PS 94 Nephropathy - clinical 1

1055

Renal hyperfiltration in relation to low renal function and incidence of overt nephropathy among individuals with type 1 diabetes

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Background and aims: It has been suggested that renal hyperfiltration predicts later deterioration of renal function, though the evidence is conflicting. We thus assessed this hypothesis in a large cohort of individuals with childhood onset type 1 diabetes (diagnosed <17 years of age).
Materials and methods: Participants of the Pittsburgh Epidemiology of Diabetes Complications study cohort with estimated (by the Cockcroft-Gault formula) glomerular filtration rate (eGFR) ≥60 ml/min/1.73 m^2 and free of overt nephropathy (ON, albumin excretion rate<200 μg/min in 2/3 timed urine collections) were selected (n=423). At study entry, mean age was 26 years and diabetes duration 18 years. Hyperfiltration was defined as eGFR ≥130 ml/min/1.73 m^2. The outcomes studied were low renal function (LRF, below 60 ml/min/1.73 m^2) and incidence of ON (albumin excretion rate ≥200 μg/min in 2/3 validated timed urine collections, renal dialysis or transplantation). Descriptive analyses (t-tests for continues variables and chi-square tests for categorical variables) were conducted to assess univariate associations between study participant characteristics at study entry and subsequent LRF and ON incidence. Multivariable Cox proportional hazard models were constructed to assess the value of hyperfiltration in predicting subsequent LRF and ON.
Results: During 18 years of follow-up, 9.3% (39/381) progressed to LRF, whereas 16.5% (70/423) developed ON. No univariate association was observed between presence of hyperfiltration at study entry and either LRF (25.6% vs. 33.6%, p=0.31) or ON incidence (34.3% vs. 33.1%, p=0.85). However, in multivariable Cox proportional hazard models, adjusting for univariately significant variables, including baseline eGFR level and diabetes duration, hyperfiltration emerged as a strong risk factor for LRF (HR=4.58, 95% CI=1.27-16.45). Nevertheless, the increased risk associated with baseline hyperfiltration in relation to incident ON, did not reach statistical significance (HR=1.44, 95% CI=0.85-2.44). When analyses were repeated using the MDRD study formula to estimate GFR, there was no increased risk for LRF associated with hyperfiltration (HR=1.19, 95% CI=0.76-1.87), whereas a borderline increased risk was observed for ON incidence (HR=1.82, 95% CI=0.92-3.62, p-value=0.08), which became significant (p-value=0.02) with further adjustment for weight (as is included in the Cockcroft-Gault formula).
Conclusion: These data suggest that hyperfiltration may increase the risk of low renal function and overt nephropathy, although the strength of such associations appears dependent on the formula used to estimate glomerular filtration rate.
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1056

Prognostic value of contrast-induced nephropathy in patients with diabetes mellitus type 2

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Background and aims: To determine the incidence of contrast-induced nephropathy (CIN) after percutaneous coronary intervention (PCI), to reveal independent predictors and determine the prognostic value of CIN among patients with and without diabetes mellitus (DM) type 2.
Materials and methods: We have studied 151 patients with DM and 50 patients without DM who were under coronarography to determine further intervention on the heart vessels. The CIN defined as an increase in serum creatinine (SCr) of at least 25% or 44μmol/l over baseline within 48 hours of PCI.
Results: Baseline SCr, glomerular filtration rate were similar in diabetic and non-diabetic patients. The incidence of CIN was significantly higher among the diabetic, than non-diabetic patients (40.4±4.4% vs. 16.0±5.5% respectively, p<.01). CIN was associated with anaemia -OR=2.19 with 95%CI (0.96,4.99), contrast volume administrated -OR=2.01 per 100ml of medium 95%CI (1.35,2.98), low (<40%) ejection fraction -OR=3.25 with 95%CI (1.15,9.05), diuretics use -OR=2.59 with 95%CI (1.002,1.017), multivessel coronary involvement -OR=2.97 with 95%CI (1.34,6.59), congestive heart failure (CHF, NYHA functional classification III/IV) - OR=4.68 with 95%CI(1.69,12.95). Duration of hospital stay was 17.7±1.3 days in diabetics with CIN, 11.9±0.9 day without CIN (p<0.0001). Importance of CIN was confirmed in Cox time-dependent Proportional-Hazards Model, that had defined survival factors - RR=2.64 with 95%CI (1.17,5.97). CIN was determined as a significant predictor of death, new or hospitalization for CHF, myocardial infarction, stroke, doubling of SCr, coronary artery bypass surgery within 24 months of follow up according to the Kaplan-Meier survival method (p<0.0001).
Conclusion: The patients with CIN had poor prognosis. Control risk factors should be intensified to protect them from future adverse events.

1057

The relevance between endogenous creatinine clearance rate and islet beta cell function in type 2 diabetic patients

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Background and aims: To investigate the relevance between islet β cell insulin secretion, insulin sensitivity and endogenous creatinine clearance rate (Ccr) in type 2 diabetic patients.
Materials and methods: 356 cases of type 2 diabetic patients were divided into 125 cases with normal renal function(90≤Ccr<130 ml/min*1.73^2), 140 cases with mild renal dysfunction(60≤Ccr<90 ml/min*1.73^2) and 91 cases with moderate and severe renal dysfunction (Ccr<60 ml/min*1.73^2). Oral glucose tolerance (OGTT) and insulin releasing test were performed. The insulin resistance (IR) was evaluated by the ratio of area under curve of insulin to area under curve of glucose (AUC_I/AUC_G) and HOMA-IR(HOMA-IR = FG×FI/22.5. The insulin sensitivity was reflected by insulin sensitivity index (ISI) [ISI=-ln(FI×FG)] and Matsuda ISI[Matsuda ISI =10,000/√(FG×FI×G_{mean}×I_{mean})]. The HOMA-β, early insulin secretion index (ΔI30/ΔG30), the second-phase insulin secretion index (AUC_I) and Disposition Index (DI)[DI=ΔI30/ΔG30/ HOMA-IR] were calculated to estimate islet β cell secretary function. The difference of clinical and biochemical parameters were compared among the three groups and the correlation between Ccr and IR was analyzed.
Results: (1) Compared with the normal renal function group, the HOMA-IR of the mild, moderate and severe renal dysfunction groups was significantly higher(P< 0.01), while the ISI and Matsuda ISI were significantly lower(P<0.01); The AUC_I/AUC_G of the moderate and severe renal dysfunction group was higher than that of the other two groups(P<0.05). (2) The AUC_I of the moderate and severe renal dysfunction group was higher than that of the other two groups (P<0.05); the DI of the mild, moderate and severe renal dysfunction groups was significantly higher than that of the normal renal function group (P<0.05). (3) Ccr was negatively correlated with the course of diabetes, systolic blood pressure, diastolic blood pressure, AUC_I, HOMA-IR, AUC_I/AUC_G (P<0.05) and was positively correlated with ISI, Matsuda ISI (P<0.01); Multiple stepwise regression analysis showed that Ccr was negatively correlated with systolic blood pressure,course of diabetes and AUC_I/AUC_G (P<0.05).
Conclusion: Ccr and IR were negatively correlated, so IR may be independent risk factor of renal dysfunction among type 2 diabetes patients.

1058

The early diagnosis of renal disorders in diabetes mellitus patients

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Background and aims: Diabetic nephropathy is one of the heaviest complications of diabetes mellitus - occupies a leading place among the main reasons of death rate in such patients. People with type 1 diabetes make more than half of all hemodialis patients. Diabetic nephropathy develops gradually and it clinical signs shows up already on the expressed stage of renal defeat.
Materials and methods: The examination was conducted on the gamma-chamber using renotrophic substance - dietilen-triaminpentaacetate (DTPA), marked

with short-life radionuklide of 99m-Ts(technetium), which hatches from an organism by urinary system and its leading out speed depends on glomerular filtration.Depending on the level of Hba1c, indexes of serum glucose levels and clinical signs of disease we estimated the state of compensation and decompensation of type 1 diabetes. Compensation of diabetes was considered at the indexes of serum glucose level from 6 to 8 mmol/l for a day long, level of HbA1c up to 6.5%, and also practical absence of hypoglycemia and ketoacidosis.
Results: We examined 50 patients with type 1 diabetes in the stage of compensation (29.7 + 3.4 years): in 38 of them (diabetic nephropathy of the II - III stage, mikroalbuminuriya - 30-205 mg/day) under act of angiotensin-trasforming factor inhibitors there was a substantial improvement of lauter and egestiv renal functions, in 8 patients (diabetic nephropathy of the III - IV stage, mikroalbuminuriya - 280-450 mg/day) - we did not look after the substantial changes of scintigraphic indexes, in 4 patients (diabetic nephropathy of the IV stage, stable proteinuriya) - we observed worsening renal functions in spite of reception of preparations.
Conclusion: The received results testify that radioisotope dynamic renal scintigraphy allows to diagnose diabetic nephropathy on the early stages of development, when a process is reversible. Reliable improvement of renal functions under act of angiotensin-trasforming factor inhibitors and at the compensated diabetes mellitus it is observed only for patients with diabetic nephropathy of I - III stages.

1059

WITHDRAWN

1060

The occurrence of impaired renal function and hypoalbuminaemia in the absence of macroalbuminuria in geriatric diabetic patients
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Background and aims: Albuminuria is considered as an early feature of diabetic nephropathy and a strong cardio-vascular risk marker. Yearly screening for increased urinary albumin excretion rate is currently recommended. However, the capacity of albuminuria to predict impaired renal function in elderly diabetic persons is unclear.
Materials and methods: We monitored renal function (serum creatinine and clearance according to the Cockcroft-Gault formula), urinary albumin excretion, albuminemia and nutritional markers in a survey of very old diabetic patients admitted to a geriatric service.
Results: 80 diabetic patients (mean age 82.8±7.4 years) have been included in this ongoing survey. Moderate to severe renal impairment (creatinine clearance <40 ml/min) and severe hypoalbuminemia (<30 g/l) were observed in 38% and 48% patients respectively. Age was associated with renal failure, hypoalbuminemia and high urinary albumin/creatinine ratio (ACR). The ACR was inversely associated with creatinine clearance (Spearman rho=-0.43 p=0.016) and albuminemia (rho=-0.42, p=0.16). However, macroalbuminuria (ACR>300 mg/g) was rarely observed in renal failure (23%) or hypoalbuminemia (20%). Albuminemia was more strongly related to malnutrition markers than to the ACR.
Conclusion: Impaired renal function and hypoalbuminemia are frequent in diabetic geriatric patients, but are only rarely accounted for by diabetic nephropathy as defined by macroalbuminuria. Hypoalbuminemia indicates associated malnutrition more often than diabetic nephropathy. Low creatinine clearance should be screened for along with albuminuria to detect impaired renal function in these patients.

1061

Increased intact urinary albumin excretion predicts development of microalbuminuria in normoalbuminuric type 2 diabetic patients - six-year follow-up study
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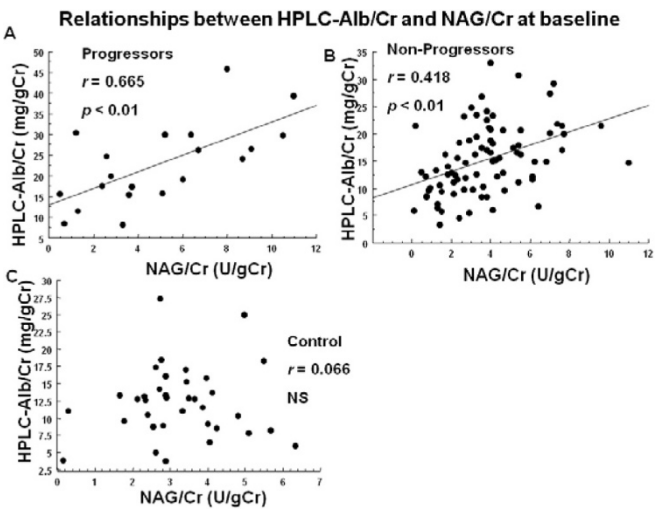
Background and aims: Conventional immunochemical urinary albumin assay underestimates certain portions of intact (not fragmented) albumin. High performance liquid chromatography (HPLC) method can detect total intact urinary albumin accurately. However a few studies reported advantage of HPLC method compared with immunochemical ones. This study evaluated whether increased intact urinary albumin excretion measured by HPLC (HPLC-U-Alb) method in normoalbuminuric type 2 diabetic patients would predict future development of microalbuminuria (MA) and how are the clinical characteristics of MA progressors with high HPLC-U-Alb level at baseline.
Materials and methods: We analyzed HPLC-U-Alb levels of first voided morning urine samples (geometric means of three serial samples) in 105 normoalbuninuric type 2 diabetic out-patients (mean age of 58.8 years; 51 males and 54 females) defined as normoalbuminuria (turbidmetric immunoassay urinary albumin [TIA-U-Alb] < 30mg/gCr in all three serial spot urine samples measured within one year prior to the registration) and followed during six years. Development of microalbuminuria were defined as two consecutive spot TIA-U-Alb > = 30mg/gCr in our outpatients clinic.
Results: Of 105 patients, 20 progressed to MA. Baseline HPLC-U-Alb in progressors were significantly higher (ANOVA followed by post-hoc Scheffe method) than in non-progressors and 36 control subjects (HPLC-U-Alb: 22.8 +/- 9.8, 15.5 +/- 6.7 and 12.3 +/-5.2 mg/gCr, respectively). Baseline TIA-U-Alb in progressors was also significantly higher than in non-progressors and controls. However, HPLC-U-Alb did not significantly correlated with TIA-U-Alb in progressors (r = 0.015). In contrast, HPLC-U-Alb significantly correlated in non-progressors and controls (r = 0.391 and 0.401, respectively). Urinary N-acetylglucosaminidase (NAG) at baseline significantly correlated with HPLC-U-Alb in diabetic patients but not in controls (Fig). No correlations between NAG and TIA-U-Alb were found in either diabetic patients or controls. At baseline, progressors had higher HbA1C level, higher rates of having retinopathy and higher rate of insulin usage. Cox's proportional hazards model including several clinical characteristics of patients as variables indicated that higher HPLC-U-Alb at baseline was an independent predictor of MA development as well as having retinopathy and high age in our patients.
Conclusion: Increased HPLC-U-Alb in normoalbuminuric diabetic patients predicts future development of MA with relation with urinary NAG elevation, indicating tubular dysfunction of diabetic patients. Immuno-chemical measurement of U-Alb cannot detect this renal dysfunction in normoalbuminuric diabetic patients.



1062

Serum bilirubin level is associated with susceptibility to diabetic microvascular complications
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Background and aims: Bilirubin is an antioxidant and antiinflammatory substrate and the inverse association of serum bilirubin levels with the risk of coronary artery disease has been reported. We hypothesized that higher levels of bilirubin would reduce susceptibility to diabetic microvasculer complications.

Materials and methods: We compared serum total birilubin levels and the prevalence of nephropathy and retinopathy in 532 type 2 diabetic patients. Nephropathy positive was defined by the presence of microalbuminuria, overt proteinuria, or an elevated serum creatinine level.
Results: Bilirubin levels were higher in patients without than in those with complications (0.74±0.30 vs. 0.65±0.28 mg/dl, p<0.001, for nephropathy, 0.74±0.29 vs. 0.66±0.29 mg/dl, p=0.002, for retinopathy, mean±SD) Multiple logistic regression analysis, adjusted for other risk factors (sex, duration of diabetes, body mass index, hemoglobin A1c, hypertension, dyslipemia, smoking), showed a inverse association between the serum bilirubin levels and the prevalence of nephropathy (odds ration [OR] 0.35, 95% confidence interval [CI], 0.17-0.72, p=0.005) or the prevalence of retinopathy (OR 0.51, 95% CI, 0.25-1.02, p=0.057). We also found a positive correlation between bilirubin levels and estimated glomerular filtration rate (r=0.149, p<0.001) in multiple regression analysis.
Conclusion: Higher serum total bilirubin level is associated with reduced susceptibility to diabetic microvascular complications.

1063

Visceral adiposity and progression of diabetic nephropathy in patients with type 2 diabetes
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Background and aims: Visceral adiposity is strongly associated with insulin resistance. Many studies demonstrated that obesity and insulin resistance were known as one of progression factors for chronic kidney disease (CKD). Furthermore, there is a strong correlation between body mass index (BMI) and the relative risk of progression of CKD. Although waist circumference (WC) is a fairly good correlate of the amount of total abdominal fat, it cannot distinguish visceral adiposity from the amount of subcutaneous abdominal fat. Ultrasound (US) measurements have been recently shown to correlate better with visceral adiposity than anthropometric measurements. The aim of our study was to elucidate whether visceral fat thickness (VFT) measured by ultrasonography is associated with progression of diabetic nephropathy in type 2 diabetic patients.
Materials and methods: We recruited a total of 249 type 2 diabetic patients (116 men and 133 women) with CKD (≥ stage 2) by diabetic nephropathy and followed for 2.6±1.4 years. Renal function was evaluated by serum creatinine levels, estimated glomerular filtration rate (eGFR) (calculated by the Cockcroft-Gault equation) and urinary albumin/creatinine ratio (ACR). Anthropometric, clinical, and laboratory data were measured. US procedures were performed by the same examiner using a 3.5-MHz probe. US-determined VFT was defined as the distance between the internal face of the rectus abdominis muscle and the anterior wall of the aorta.
Results: Overall, the mean age was 56.3±10.9 years, duration of diabetes 11.5±7.0 years, HbA_{1C} 7.7±2.9%, ACR 859.4±562.7 mg/gCr, serum creatinine 2.0±1.2 mg/dL, and systemic blood pressure (BP) 149.5±25.3/91.6±15.2 mmHg. Mean calculated GFR was 36.1 ±29.6 mL/min/1.73m^2. Of the study population, 75 patients (30.1%) were smokers or exsmokers, 223 patients (89.6%) were having hypertension, and 237 patients (95.2%) were taking angiotensin converting enzyme inhibitor or angiotensin receptor blocker. BMI (r = 0.463; p< 0.001), WC (r = 0.511; p< 0.001), age (r = 0.356; p< 0.05), duration of diabetes (r = 0.216; p< 0.05), and HOMA-IR (r = 0.264; p< 0.05) were significantly correlated with US-determined VFT. But serum creatinine, eGFR, ACR, lipid profiles, HbA_{1C}, and systolic and diastolic BP were not correlated with VFT. Mean yearly change of eGFR was 5.8±4.1 mL/min/year. Yearly change of eGFR was positively correlated with baseline US-determined VFT (r = 0.271; p< 0.05). Changes of ACR or serum creatinine were not significantly correlated with VFT.
Conclusion: Our data suggests that chronic kidney disease is associated with an increased prevalence of visceral obesity and visceral adiposity measured by ultrasonography may be predictor of progression of diabetic nephropathy in patients with type 2 diabetes.

1064

Association between diabetic nephropathy and atherosclerosis
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Background and aims: The link between nephropathy, particularly microalbuminuria, and cardiovascular disease, is becoming increasingly apparent. Diabetic nephropathy and cardiovascular disease share many risk factors. We postulated that insulin resistance may have a role in the contributor to microalbuminuria and cardiovascular disease. The aim of this study was to assess the associations between diabetic nephropathy and structural changes in arteries, such as carotid intima-media thickness (IMT) in type 2 diabetic patients, and determine the pathogenesis.
Materials and methods: The subjects of this study were 56 type 2 diabetic patients (age 57.5±10.7 years, 34 males and 22 females). They were divided into 3 groups according to level of urinary albumin excretion (UAE); a group of 29 patients with normoalbuminuria (N-group), a group of 13 patients with microalbuminuria (M-group), and a group of 14 patients with macroalbuminuria (MA-group). The IMT of the common carotid artery was measured by ultrasonography. The following parameters were compared among the 3 groups; blood pressure, plasma glucose, insulin and lipid levels, and serum adiponectin levels. We assessed insulin resistance using fasting IRI levels and the products of insulin and free fatty acid (FFA) levels (IRI × FFA). Carnitine is required in mammalian tissue to transfer long-chain acyl CoAs across the inner mitochondrial membrane for β-oxidation. Plasma acylcarnitine level (ACAT) is a marker of mitochondrial β-oxidation. ACAT levels were measured in 18 out of 56 patients.
Results: No significant differences in age, duration of diabetes, BMI, smoking, or kind of antihypertensive agent used were found between the 3 groups. Systolic blood pressure in the M-group and MA-group were significantly higher (p<0.01) than in the N-group. There were no significant differences in HbA1c, lipid, serum creatinine or adiponectin levels among the 3 groups. IMT level in the M-group was 1.13±0.32 mm which was significantly higher than those in the N-group and MA-group (0.89±0.26 mm, 0.92±0.18 mm, respectively). Serum IRI level in M-group was 16.1±19.6μU/ml which was significantly higher (p<0.05) than those in the N-group and MA-group. A significant positive correlation was found between log UAE and serum IRI (r=0.46, p<0.05), and it was found, furthermore, that serum IRI level showed a significant positive correlation with IMT. Log UAE showed positive correlations with IRI×FFA (r=0.57, p<0.05) and ACAT levels (r=0.57, p<0.05), respectively.
Conclusion: Microalbuminuria was associated with structural changes in arteries, such as IMT in this study. Insulin resistance might contribute to the development of both macroangiopathy and diabetic nephropathy. Furthermore, ACAT as a marker of mitochondrial β-oxidation may contribute to the development of macroangiopathy in diabetic nephropathy.

PS 95 Nephropathy and treatment effects

1065

Do analogue insulins have nephroprotective effects?

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Background and aims: In diabetic patients with nephropathy an improved metabolic activity as well as a decrease of the postprandial hyperperfusion have been shown in case of the analogue insulin (AI) Lispro compared to human insulins (HI). Both factors could positively influence the nephropathy course through improved metabolic control and favourable renal hemodynamics. We investigated the influence of the treatment with AI or HI on renal functional parameters.
Materials and methods: The medical files of all outpatient type 1 diabetics between June 1, 2004 and December 31, 2006 have been reviewed and stratified according to the type of insulin therapy (AI, HI, mixed therapy). Exclusion criteria were pregnancy, dialysis, erythropoietin therapy, severe systemic disease, mixed insulin therapy (AI plus HI), insulin therapy shorter than 24 months. 68 diabetic patients received human insulin and 117 received analogue insulin (glargin 65%, lispro 59%, aspart 34%, detemir 22%, glusiline 4%). Besides demographic and clinical findings the following parameters were methods using standard methods: e GFR (CCL), albumin excretion (AE), Hb, HbA1c, hsCRP.
Results: Concerning the demographic parameters (age, gender, diabetes duration, BMI, prevalence of hypertension, antihypertensive therapy) patients with or without renal insufficiency (CCL >or< 90 ml/min) receiving AI and HI did not differ from each other. However, patients with AI and CCL < 90 ml/min showed significantly better renal function parameters than patients with HI: CCL 69 ml/min vs. < 55.6 ml/min; AE 50.5 vs. 143 mg/l. The Hb levels were also significantly higher with AI: 14.0 vs. 13.2 g/dl. In case of CCL < 60 ml/min the differences were most distinctive. The metabolic control was not different (Hba1c 7.7% vs. 7.9%). In a regression analysis of the treatment effect considering numerous confounders (age, gender, diabetes duration, BMI, hypertension, ACE-AT1-therapy) the positive effect of an AI-therapy did remain. In consideration of the better renal function of the patients treated with AI this positive effect was also measurable in a detailed analysis of the therapeutical effect on the Hb level.
Conclusion: Type 1 diabetics with renal insufficiency had better renal values and a higher Hb level under AI therapy than patients under HI therapy. The cause for this finding described for the first time is unclear; a higher effect on the Hb synthesis is to be discussed so that the negative effects of an anaemia on the nephropathy progression are reduced. A positive influence on the renal haemodynamics through AI with consecutively lower loss of filtrate and higher Hb levels can not be excluded either. Prospective studies are necessary for confirming and clarifying this positive effect of AI.
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1066

Cholecystokinin ameliorates diabetic renal injuries via anti-inflammatory effects

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Background and aims: We previously reported that blockade of inflammatory axis via ICAM-1 protects against renal injuries after induction of diabetes, suggesting that low grade inflammation, i.e. "microinflammation", plays a pivotal role in the pathogenesis of diabetic nephropathy. DNA microarray analysis revealed that cholecystokinin (CCK) is up-regulated in the kidneys of diabetic wild type mice but not increased in diabetic ICAM-1 deficient mice. CCK is a peptide with various functions including gastrointestinal homeostasis and neurotransmission, yet little is known about renal effects of CCK. Two types of CCK receptors, types A and B, have been identified in kidney tissues (glomeruli, tubules) and cultured macrophages. We have recently shown that deficiency of both CCK-A and B receptors accelerates renal injuries in streptozotocin (STZ) -induced diabetic mice. The aim of this study is to investigate the protective effects of CCK against progression of diabetic nephropathy.
Materials and methods: (1) Sprague-Dawley rats were divided into three groups: non-diabetic controls received continuous saline infusion, diabetic group received continuous saline infusion (DM), and diabetic group received continuous CCK octapeptide (CCK-8s) infusion (DM-CCK). Diabetes was induced by STZ, and CCK-8s or saline were infused subcutaneously via osmotic pumps over a two months period. Glomerular size and mesangial matrix area were assessed by morphometric analysis. Total RNA was extracted from kidneys, and expressions of proinflammatory genes in kidney tissues were examined by quantitative real-time RT-PCR. Macrophage infiltration into glomeruli and the expression of type-IV collagen were assessed by immunostaining.
(2) Cultured human macrophages (PMA-treated THP-1 cells) were treated with CCK-8s, control peptide or vehicle for 24h. Expressions of proinflammatory genes were examined by quantitative real-time RT-PCR.
Results: (1) DM-CCK showed reduced albuminuria as compared with DM (Mean value; 416 vs. 1160 μg/day, p<0.01), whereas there was no significant difference in HbA1c and systolic blood pressure between both diabetic groups. Glomerular size was similar between DM-CCK and DM, but mesangial matrix area was significantly reduced in DM-CCK than in DM (6.3 vs. 13.9 %, p<0.0001). Gene expression of ICAM-1, CD68 and TGF-β were significantly inhibited in both kidney cortex and isolated glomeruli of DM-CCK as compared with DM (p<0.05). The intensity of type-IV collagen in glomeruli was decreased in DM-CCK as compared with DM (P<0.01). The number of macrophage infiltrated into glomeruli was markedly decreased in DM-CCK than in DM (P<0.0001).
(2) Cultured macrophages treated with CCK-8s suppressed mRNA expression of TNF-α and IP-10 as compared with control peptide and vehicle (P<0.05).
Conclusion: Our results have shown that CCK-8s ameliorates inflammatory reactions and tissue injuries in diabetic kidneys, and that CCK-8s inhibits the production of proinflammatory cytokines from cultured macrophages. These findings suggest that CCK exerts protective effects against progression of diabetic nephropathy via anti-inflammatory effects. CCK may be beneficial for the treatment of diabetic nephropathy.
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1067

α-linolenic acid protects against palmitic acid toxicity via inhibition of endoplasmic reticulum stress in renal proximal tubular cells

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Background and aims: The saturated fatty acid palmitic acid (PA) causes cell death and induces endoplasmic reticulum (ER) stress in vitro. The aim of this study was to evaluate the protective effects of a polyunsaturated fatty acid, α-linolenic acid (ALA) against PA-induced ER stress and loss of renal cell viability and to examine the signaling mechanism.
Materials and methods: Cytotoxicity was assessed by measuring MTT reduction, LDH release or Hoechst-propidium iodide (HPI) staining for apoptosis and necrosis in renal proximal tubular NRK-52E cells. ER stress was evaluated by Western blotting against the markers CHOP, phosphorylated eIF2α (p-eIF2α) and the protein chaperone glucose regulated protein 78 (GRP78).
Results: Co-treatment with 100μmol/L ALA prevented palmitic acid induced cellular release of LDH and increased MTT reduction from 43.8±3.47 to 75.31±3.81% (P<0.01). α-linolenic acid (ALA) decreased palmitic acid-induced apoptosis from 17.1 ± 2.6 to 4.2 ± 0.7% and necrosis from 9.6 ± 1.7 to 2.1 ± 0.4%. Addition of wortmannin (100nmol/L), a phosphatidylinositol 3-kinase (PI3K) inhibitor, did not inhibit the protective effects of ALA against palmitic acid-induced LDH release and MTT reduction. PA (300 μmol/L) increased GRP78 expression from 0.33±0.02 to 0.41±0.01 arbitrary units (n = 3, P < 0.05), CHOP from 0.19±0.04 to 0.88±0.14 (n = 6, P < 0.05) and p-eIF2α from 0.32±0.03 to 0.70±0.03 (n = 5, P < 0.05). Expression of the latter two proteins was reduced in the presence of ALA to levels in untreated cells. In the absence of palmitic acid, but the presence of salubrinal, which maintains eIF2α in the phosphorylated state, ALA also decreased salubrinal-induced p-eIF2α and CHOP levels from 0.69±0.02 and 0.73±0.03 to 0.54±0.04 (n = 6, P < 0.01) and 0.52±0.07, respectively (n = 10, P < 0.05).
Conclusion: These results suggest that α-linolenic acid protects renal proximal tubular cells against palmitic acid induced apoptosis. This protection may involve reduction of ER stress caused by palmitic acid, at the level of eIF2α phosphorylation.
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1068

Effects of CB1 blockade in experimental diabetic nephropathy

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Background and aims: Diabetic nephropathy is characterized by increased glomerular permeability to proteins. Podocyte abnormalities have been implicated in the pathogenesis of the diabetic proteinuria, but the underlying mechanism/s remains unclear. The CB1 endocannabinoid receptor is predominantly localised in the central nervous system; however, recent studies have shown that it is also expressed by peripheral tissues and plays a role in the pathogenesis of chronic degenerative diseases. Our aim was to assess whether the CB1 receptor is expressed in the glomeruli in both normal and diabetic mice and if CB1 blockade affects expression of podocyte slit diaphragm proteins and proteinuria in experimental diabetes.
Materials and methods: Male C57Bl6 mice were made diabetic by intraperitoneal (IP) injection of streptozotocin in citrate buffer. Control mice were injected with citrate buffer alone. After the onset of diabetes both control (ND n=10) and diabetic mice (DM n=14) were treated with either AM251, a selective CB1-receptor antagonist (1 mg/kg IP daily), or vehicle. Fourteen weeks after the induction of diabetes, urine were collected to perform measurement of the urinary excretion of albumin and a blood sample taken for blood glucose and glycated haemoglobin measurements. Mice were sacrificed, kidneys removed, and both immunofluorescence and immunohistochemistry were performed to examine CB1, nephrin, synaptopodin, and zonula occludens-1 (ZO-1) glomerular expression. Quantification of the percentage area of staining of positive cells was performed in 20 glomeruli for each section in a blinded manner.
Results: Diabetic mice showed a significant increase in both plasma glucose and glycated haemoglobin levels. Within the glomeruli CB1 receptor expression localised predominantly to podocytes and was significant greater in the diabetic than in the control animals (DM: 4.32 ± 0.28 vs ND: 2.76 ± 0.45, percentage area, p<0.02). Treatment with AM251 reduced CB1 expression in the controls and normalised it in the diabetic mice (ND+AM251: 1.11 ± 0.62, DM+AM251: 2.16 ± 0.23, p=ns ND vs DM+AM251). Albuminuria was significantly (p<0.001) increased in the diabetic animals [DM: 372.34 (250.61-650.84) μg/18hrs, geometric mean (25^{th}-75^{th} percentile)] as compared to the controls [ND: 33.41 (27.48-39.06)] and ameliorated by treatment with AM251 [ND+AM251: 24.15 (15.99-38.91); DM+AM251: 167.71 (125.78-250.74); p<0.01 DM vs DM+AM251]. In the diabetic mice the increase in albuminuria was paralleled a significant reduction in nephrin expression (ND: 26 ± 6.1; DM: 12.8 ± 2.2; p<0.01). This effect was completely prevented in the diabetic mice treated with AM251 (ND+AM251: 28 ± 3.3; DM+AM251: 21.2 ± 1.9, p<0.05 DM+AM251 vs DM; p=ns DM+AM251 vs ND). Similarly, diabetes-induced ZO-1 downregulation was entirely abolished in AM251-treated diabetic mice (ND: 23.1 ± 2.2; ND+AM251: 24.3 ± 3; DM: 6.7 ± 1.4; DM+AM251: 22.9 ± 2.3, p<0.001 DM vs ND and ND+AM251; DM+AM251 vs DM). No significant changes in synaptopodin expression were observed among groups.
Conclusion: These findings demonstrate that in experimental diabetes there is CB1 receptor overexpression and that CB1 blockade ameliorate diabetic proteinuria, possibly via prevention of podocyte slit diaphragm protein loss.
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1069

Exendin-4 ameliorates renal injuries through anti-oxidative and anti-inflammatory effects in type 1 diabetic rats

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Background and aims: We have recently shown the renoprotective effects of exendin-4 independent of blood glucose lowering actions using streptozotocin induced diabetic rats. In addition, we have demonstrated that GLP-1 receptor is expressed in glomeruli, tubular epithelium, arterioles and cultured macrophage (THP-1 cell). The aim of this study is to clarify the mechanism of protective effects of exendin-4 against diabetic nephropathy.
Materials and methods: Five-week old male Sprague-Dawley rats were divided into four groups: non-diabetic: ND, non-diabetic rats treated with exendin-4: ND+EX, diabetic rats without treatment: DM, diabetic rats treated with exendin-4: DM+EX. Rats were administered intraperitoneally with exendin-4 (10μg/kg/day, ND+EX and DM+EX) or vehicle (ND and DM) every day for 8 weeks. To examine the effects of exendin-4, we measured urinary albumin excretion and creatinine clearance and evaluated the renal histological morphometry. We also examined the expression of CD14, MCP-1, TGF-β1, ICAM-1 in kidney tissues by quantitative real-time RT-PCR and immunostaining. In addition, to clarify the locarization of GLP-1 receptor in glomerulus, we examined gene expression of GLP-1 receptor in cultured human glomerular endothelial cell, murine mesangial cell and epithelial cell. To investigate the mechanism for renoprotective effects of exendin-4, we measured the urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) and examined nonphagocytic NADPH oxidase 4(NOX4) in the kidney.
Results: Exendin-4 significantly reduced urinary albumin excretion (Mean values; DM 2,215μg/day, DM+EX 713μg/day, DM vs DM+EX: P<0.05) without change of blood glucose levels and blood pressure. Exendin-4 significantly attenuated glomerular size (glomerular size; ND: $6.7\times10^3\mu m^2$, ND+EX: $7.0\times10^3\mu m^2$, DM: $9.0\times10^3\mu m^2$, DM+EX: $7.7\times10^3\mu m^2$ DM vs DM+EX: P<0.01) and mesangial matrix expansion (mesangial matrix index; ND: 2.9%, ND+EX: 2.8%, DM: 8.0%, DM+EX: 2.9%, DM vs DM+EX: P<0.05). Exendin-4 also significantly reduced mRNA expression of CD14, TGF-β1 and ICAM-1(P<0.05) in kidney tissues. Expression of MCP-1 was reduced by exendin-4, however, there was no statistical significance. Immunohistochemical analysis revealed that exendin-4 suppressed macrophage infiltration and expression of type IV collagen and ICAM-1 in the kidney of diabetic rats. In addition, exendin-4 significantly reduced the urinary excretion of 8-OHdG and the expression of 8-OHdG in the glomeruli in diabetic rats. NOX4 was significantly reduced in the glomeruli of diabetic rats by exendin-4 treatment. GLP-1 receptor was expressed in glomerular endothelial cell.
Conclusion: The current results suggest that exendin-4 decreased the expressions of NOX4 and 8-OHdG in the glomeruli via GLP-1 receptor expressed on glomerular endothelial cell in diabetic rats. Exendin-4 also prevented the expression of ICAM-1 and macrophage infiltration in diabetic glomeruli. Exendin-4 may ameliorate diabetic nephropathy through anti-oxidative and anti-inflammatory effects.
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1070

Aldosterone blockade improves nephropathy by reducing renal NADPH oxidase induced oxidative stress in the diabetic hypertensive rats

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Background and aims: It has been shown that the blockade of aldosterone ameliorates nephropathy in patients and in animal models of diabetes mellitus (DM). Reduction of oxidative stress and/or renal inflammation had been implicated in the mechanism by which aldosterone blockade improves nephropathy. However, in these studies blood pressure reduction with aldosterone blockade could not be ruled out as a contributor to this beneficial effect. The aim of this study was to investigate the hypothesis that aldosterone blockade may improve nephropathy by reducing oxidative stress and independently of its antihypertensive effect.
Materials and methods: Spontaneously hypertensive rats (SHR), with 4 weeks of age, were rendered diabetic by intravenous injection of streptozotocin. Blood glucose levels of ≥ 270 mg/dL were considered diabetic. The diabetic animals were randomized to receive or not an aldosterone blocker, spironolactone (50mg/kg/day), in drinking water. The albumin excretion rate was determined in 24 h urine by radial immunodiffusion. After 20 days or 8 weeks of treatment, the rats were euthanized and kidneys collected. Changes induced in DNA by oxidative stress and infiltration of macrophages were evaluated by immunohistochemistry for 8-OHdG and ED1, respectively. NADPH-induced superoxide generation was assessed by lucigenin-enhanced chemiluminescence. NADPH oxidase subunit p47phox and fibronectin were assessed by Western Blot.
Results: Plasma glucose levels were significantly (p<0.0001) higher in untreated diabetic rats and it was not modified by spironolactone. Likewise, systolic blood pressure was unaltered by diabetes or by treatment with spironolactone (control=159±10, untreated diabetic=156±17, spironolactone diabetic=151±2 mmHg). Albuminuria was higher in the diabetic group compared to control (216(129-265) *vs* 625(412-752) μg/24h, p=0.0062) and it was reduced to 474(249-547), p=0.0283, with aldosterone blockade. Tubulointer-

stitial expression of 8-OHdG was higher in diabetic rats when compared to controls (292±112 *vs* 555±86 positive cells/50 high field, p=0.0008, and decreased in rats treated with spironolactone for 20 days (457±54, p=0.0143). There was no increase in the expression of ED1 in diabetic animals when compared to controls in treatment for 20 days and 8 weeks (59±18 *vs* 53±19 *vs* 55±22 positive cells/50 high field, 20 days of treatment; 113±35 *vs* 133±71 *vs* 158±31, 8 weeks of treatment). The production of superoxide induced by NADPH oxidase was higher in diabetic rats when compared to controls (3.0±0.52 *vs* 6.8±2.28 RLU/20s/mg, p=0.0143, 20 days of treatment; 4.7±2.70 *vs* 9.4±2.46, p=0.025, 8 weeks of treatment) and was reduced in 8 weeks-treated rats (4.8±1.9, p=0.0389). Western blotting analysis showed an increase in expression of the p47phox subunit of NADPH oxidase in the diabetic group *versus* control group (0.43±0.24 *vs* 1.15±0.3 arbitrary units, p=0.0004) and reduction with 20 days of treatment (0.33±0.11, p<0.0001). Fibronectin expression was enhanced in 8-week diabetic rats compared to controls (1.45±0.71 *vs* 2.54±1.27 arbitrary units, p=0.062) and the treatment reduced to normal levels (1.07±0.62, p=0.0211).
Conclusion: These results suggest that spironolactone ameliorates nephropathy in the diabetic hypertensive rats by diminish NADPH oxidase-induced oxidative stress and without affecting blood pressure.
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1071

Polymorphism in the angiotensin II type-1 receptor gene and renoprotective effect of losartan
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Background and aims: The angiotensin II type-1 receptor (AT-1) mediates the major hemodynamic and trophic actions of angiotensin II. The C allele of the 1166 A/C gene polymorphism in the AT-1 receptor has been associated with severe hypertension and previous studies have suggested that the renal and systemic responses to angiotensin-II receptor-blockers (ARB) are related to this polymorphism. We investigated whether the 1166 A/C polymorphism is related to reductions in blood pressure, albuminuria and preservation of glomerular filtration rate (GFR) during short-term and long-term treatment with losartan 100 mg in 61 hypertensive type 1 diabetic patients with diabetic nephropathy.
Materials and methods: After a 4-week wash-out period, patients received losartan (100 mg o.d.) and were followed prospectively with a mean follow-up of 36 months. After 4 months with losartan as monotherapy, other antihypertensive medication was added to achieve a blood pressure target below 135/85 mmHg. At baseline, after 4 months and every 6 months thereafter, GFR (51Cr-EDTA-clearance), albuminuria and 24 h blood pressure were determined. The 1166 A/C polymorphism was determined by standard polymerase chain reaction (PCR). Investigators and patients were masked to genotypes.
Results: The AA, AC and CC genotypes were found in 46%, 44 % and 10 % of the patients, respectively. Patients homozygous or heterozygous for the C allele were combined in one group as in previous studies. At baseline, GFR, albuminuria and blood pressure were similar in the two groups, AA vs. AC/CC: (mean (SE)), 86 (5) vs. 88 (4) ml/min/1.73m^2; geometric mean (95 % CI) 1080 (778 - 1498) vs. 1291 (940-1775) mg/24 h; mean (SE) 148/80 (4/2) vs. 154/81 (3/2) mm Hg. After 4 months, blood pressure lowering response was more pronounced in the AA compared to the AC/CC group and lowered by (mean (SE)) 13/9 (4/1) vs. 8/4 (2/1) mmHg, respectively (systolic and diastolic p < 0.05). Blood pressure reduction was comparable in both groups during long-term follow-up. Equalisation of blood pressure reduction may be explained by additional antihypertensive medication during follow-up, which was given to 53 % of patients in the AA group as compared to 76 % in the AC/CC group (p = 0.07). No significant differences between groups were seen in rate of decline in GFR (median (range)): 4 .0 (0-16) vs. 3.6 (0-16 ml/min/year (p=0.5). Albuminuria was reduced to the same extend in both groups during the initial 4 months and long-term treatment (p>0.1 AA vs. AC/CC).
Conclusion: Our study indicates that presence of the C allele of the 1166 A/C gene polymorphism in the AT-1 receptor reduces the short-term blood pressure lowering response to losartan. Additional antihypertensive medication may compensate for this and contribute to a similar long-term blood pressure lowering effect and comparable decline in kidney function in both genotype groups.

PS 96 Predictors of nephropathy

1072

Leptin is a crucial factor responsible for the relationship between abdominal obesity and microalbuminuria in patients with type 2 diabetes
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Background and aims: Abdominal obesity has been implicated in the pathogenesis of microalbuminuria in patients with diabetes; however, the mechanisms remain unclear. Increased leptin secretion by adipocytes may be a candidate for responsible factors for the relationship between abdominal obesity and microalbuminuria, because increased leptin secretion has been demonstrated to contribute to renal impairment through glomerular hyperfiltration by activating sympathetic nervous system or direct injury to glomerular endothelial and mesangial cells. However, it is controversial whether serum leptin levels are associated with microalbuminuria in patients with diabetes. We, therefore, conducted a cross-sectional study to clarify the relationship between increased serum leptin levels associated with abdominal obesity and albuminuria in patients with type 2 diabetes mellitus (T2DM).
Materials and methods: We studied a total of 463 adult Japanese patients with T2DM, 212 women and 251 men, with a mean (± SD) age of 61 ± 13 years. Patients with macroalbuminuria, defined as a urinary albumin-to-creatinine ratio (ACR) ≥ 300 mg/g Cr and those with an estimated glomerular filtration rate (eGFR) < 15 mL/min/1.73 m^2 were excluded. Normo- and microalbuminuria was defined as an ACR less than 30 mg/g Cr and 30-299 mg/g Cr, respectively. Waist circumference was used as a surrogate marker of abdominal adiposity. Serum leptin levels were measured by radioimmunoassay.
Results: Geometric mean (95% confidence interval [CI]) of serum leptin levels were 5.9 (5.5 - 6.4) ng/mL, overall. Serum leptin levels in patients with microalbuminuria (N=111) were significantly higher than those with normoalbuminuria (N=352, 5.6 [5.2 - 6.1] *versus* 7.0 [6.0 - 8.2] ng/mL, p= 0.012). In the Spearman's correlational analysis, waist circumference was strongly associated with serum leptin levels (rs= 0.556, p< 0.001). Urinary ACR was significantly associated with both waist circumference (rs= 0.187, p< 0.001) and serum leptin levels (rs=0.212, p<0.001). In the multiple regression analysis adjusted for age, sex, HbA1C, presence of hypertension, presence of dyslipidemia and eGFR, the association between waist circumference and urinary ACR remained statistically significant (standardized parameter estimate= 0.167, p< 0.001). When serum leptin level was incorporated in the model as an independent variable, urinary ACR was also significantly associated with serum leptin levels (standardized parameter estimate= 0.176, p= 0.017), whereas statistical significance between urinary ACR and waist circumference disappeared (p= 0.230), indicating that effects of waist circumference on the urinary ACR may be explained by increased serum leptin levels associated with abdominal obesity.
Conclusion: Leptin may be a crucial factor responsible for the relationship between abdominal obesity and microalbuminuria in patients with T2DM.

1073

Lipid profiles predict progression of renal disease
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Background and aims: Multiple lipid abnormalities have been observed in cross sectional studies and earlier small scale studies have suggested that lipid variables might be involved in the progression of diabetic nephropathy. However, whether lipid abnormalities play a true role in the pathogenesis of diabetic nephropathy is still a matter of debate. Therefore, we decided to systematically study the impact of baseline lipid variables on the progression of renal disease in a large nation-wide prospective cohort of patients with type 1 diabetes.
Materials and methods: A total of 2,304 adult patients with type 1 diabetes and centrally measured lipid profiles participating in the Finnish Diabetic

Nephropathy Study (FinnDiane). Data on progression of renal disease were verified from medical files and patients were followed for 5.4 ± 2.0 years. The lipid profile included total cholesterol, LDL-cholesterol, triglycerides, total HDL (HDL_2, HDL_3) -cholesterol, apolipoproteins (apoA-I, A-II, B) and ratios. Albuminuria status was defined based on AER in at least two out of three collections.
Results: Patients that developed microalbuminuria (100 out of 1449), had higher total cholesterol, non-HDL-cholesterol, triglycerides, ApoB, and triglyceride/HDL-cholesterol ratio at baseline than patients that did not progress (p<0.001). In a Cox regression analysis with traditional risk factors (sex, duration, HbA1c, systolic blood pressure, BMI, and smoking), ln-triglycerides [HR 1.71 (95% CI 1.17-2.50)] predicted development of incident microalbuminuria. When the combined effect of above median triglycerides and AER was studied, the risk of microalbuminuria rose from 8.97 (2.73-39.50), in patients with above median AER alone, to 14.57 (4.52-47.02), in patients with both above median triglycerides and AER. Progressors from micro- to macroalbuminuria (50 out of 303) had higher total cholesterol,non-HDL-cholesterol, triglycerides, ApoB, ApoB/ApoA-I and triglyceride/HDL-cholesterol ratio at baseline than patients that did not progress (p<0.001). In a Cox regression analysis, ln-triglycerides [2.69 (1.53-4.71)] independently predicted progression to macroalbuminuria. The risk of macroalbuminuria rose from 7.65 (1.69-34.55) in patients with above median AER alone, to 16.61 (3.96-69.71), in patients with both above median triglycerides and AER. Progressors from macroalbuminuria to end stage renal disease (ESRD, 92 out of 310) had higher total cholesterol, non-HDL-cholesterol, triglycerides, ApoB, ApoB/ApoA-I and triglyceride/HDL-cholesterol ratio than patients that did not progress (p<0.001). In a Cox regression analysis, ln-triglycerides [2.30 (1.50-3.53)] independently predicted progression to ESRD. When triglycerides were replaced with the other lipid variables separately, high total cholesterol, LDL-cholesterol, non-HDL-cholesterol as well as low HDL-, HDL_2-cholesterol and ApoA-II concentrations were also predictive of progression to ESRD.
Conclusion: Lipid abnormalities, particularly high triglyceride concentrations, increased the risk of progression of renal disease and had an additive effect beyond albuminuria.
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1074

Incidence of microalbuminuria in patients with type 1 diabetes mellitus diagnosed in childhood and adolescence
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Background and aims: Type 1 diabetes mellitus (T1DM) is increasing worldwide and diabetic nephropathy (DN) is a life-threatening long-term complication. Microalbuminuria (MA) is a precocious marker of DN and its detection is recommended for screening even since adolescence.
Methods: We estimated the incidence of MA using overnight albumin excretion rate (AER) in 280 T1DM patients. According to ISPAD Guidelines, AER values between 20-200 μg/min/m^2 (in two of three samples in a 6 months period) were considered positive. At screening evaluation, we collected data for age, disease duration, pubertal stage, body mass index (BMI-SDS), metabolic control (HbA1c), coexistence of autoimmune thyroid and celiac disease, and retinopathy screening results.
Results: 164/280 patients were males, age at diagnosis was 1-16.5 yrs (M 7.6± SD 3.9), follow-up mean period was 10.7±7.0 yrs and mean age at the end of follow-up was 18.2 years (SD 7.6). MA was found in 22/280 patients (7.8%), with an incidence rate of 7.36/1000 pts-yr (IC 95%: 4.84-11.17). We found a relationship between MA incidence and disease duration <10 years (p = <0.0001) and coexistence of retinopathy (p=0.047). Moreover we found a relationship between MA and either mean HbA1c levels in the last year of follow-up ≥ 7.5% (p=0.04) or a high number of follow-up yrs (≥ 85%) with poor metabolic control (cut off ≥ 85% according to ROC curve analysis). In 12/22 patients MA disappeared during follow-up, therefore angiotensin converting enzyme inhibitor treatment was started in 10/22 patients with persistent MA.
Conclusion: Diabetic nephropathy is the most important cause of end stage renal disease in adulthood worldwide. DN screening is mandatory in pediatric T1DM patients to identify patients at risk because of genetic background or poor metabolic control.

1075

Determinants of decline in glomerular filtration rate in normoalbuminuric subjects with and without diabetes
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Background and aims: We investigated whether the slope of estimated glomerular filtration rate (GFR) is different between non-proteinuric subjects with and without diabetes, and what clinical factors are associated with the GFR slope.
Materials and methods: An observational cohort study was performed in 923 subjects, and the predictive value of baseline variables on the GFR slope was investigated.
Results: Based on the median 3-year follow-up and 7 measurements of GFR, GFR slope (% per year, median and interquartile range) was significantly larger in subjects with diabetes (-2.39 (-4.86- 0.15), N=729) than in those without diabetes (-1.02 (-4.28- 1.37), N=194), and this difference remained significant with or without presence of hypertension. After adjustments for confounding factors, progression promoters of GFR decline were found to be baseline high values of HbA1C, GFR, systolic blood pressure and low plasma total protein in subjects with diabetes, while the latter two were significant in subjects without diabetes. In subjects with diabetes the high GFR was accounted for by high HbA1C at baseline, and the progression promoters differed between those with and without hypertension, or with high and low baseline GFR. Any combination of the promoters showed increased risk for GFR decline.
Conclusion: GFR slope is substantially affected by multiple factors at various stages. The degree of chronic hyperglycemia is likely to play a crucial role in elevating GFR and accelerating the decline in patients with type 2 diabetes even from the normoalbuminuric stage.

1076

***RAGE* polymorphism and serum sRAGE levels are associated with diabetic nephropathy in Japanese type 2 diabetic patients**
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Background and aims: Diabetic microangiopathy such as retinopathy, nephropathy, and neuropathy are the major causes of lowering quality of life in diabetic patients. The ligand-receptor for advanced glycation end products (RAGE) axis has emerged as a novel pathway involved in diabetes mellitus itself and its vascular complications. As circulating soluble forms of RAGE (sRAGE) may reflect the activity of AGE-RAGE axis, it has been proposed as a potential mechanism linking hyperglycemia and diabetic microvascular complications. Also some previous papers reported the positive relationship between RAGE polymorphisms and its blood concentrations. In this cross-sectional study, therefore, we explored its single nucleotide polymorphisms (SNPs) especially *AGER*-374T/A in correlation with its blood levels and examined the *RAGE* SNPs for the possibility as a risk marker of diabetic microangiopathy in Japanese type 2 diabetic patients.
Methods: A total of 289 type 2 diabetic outpatients were enrolled in this study. Soluble RAGE was measured by an enzyme-linked immunoassay kit. Genotyping of 3 polymorphisms in *AGER* were performed by polymerase chain reaction restriction fragment length polymorphism method. Diabetic nephropathy was defined by urine albumin extraction rate (≥ 30 mg/g Cr/day). Statistical analyses were performed using the program SPSS. Data are presented as means ± SD.
Results: There were no significant differences in genotypic or allelic distribution among patients with or without diabetic retinopathy and neuropathy, but there was a significant increase in the frequency of the TA+AA genotypes at -374T/A in the patients with diabetic nephropathy (P=0.009). The serum sRAGE levels were increased in a genotype-dependent fashion only at -374T/A (TT<TA<AA, Kruskull-Wallis test, P=0.045). Also serum sRAGE levels were higher in the patients with diabetic nephropathy than in those without this disease (500±349 ng/ml vs. 417±263 p=0.026), but there were no significant differences in patients with or without diabetic retinopathy or neuropathy. In Spearman's correlations, serum RAGE levels were correlated

with fasting blood glucose (r=-0.221, P<0.01) and estimated GFR (eGFR) (r=-0.221, P<0.01). After adjustment for sex, body mass index, systolic blood pressure, diastolic blood pressure, duration of diabetes, and serum levels of triglycerides, RAGE, HbA1c and eGFR in multivariate logistic-regression analysis, individuals with the TA or TT genotypes were significantly more likely to have had a diabetic nephropathy than individuals with the TT genotype (Odds ratio 2.01, 95% Confidence Interval 1.13-3.55, P=0.017).
Conclusion: The serum sRAGE levels and its genotype were associated with the prevalence of diabetic nephropathy in Japanese type 2 diabetic patients. Our results suggest that the *AGER* gene polymorphism (-374T/A) and serum sRAGE levels might be a risk marker of susceptibility to diabetic nephropathy among those patients. Further studies are needed to determine the exact role of sRAGE between the relationship of AGE-RAGE axis and the development of diabetic nephropathy.
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1077

Association of a single nucleotide polymorphism in *KCNQ1* with diabetic nephropathy in Japanese subjects with type 2 diabetes
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Background and aims: It has been suggested that genetic susceptibility plays an important role in the pathogenesis of diabetic nephropathy, but most of genes responsible for the development and progression of diabetic nephropathy remain to be identified. In this study we focus on the *KCNQ1* at chromosome 11p15.5, recently identified as a strong susceptibility gene to type 2 diabetes, as a plausible candidate gene for diabetic nephropathy, and examined the association of SNPs within the *KCNQ1* with diabetic nephropathy in Japanese populations.
Materials and methods: We first examined the association of 33 SNPs within the *KCNQ1* in 1,304 Japanese subjects with type 2 diabetes having diabetic retinopathy (Study 1: 747 with overt nephropathy, 557 controls with normoalbuminuria) by using a invader assay. We further examined additional 2 independent Japanese cohorts for replication studies (Study 2: 300 type 2 diabetes with end-stage renal disease, 325 type 2 diabetes with normoalbuminuria, Study 3: 6 years longitudinal study, 31 cases progressed to overt nephropathy and 168 controls).
Results: Among the 33 SNPs examined, T allele of rs2237897 was nominally associated with susceptibility to diabetic nephropathy (OR = 1.29, 95% CI 1.09 - 1.53, nominal p = 0.002, corrected p = 0.07). Subsequent haplotype analyses revealed that common haplotype consisted of 2 SNPs including rs2237897 showed nominal association with the disease, but the association was not stronger than that of rs2237897 alone. The same trend of the association of rs2237897 could be observed in two independent Japanese cohorts (Study 2; OR = 1.19, Study 3; OR = 1.16), and combined analysis for all 3 tests with a fixed model meta-analysis gave nominal p value of 0.0009 (OR = 1.26, 95% CI 1.10 - 1.44, corrected p = 0.03).
Conclusion: We have identified significant association of rs2237897 with diabetic nephropathy in 3 independent Japanese populations. The results suggest that *KCNQ1* may play some roles in the pathogenesis of diabetic nephropathy, and can be considered as a new candidate gene for conferring susceptibility to diabetic nephropathy.

1078

Prevalence and determinants of chronic kidney diseases in a Bangladeshi population
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Background and aims: Chronic kidney diseases (CKDs) are now creating substantial burden on health care resources in developing countries and it is closely linked with the epidemic of diabetes mellitus. Data on burden of diseases and its determinants in a specific population are of vital importance in designing proper management and preventive strategies. The present study aimed to explore the prevalence and determinants of CKDs in a semi-urban and rural population of Bangladesh.
Materials and methods: A total number of 803 adult subjects (male 449, female 354), aged between 25-67 years, were screened for diabetes in 3 outreach camps. Diabetes mellitus was diagnosed by OGTT following WHO criteria and nephropathy was diagnosed by urine testing through dipstick, eGFR (calculated from serum creatinine) and measurement of albumin-creatinine ratio (ACR). Anthropometric measurements were done by standardized techniques. Blood glucose was measured by glucose-oxidase method, lipids by enzymatic method, creatinine by alkaline picrate method and albumin was measured by immunonephelometry. Following a nested case-control design an age-matched control group was selected from the non-diabetic subjects to compare with cases having abnormal glucose tolerance (AGT). Data were analyzed by univariate as well as multivariate statistics.
Results: Out of 803 subjects 113 (14.1%) showed AGT (7.5% diabetes, 5.1% IGT and 1.5% IFG). The age-matched 106 cases, who were selected as control, did not show proteinuria in the dipstick method, but in the diabetic group 17 (15.1%) subjects were found to have proteinuria of verifying degrees. As judged by eGFR only 9.4% cases in the control group showed normal eGFR; the corresponding value in the Diabetic group was even lower (3.5%). Using the ACR criteria the prevalence of nephropathy (including incipient nephropathy) in the control group was about 46% (with 41% at stage 3 and 1.9% at stage 4) and that in the Diabetic group was 65% (with 51% at stage 3 and 12.5% at stage 4). On logistic regression analysis nephropathy was found to be strongly associated with diabetes even when the confounding variables were adjusted.
Conclusion: A large population of adult Bangladeshis, even in semi-urban and rural population, are suffering from nephropathy (frank and incipient), but those can not be detected by dipstick (under diagnosis) or eGFR (over diagnosis). Measurement of ACR, along with OGTT, can detect a large number of incipient diabetic nephropathy cases at the field level and this can be used as a public health tool for prevention.
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1079

Thiamine levels and transketolase genetic variants as modifiers of progression of diabetic nephropathy
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Background and aims: Diabetic complications including diabetic nephropathy (DN) develop due to the complex dysregulation of cellular metabolism during hyperglycemia. Accumulation of proximal glycolytic intermediates provides substrates for several alternative metabolic pathways producing harmful moieties such as advanced glycation end-products, dicarbonyls, sorbitol, hexosamines, reactive oxygen and nitrogen species etc. Pentose phosphate pathway (PPP) represents potentially "protective" mechanism in hyperglycemia since shunting of cumulated glycolytic intermediates (esp. triosephosphates) into the PPP reactions quantitatively limits their processing elsewhere. Thiamin status (thiamine and its esters) as cofactors of transketolase (TKT) and genetic variability in the TKT locus - potentially together with other genes encoding enzymes of PPP such as transaldolase, TKT-like and glucose-6-phosphate dehydrogenase - might contribute to an interindi-

vidual variability in the onset and progression of DN. The specific aims of the study were (i) quantification of plasma and erythrocyte levels of thiamines (Th, ThMP, ThDP), (ii) haplotype-based association study of DN with TKT variability, (iii) genotype vs. phenotype correlation of TKT variability and enzyme erythrocyte activity and (iv) evaluation of prognostic value of those biochemical and genetic parameters for the progression of DN.
Materials and methods: A total of 231 diabetic subjects were included in the case (DN) - control (non-DN) study (type 1 or 2 diabetics with parallel DN and gender- and age-matched diabetics without organ complications, respectively, in ~1:1 ratio), DN cohort followed prospectively for the average period of 24 months. SNPs (total n = 15) were genotyped by means of PCR using fluorescent-labelled probes (TaqMan®, Applied Biosystems). Haplotypes were inferred using Bayesian-based algorithm (PHASE). Concentration of thiamines (plasma and erythrocyte) and TKT catalyzed reaction were determined by HPLC.
Results: P-Th, ery- and p-ThDP and ery. activity of TKT differed significantly between diabetics with and without DN (P<0.05, Mann-Whitney). There was a signif. negative correlation between fasting glycemia and ery-Th and ery-ThDP (P<0.05, Spearman) and signif. positive correlation between serum creatinin and most of the parameters of thiamine metabolism. Haplotype distribution of TKT differed significantly between DN vs. non-DN groups (P<0.05, 10 000 permutations). Common haplotype with frequency 21.3% in the whole study population (risk-haplotype) exhibited the greatest difference. Carrier state of the risk-haplotype was associated with significantly accelerated onset of DN (P<0.05) and lower thiamin concentrations but not with TKT activity.
Conclusion: Results suggest that TKT variability and thiamine status modify susceptibility and progression of DN.
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1080

The effect of alcohol consumption on nephropathy in type 1 diabetic patients
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Objective: To study the effect of alcohol consumption on the initiation and progression of diabetic nephropathy
Research design and methods: The study included 3,468 type 1 diabetic patients from the FinnDiane (Finnish Diabetic Nephropathy) study. We studied the cross sectional association between alcohol consumption and nephropathy ass well as the effect of alcohol consumption on the progression of nephropathy during follow-up. The alcohol data were collected with questionnaires during the base-line visit. The patients were divided in five different groups according to their alcohol consumption in grams per week. Because the limit of heavy drinking is classified differently for men (≥280g/week) and women (≥140g/week) also the groups were formed differently for men (0 g/week, 0-139,9 g/week, 140-279,9 g/week, ≥280 g/week and former users) and women (0 g/week, 0-69,9 g/week, 70-139,9g/week, ≥140g/week and former users). In the cross sectional analysis the women consuming more than 70g/week were pooled due to the small number of heavy consuming women. The renal status was defined according to the albumin excretion in three urine collections. Data were analysed with logistic regression analyses. In multivariate analyses the data were adjusted for duration of diabetes, $HbA1_c$, systolic blood pressure and smoking status. In the analysis of follow up data men and women were pooled and data were adjusted for sex.
Results: According to the cross sectional data ORs for the risk of nephropathy in each increasing alcohol consumption group compared with abstainers were for men 0.71 (CI 0.50-0.99), 0.61 (CI 0.38-0.98), 0.50 (CI 0.27-0.95) and for former users 1.85 (CI 1.01-3.41). Correspoding ORs for women were 0.51 (CI 0.37-0.71), 0.48 (CI 0.29-0.79) and for former users 0.87 (CI 0.41-1.84). ORs for the progression of nephropathy in the follow up data were 1.04 (CI 0.76-1.44), 0.86 (CI 0.52-1.42), 1.67 (CI 0.91-3.05) and for former users 1.85 (CI 1.04-3.31).
Conclusion: In the cross sectional data we could observe a smaller risk of nephropathy in men and women with present alcohol consumption compared with the abstainers. The follow-up data, however, showed that the progression of nephropathy was equal among alcohol consumers and abstainers. The risk of nephropathy was highest in the former alcohol users. This was seen in the cross sectional data for men and in the follow up data for both men and women. Former heavy alcohol consumption seems to be associated with a higher risk of nephropathy. However, it is unclear if alcohol consumption per se is a risk factor for nephropathy or could the increased risk of nephropathy be explaned with other factors related to higher alcohol consumption.
Supported by: FinnDiane Study Group

1081

The association between increased urinary albumin excretion and insulin resistance in subjects at high-risk of type 2 diabetes
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Background and aims: Insulin resistance has been implicated in the pathogenesis of increased urinary albumin excretion (UAE) in diabetes. In the current study, we investigate the association between increased UAE and insulin resistance in a large cohort of subjects at high-risk of type 2 diabetes.
Materials and methods: A total of 471 men and 874 women from the High-risk for Diabetes Changfen Study, aged 61±10 years with at least one of the risk factors for type 2 diabetes which included positive family history of diabetes, overweight or obesity, hypertension, dyslipidemia, history of vascular disease or history of delivery a big baby(only for women) were included. Insulin resistance was derived from the hemeostasis model assessment of insulin resistance ($HOMA_{IR}$) and UAE was derived from the albumin-to-creatinine ratio (ACR) defined as increased if the value was ≥ 40 μg/mg. Abnormal glucose metabolism was defined as fasting glucose≥5.6 mmo/L or 2h post prandial glucose≥7.8mmo/L in oral glucose tolerance test.
Results: 54 men (11.5%) and 201 women (23.0%) had increased UAE. After adjusting for age, gender, overweight or obesity, hypertension, dyslipidemia and abnormal glucose metabolism simultaneously, subjects with highest $HOMA_{IR}$ (the most insulin resistance) were more often having increased UAE than those with the lowest $HOMA_{IR}$ (the least insulin resistance) [Odds ratio (OR) 1.78, 95%CI (1.08-2.92), P=0.023), highest vs. lowest quintile]. For women, subjects with highest $HOMA_{IR}$ were more often having increased UAE [OR 2.1, 95%CI 1.1-3.9, P<0.05; highest vs. lowest quintile], but for men the risk to have increased UAE was not increased [OR 1.2, 95%CI 0.5-2.9, P>0.05; highest vs. lowest quintile]. In multivariate analyses, increased UAE was associated with $HOMA_{IR}$ independent of age, gender, overweight or obesity, hypertension, dyslipidemia and abnormal glucose metabolism (OR 1.76, P=0.000). The correlation between increased UAE and $HOMA_{IR}$ was still significant in women (OR 1.66, P=0.009), but was less pronounced and non-significant in men (OR 1.06, P>0.05).
Conclusion: In subjects at high-risk of type 2 diabetes, increased UAE is strongly associated with insulin resistance. This association seems to be stronger in women than in men.

Risk of having increased UAE according to quartiles of HOMAIR in subjects at high-risk of diabetes

Quartiles of $HOMA_{IR}$ (range)	Whole population (n=1345)	Men (n=471)	Women (n= 874)
1. (0.18-2.37)	1.0	1.0	1.0
2.(2.38-3.27)			
A	1.3 (0.8-2.0)	1.1 (0.5-2.7)	1.1 (0.6-1.9)
B	1.0 (0.6-1.7)	1.9 (0.9-4.3)	1.0 (0.5-1.9)
3.(3.28-4.42)			
A	1.7 (1.07-2.65)*	1.1 (0.5-2.8)	1.5 (0.8-2.6)
B	1.4 (0.84-2.23)	2.1 (0.9-4.6)	1.4 (0.8-2.6)
4.(4.43-31.6)			
A	3.0 (2.0-4.6)***	1.0 (0.4-2.5)	2.7 (1.6-4.6)***
B	1.8 (1.1-2.9)*	1.2 (0.5-2.9)	2.1 (1.1-3.9)*

Date are OR (95% CI). A: unadjusted; B: adjusted for age, gender, overweight or obesity, hypertension, dyslipidemia and abnormal glucose metabolism. *P<0.05, **P<0.01, ***P<0.001.

Supported by: STCSM

PS 97 Nephropathy - clinical 2

1082

Fibrinocoagulopathy in chronic kidney disease in patients with elderly type 2 diabetes

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Background and aims: Chronic kidney disease (CKD) is now widely accepted as a risk factor for coronary vascular disease (CVD) and mortality. Diabetes mellitus (DM) is a leading cause of CKD in patients with established CKD in the world. Macrovascular events such as CVD, especially in elderly DM, are most likely due to thrombotic process of the large arteries. The aim of this study was to evaluate the relationships between the blood coagulation and fibrinolytic function and the degree of CKD stages in patients with elderly type 2 diabetes in terms of CKD.
Materials and methods: We studied 139 elderly type 2 diabetic outpatients of 65 to 86 years of age[73.0+/-6.7 years(mean+/-SD)]. All of subjects had no evidence of cerebral, cardiac or peripheral vascular complications. None of them received the treatment of dialysis. At overnight fast, blood pressure (BP) was measured, and blood sampling for plasma glucose (PG), HbA1c, serum lipids, creatinine, plasma thrombomodulin (TM): a marker of vascular endothelial damage, prothrombinfragment 1+2 (F1+2), a direct marker of thrombin generation, α2 plasmin-inhibitor plasmin complex (PIC), a marker of fibrinolytic activity and fibrinogen (Fbg) were performed. Urinary albumin excretion ratio (UAE) was obtained in morning spot urine sample. The estimated glomerular filtration rate (eGFR) was estimated by using the following equations: $eGFR=0.741\times175\times Cr^{-1.154}\times Age^{-0.203}\times0.742$ (for females). All patients were divided into five CKD stages based on eGFR: stage 1 (GFR> 90 ml/min/1.73m^2), stage 2 (GFR: 60-89 ml/min/1.73m^2), stage 3 (GFR 30-59 ml/min/1.73m^2), stage 4 (GFR 15-29 ml/min/1.73m^2), and stage 5 (GFR<15 ml/min/1.73m^2).
Results: Number of each CKD stage was stage 1; 13, stage 2; 68, stage 3; 37, stage 4; 12 and stage 5; 9 cases, respectively. No differences in gender and age were present among 5 CKD stages. However, the duration of DM was significantly longer in stage 4 and 5 than other three stages. There were no differences among 5 stages in BP, PG, HbA1c and serum lipids. Concentration of plasma TM in stage 4 and 5 were significantly higher than stage 1, 2 and 3 ($p<0.01$, $p<0.01$, $p<0.01$ in stage 4 and 5, respectively). Similarly, concentration of F1+2 in stage 4 was higher than stage 2 ($p<0.05$) and that in stage 5 was higher than stage 1 and 2 ($p<0.01$, $p<0.05$, respectively). PIC concentration in stage 4 was significantly higher than stage 1, 2 and 3 ($p<0.01$, $p<0.05$, $p<0.05$, respectively). Moreover, PIC in stage 5 was significantly higher than stage 1, 2 and 3 ($p<0.01$, $p<0.01$, $p<0.01$, respectively). Plasma Fbg levels in stage 4 was significantly higher than stage 2 ($p<0.05$) and that in stage 5 was higher than stage 1 and 2 ($p<0.01$, $p<0.05$, respectively). eGFR was inversely correlated with TM ($r=-0.668$, $p<0.01$), F1+2 ($r=-0.400$, $p<0.01$), PIC ($r=-0.379$, $p<0.01$) and Fbg ($r=-0.323$, $p<0.01$), respectively. Moreover, significant linear correlation was observed between log UAE and TM ($r=0.472$, $p<0.01$). Furthermore, log UAE was correlated with F1+2 ($r=0.353$, $p<0.01$), PIC ($r=0.363$, $p<0.01$) and Fbg ($r=0.443$, $p<0.01$), respectively.
Conclusion: The vascular endothelial damage was highly progressed and the thrombin generation was accelerated, and the plasma fibrino-coagulation resulted in strongly reinforced accompanied with the secondary increased of fibrinolysis in advanced CKD stages with elderly type 2 diabetes. These abnormal coagulation and fibrinolytic function may contribute to the increased cardiovascular risk in CKD patients with elderly type 2 diabetes.

1083

Change in body composition but not glomerular filtration is related to altered insulin sensitivity in type 1 diabetes patients with or without diabetic nephropathy

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Background and aims: Insulin resistance has been recognised in patients with albuminuria and in chronic renal failure. Recent studies have shown that insulin resistance is present already in mild renal impairment. It could therefore be argued that loss of glomerular filtration rate (GFR) over time could contribute to a decline in insulin sensitivity. The aim of this study was to evaluate if a decline in GFR over time is associated with changes in insulin-antagonistic hormones, cytokines and insulin sensitivity in patients with T1D with different degrees of renal impairment at baseline over time.
Patients and methods: In a prospective follow-up study 20 patients with type 1 diabetes with or without diabetic nephropathy at baseline were re-examined after 5.0±0.4 (range 4.4-6.1) years. 8/20 (40%) patients had no signs of diabetic nephropathy (DN) and 12/20 had DN at baseline defined as micro- (≥20 μg/min), or macroalbuminuria (≥200 μg/min) and no sign of any other renal disease. 14/20 (70 %) patients displayed a decline in GFR during the follow-up period. GFR was determined by ^{51}Cr-EDTA clearance. Insulin sensitivity was assessed as hyperinsulinemic (56 mU/m^2/min), euglycemic glucose uptake (M-value) at steady state during clamp and calculated per lean body mass. Body composition was determined by bioelectrical impedance analysis.
Results: In all patients taken together, a significant decline in GFR over time was found (-13±21 ml/min/1.73m^2, $p<0.01$). There was no change in BMI ($p=0.68$) but a significant change in body composition, with a decrease in lean body mass (%) and an increase in fat mass (%) (both $p<0.01$). In all patients taken together and in patients without DN at baseline there was no significant change in insulin sensitivity during the study period (M-value +0.9±3.1 mg/kg lbm/min, $p=0.18$ and -0.6±2.9, $p=0.59$). In contrast, a significant increase in insulin sensitivity was found in patients with DN at baseline (+2.0±2.9 mg/kg lbm/min, $p=0.04$). In univariate analysis no significant association was found between change in GFR and change in M-value over time in all patients taken together or in patients without DN at baseline or with DN at baseline ($r=-0.33$, $p=0.15$; $r=-0.43$, $p=0.28$ and $r=-0.39$, $p=0.21$, respectively). Instead, change in M-value was significantly associated to change in fat mass (%) and IL-6 levels ($r=-0.55$, $p=0.012$ and $r=-0.62$, $p=0.006$). When fat mass and IL-6 were included in a multivariate regression model these variables explained approx 53% (adj $r^2=0.53$, $p=0.001$) of the change in M-value, and the changes in fat mass and IL-6 both contributed significantly ($p=0.015$ and $p=0.009$) to the model.
Conclusion: In patients with type 1 diabetes a decline in GFR over five years does not influence insulin sensitivity. This may suggest that the insulin resistance found in type 1 diabetes patients with nephropathy may be genetically determined and not an effect of the reduction in GFR per se. Instead, this study indicates that increase in body fat and elevated levels of inflammatory cytokines, such as IL-6, are major determinants of insulin resistance in these patients.

1084

Acute hyperglycaemia decreases serum angiotensin converting enzyme 2 (ACE2) activity

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Background and aims: Activation of the renin-angiotensin system (RAS) contributes to diabetic kidney and vascular injury. Angiotensin converting enzyme 2 (ACE2) is a newly identified homolog of ACE that converts angiotensin (AT) II to AT-1-7 and potentially promotes vasodilatation. ACE2 activity levels are shown to be increased in early diabetes in experimental models. However, the role of circulating ACE2 activity in human physiology and disease is unknown. The purpose of this study was to test whether ACE2 activity is modified by diabetes or hyperglycaemia in patients with type 1 diabetes, with and without microvascular complications.
Materials and methods: Quantitative ACE2 activity was measured by a fluorometric assay in 546 adult patients with type 1 diabetes participating in The Finnish Diabetic Nephropathy (FinnDiane) study and in 205 healthy control subjects. In addition, ACE2 activity was determined in 22 type I diabetic patients and 13 controls before and after a 2 hour hyperglycemic clamp.
Results: Acute hyperglycemia during a 2-hour hyperglycemic clamp resulted in an 8% reduction in the ACE2 activity with simultaneous stiffening of the arteries in diabetic patients as well as in healthy controls ($n=35$, $P<0.05$). Baseline ACE2 activity was similar in patients with type 1 diabetes and healthy control subjects (461±20 vs 494±21, P=n.s., data presented in arbitrary fluorescence units). Chronic hyperglycemia as measured by HbA1C (%) or presence of albuminuria was not associated to the ACE2 activity. Among men with diabetes, ACE2 activity level was increased in patients with increased glomerular filtration rate (GFR) ($r=0.156$, $P=0.034$).

Conclusion: Circulating ACE2 activity is modified by acute hyperglycemia, potentially contributing to the hyperglycemia-associated increased vascular tone. Chronic hyperglycemia appears to have no effect on circulating ACE2 activity levels. ACE2 is increased when the patients show signs of renal hyperfiltration, which indicates vasodilatory changes in the kidney in early diabetes.

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1085

Increased urinary excretion of fibrogenic growth factors is associated with initial structural changes in the kidneys in type 1 diabetic patients

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Background and aims: Transforming growth factor beta (TGF-beta), insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) are believed to be involved in pathogenesis of diabetic nephropathy (DN). Recent experimental studies have shown that these factors enhance the extracellular matrix production in glomerular and tubular cells and, therefore, contribute to progression of glomerular and interstitial sclerosis in diabetes. The aim of our study was to assess the relation between urinary excretion of fibrogenic growth factors and development of nephropathy in type 1 diabetic patients.

Materials and methods: 57 patients with type 1 diabetes were examined, including 22 normoalbuminuric (group DN0), 23 microalbuminuric (group DN1) and 12 macroalbuminuric ones (group DN2). Urinary excretion of TGF-beta, IGF-1 and VEGF was determined by ELISA and compared to control (10 healthy subjects, group C). A kidney biopsy was performed in 8 normoalbuminuric and 10 microalbuminuric patients. The glomerular and tubular basement membrane (GBM and TBM) width, mesangial fractional volume [Vv(mes/glom)] and mean podocyte foot processes number was estimated by morphometric analysis.

Results: (median, percentile 25-75)**:** TGF-beta excretion was increased significantly in patients with micro- and macroalbuminuria as compared to control (DN0: 2.0, 1.2-2.5, p=0.3, DN1: 3.0, 2.1-5.1, p=0.003, DN2: 6.3, 4.0-8.2, p=0.003 vs C: 1.6, 0.9-2.2 pg/μmol creatinine). IGF-1 excretion exceeded control in micro- and macroalbuminuric patients also (DN0: 2.5, 2.1-3.2, p=0.25, DN1: 3.7, 3.0-4.6, p=0.004, DN2: 7.6, 6-13, p=0.04 vs C: 2.1, 1.7-3.5 pg/μmol creatinine). VEGF excretion was increased significantly in patients with macroalbuminuria only (DN0: 6.5, 4.4-10.8, p=0.69, DN1: 10.2, 5.8-20, p=0.06, DN2: 16.7, 7.0-45.9, p=0.02 vs C: 5.4, 3-9.7 ng/μmol creatinine). There were negative correlations between TGF-beta, IGF-1 and glomerular filtration rate (TGF-beta: r=-0.32, p=0.02; IGF-1: r=-0.43, p=0.002; VEGF: r=-0.14, p=0,3). In normoalbuminuric and microalbuminuric patients excretion of all growth factors correlated positively with the width of GBM (TGF-beta: r=0.75, p=0.0003; IGF-1: r=0.53, p=0.04; VEGF: r=0.53, p=0.04) and TBM (TGF-beta: r=0.73, p=0.0004; IGF-1: r=0.59, p=0.02; VEGF: r=0.3, p=0.29). Only VEGF excretion correlated with Vv(mes/glom) significantly (r=0.69, p=0.004; TGF-beta and IGF-1: r=0.34, p=0.19). The increased excretion of TGF-beta and IGF-1 was associated with reduction of podocyte foot processes number (TGF-beta: r=-0.5, p=0.03; IGF-1: r=-0.69, p=0.004; VEGF: r=-0.48, p=0.08).

Conclusion: In type 1 diabetic patients increased urinary excretion of fibrogenic growth factors (TGF-beta, IGF-1 and VEGF) is associated with albuminuria and initial structural changes in the kidneys.

1086

Evaluation of prognostic factors affecting urinary albumin and urinary type IV collagen in early-onset type 1 diabetes mellitus

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Background and aims: To clarify factors affecting increases in urinary albumin/ creatinine ratio (ACR) and urinary type IV collagen/creatinine ratio (T4C) in early-onset type 1 diabetes mellitus (T1DM) by age at the start of observation.

Materials and methods: This study included 238 patients diagnosed with T1DM at <30 years old, and <40 years old and without overt diabetic nephropathy at the start of observation. Study endpoints were increased ACR (progression from normoalbuminuria to microalbuminuria or overt nephropathy; progression from microalbuminuria to overt nephropathy) and progression to elevated T4C (*multivariate Cox proportional* hazards analysis).

Results: Prognostic factors selected for increased ACR were baseline ACR in all patients along with diastolic blood pressure (BP) in ≥16 year and ≥18 year subgroups and Body mass index (BMI) in ≥20 year and ≥22 year subgroups. Prognostic factors selected for increased T4C were hemoglobin (Hb) A1c, diabetic retinopathy, and female sex in all patients, along with tobacco use in the ≥18 year subgroup only.

Conclusion: Prognostic factors for ACR and T4C differed, including differences based on age at the start of observation (baseline). T4C, as compared to ACR, may more sensitively reflect the effects of hyperglycemia itself on the kidneys. This difference in prognostic factors based on age at the start of observation suggests that T4C, in addition to ACR, may be an important marker to monitor disease status.

1087

Normalizing albuminuria using urinary-creatinine rather than measuring albuminuria alone leads to more accurate and less false positive or false negative results when screening patients with diabetes mellitus for nephropathy

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Background and aims: Microalbuminuria (MIA) is an early indicator of renal damage in diabetic patients. Therefore, MIA is a valuable screening tool and routinely used in quality assurance programs (e.g. the German nationwide disease management programme for diabetes). In clinical routine, for practical reasons, MIA measurements are mostly drawn from a single, "on the spot" urine specimen. A value > 20mg/l is considered pathologic. However, unstable results occur because of fluctuating urine volumes. This can lead to inaccurate diagnosis of nephropathy. We examined whether normalizing MIA to urinary-creatinine (MIA/gUCrea) ratio increases the reproducibility of the results.

Patients and methods: The electronic patient file EMIL® was used as the data source. All visits from 06/2000 to 01/2008 with a simultaneous analysis of MIA and MIA/gUCrea in patients with diabetes mellitus type 1 (DM1) and 2 (DM2) were exported. A total of 3716 data sets were retrieved (average number of visits 2.1 (span 1-14), comprising 326 patients with DM1 (age 46.1±15.6years, diabetes duration 18.3±12.8 years, BMI 26.5±4.4kg/m^2, HbA1c 8.14±1.47%, serum-creatinine 93.5±39.7μmol/l, systolic blood pressure 135.7±16.6 mmHg, diastolic blood pressure 77.0±7.4 mmHg) and 1468 patients with DM2 (age 64.6±11.4 years, diabetes duration 12.3±9.0 years, BMI 32.4±6.1 kg/m^2, HbA1c 7.80±1.53%, serum-creatinine 111.1±67.5 μmol/l, systolic blood pressure 135.2±11.2 mmHg , diastolic blood pressure 77.5±8.5mmHg). Ten patients on dialysis (DM1=1, DM2=9) were excluded from the analysis. The MIA and MIA/gCrea values per visit and the inter-visit differences were compared.

Results: Average serum-creatinine of all patients was 108.0±63.9 μmol/l. Proteinuria >1g/l was present in 58 (3.2%) patients using MIA, in 76 (4.2%) using MIA/gUCrea (p<0.001). Average MIA was significantly lower than average MIA/gUCrea (141.7±415.1 mg/l vs. 177.8±550.0 mg/g; p<0.001). MIA in the normal range and increased MIA/gUCrea occurred in 280 cases (7.5%), and increased MIA and MIA/gUCrea in the normal range occurred in 234 cases (6.3%; p=0.04). Unstable values, alternating between normal and pathological MIA or vice versa in consecutive measurements, occurred with MIA in 204 (5.5%) cases, and with MIA/gUCrea in 155 (4.2%) of the cases (p=0.010).

Conclusion: Values of urinary-creatinine normalized albuminuria (MIA/gUCrea) are, when compared to non-normalized albuminuria (MIA), significantly lower. MIA/gUCrea is less vulnerable to instability in consecutive samples because differences in urine volume and concentration are normalized. Use of MIA alone would have led to misclassification regarding the diagnosis of nephropathy in 13-14% of the cases (7.5% false negative, 6.3% false positive). In quality assurance programs where screening for renal damage plays an important role, measurement of MIA alone should be abandoned in favour of urine-creatinine normalized MIA. Quality assurance programmes, e.g. the German nationwide disease-management program for diabetes, should be adapted accordingly.

1088

Plasma proteome analysis of patients with type 1 diabetes with diabetic nephropathy

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Background and aims: At present microalbuminuria is used as a risk marker for development of diabetic nephropathy (DN); however, much debate exists about its sensitivity and specificity. The search for new and better biomarkers for DN has, with a few exceptions, previously focused on either hypothesis-driven studies or urinary based investigations. To date only two studies have investigated the proteome of blood in search for new biomarkers, and these studies were conducted in sera from patients with type 2 diabetes. This is the first reported study where the plasma from type 1 diabetic patients was investigated in depth with the goal of finding improved candidate biomarkers to predict DN.
Materials and methods: Plasma from a cross-sectional cohort of 123 type 1 diabetic patients previously diagnosed as normoalbuminuric, microalbuminuric or macroalbuminuric, was collected at Steno Diabetes Center. In order to reach lower concentration proteins in plasma a pre-fractionation step was performed prior to surface enhanced laser desorption/ionization time-of-flight mass spectrometry analysis (SELDI-TOF-MS). In addition to traditional statistical tools, we evaluated our spectral data with independent component analysis (ICA). ICA measures signals in complicated background and considerably increase the quality of the resulting data as well as improving the biological validity of subsequent examination.
Results: Mass spectrometry analysis gave rise to 290 peak clusters significantly different between the groups. 17 were selected for future examination as the most promising biomarker candidates with $p < 0.0001$ and component stability scores > 0.8. Two of the peaks that were discovered have been identified as transthyretin (difference in peak intensity between groups, $p < 0.0001$) and transferrin (difference in peak intensity between groups, $p < 0.0001$). Several other (e.g., m/z 6963.9, m/z 11351 and m/z 13584) have also been discovered as candidate biomarkers and are in the process of being identified. These proteins, especially when combined, are better able to define the stages of diabetic nephropathy.
Conclusion: These results demonstrate the capacity of proteomic analysis of plasma, by confirming the presence of known biomarkers as well as revealing new biomarkers for diabetic nephropathy in plasma in type 1 diabetic patients.

1089

Vascular diseases and mortality of patients on haemodialysis with type 2 or type 3 diabetes

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Background and aims: In rare cases, diabetic patients on dialysis therapy have pancreatic diabetes (PAD), also called type 3 diabetes. Aim of this study was to investigate possible differences in patient survival, prevalence of vascular diseases and incidence of vascular complications in dialysis patients with type 3 or type 2 diabetes.
Materials and methods: In a retrospective study we evaluated 96 diabetic patients, who started dialysis therapy between 1995-2003 in our dialysis centre. Type 3 diabetes was diagnosed in 12 patients and type 2 diabetes in 84 patients. In both groups we compared vascular risk factors, prevalence of vascular diseases, incidence of vascular complications and patient survival in the first 5 years after the initiation of dialysis therapy.
Results: At the beginning of dialysis therapy, patients with type 2 diabetes were significantly older than patients with type 3 diabetes (63 ± 8 vs. 56 ± 6 years, $P < 0.05$). Vascular risk factors were similar in both groups, only mean cholesterol was significantly lower in type 3 diabetic patients compared to those with type 2 diabetes (142 ± 42 vs. 196 ± 36, $P < 0.05$). In addition, mean BMI (22 ± 3 vs. 25 ± 3 kg/m^2, $P < 0.05$) and mean serum levels of fasting C-peptide (0.9 ± 0.3 vs. 1.9 ± 0.6 ng/ml, $P < 0.05$) and of albumin (2782 ± 348 vs. 3464 ± 342 mg/dl, $P < 0.05$) were significantly lower in type 3 diabetic patients compared to those with type 2 diabetes. At the beginning of dialysis therapy, the prevalence of vascular diseases was not significantly different between both groups, as well as the incidence of vascular complications (stroke, myocardial infarction and/or amputations) in the first 5 years. The frequency of malnutrition (BMI < 21 kg/m^2) was 50% in type 3 diabetic patients versus 9% in type 2 diabetics ($P < 0.05$). In type 2 diabetic patients 3-year survival was slightly higher (54 vs. 42%, n.s.), whereas 5-year survival was significantly higher (27 vs. 8%, $P < 0.05$).
Conclusion: In dialysis patients with type 3 and type 2 diabetes the prevalence of vascular diseases and the incidence of vascular complications was not significantly different in the first 5 years after the initiation of dialysis therapy. However, malnutrition was more common and survival was lower in type 3 diabetic patients compared to those with type 2 diabetes.

1090

Prorenin receptor expression in human kidneys with diabetic nephropathy

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Background and aims: The renin-angiotensin-aldosterone system (RAS) plays critical roles in the progression of diabetic nephropathy. (Pro)renin receptor ((P)RR), a specific receptor for renin and prorenin, has recently been identified. It is a 350 amino-acid protein with a single transmembrane domain and widely expressed in various tissues including brain, heart and kidney. When bound to (pro) renin, (P)RR activates the angiotensin I-generating activity of (pro)renin in the absence of cleavage of the prosegment, and directly stimulates the MAPK pathway independently from the RAS. Plasma levels of prorenin are elevated in diabetic patients, and thought to play important roles in the pathophysiology of diabetic nephropathy. The aim of the present study is therefore to clarify the expression of (P)RR in the kidney with diabetic nephropathy.
Materials and methods: The present study was approved by the Ethics Committee of the Tohoku University Graduate School of Medicine. The kidney tissues were obtained at autopsy from patients with and without Type 2 diabetes mellitus (n=5 without diabetes mellitus; and n=10 with diabetes mellitus). Immunocytochemistry was performed by the ABC method using paraffin-embedded sections. The antiserum against (P)RR was raised in a rabbit by injecting the peptide fragment of human (P)RR corresponding to 224-237 a.a. conjugated with bovine serum albumin. The identity of the (P)RR immunoreactivity was confirmed by Western blot analysis, which showed a band of 39 kDa in the protein extract of rat kidney and heart. Furthermore, the preabsorption of the antibody by the antigen peptide abolished the immunostaining of (P)RR in immunocytochemistry of the human kidney.
Results: (P)RR was mainly expressed in the tubular cells and collecting duct cells of the kidney without diabetic nephropathy. (P)RR was very weakly and sporadically immunostained in the cells of glomeruli. Vascular smooth muscle cells and endothelial cells were very weakly or were not immunostained with (P)RR. Adipocytes in the adipose tissue around the kidney were positively immunostained with (P)RR. Immunostaining pattern of (P)RR in the kidney with diabetic nephropathy was similar to that without diabetic nephropathy. However, most notably, (P)RR immunostaining in the tubular cells and collecting duct cells was clearly and frequently more strongly observed in the kidney with diabetic nephropathy up to the end stage renal disease. Even the atrophied tubular cells in the kidney tissue with end stage renal disease expressed (P)RR.
Conclusion: (P)RR was expressed in tubular cells and collecting duct cells of the kidney tissue with diabetic nephropathy up to end stage renal disease. (P)RR expressed in the diabetic kidney may play a pathophysiological role in angiotensin I generation and renal fibrosis found in end stage renal disease.
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1091

Diabetic nephropathy is associated more strongly with toe-brachial index than ankle-brachial index in type 2 diabetic patients

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Background and aims: Microalbuminuria is an indicator for diabetic nephropathy. It was known that increased urinary albumin/creatinine ratio (ACR) is a risk factor for peripheral vascular disease (PVD). Ankle-brachial index

(ABI) and toe-brachial index (TBI) are a simple useful method for assessing PVD. Some data suggested that ACR is associated with PVD, and ACR is significantly correlated with ABI in an inverse pattern in the type 2 diabetic patients. But the relationship between TBI and diabetic nephropathy was not well known. The present study was undertaken in type 2 diabetic patients to investigate whether ABI or TBI was more strongly associated with markers of diabetic nephropathy such as ACR, serum creatinine levels, and estimated glomerular filtration rate (eGFR).
Materials and methods: We recruited a total of 365 type 2 diabetics: 192 men (mean age 56.1±13.7 years) and 173 women (mean age 57.3±14.8 years). Renal function was evaluated by serum creatinine levels, estimated eGFR (calculated by the Cockcroft-Gault equation) and urinary ACR. Because the distribution of ACR was highly skewed, the natural logarithm of ACR [Ln(ACR)] was used for statistical analyses. ABI and TBI measurements were performed with the subject in a supine position, and were determined as the ratio of ankle or toe systolic blood pressure to the brachial systolic blood pressure, with both determined using an automatic device.
Results: Overall, the mean age was 60.5±11.2 years, duration of diabetes 12.1±6.8 years, HbA_{1C} 7.6±2.4%, ACR 826.7±629.0 mg/gCr, serum creatinine 1.4±1.2 mg/dL, and systemic blood pressure (BP) 138.4±14.8/88.1±13.4 mmHg. Mean calculated GFR was 59.2±35.9 mL/min/1.73m^2. ABI were 1.12±0.11 (Rt.) and 1.11±0.13 (Lt.). TBI were 0.84±0.15 (Rt.) and 0.83±0.16 (Lt.). The mean difference between ABI and TBI in our population was 0.28±0.15. Among all patients there was a significant linear association (r = 0.492; p< 0.001) between ABI and TBI. Ln(ACR) was inversely correlated with ABI (r=−0.193, P<0.05) and TBI (r=−0.249, P<0.05). TBI also tended to correlate negatively with age and diabetes duration. We found no significant correlation between hemolglobin A_{1C}, fasting C-peptide, serum creatinine levels or eGFR and ABI or TBI. By Univariate linear regression, right TBI but not right ABI showed a significant negative correlation with Ln(ACR) (r=-0.215, P<0.05).
Conclusion: This study demonstrated that ABI and TBI were related to urinary ACR and especially right TBI is suggested to be the more strongly related parameter than ABI for diabetic nephropathy in type 2 diabetic patients.

1092

Renal interstitial fibrosis factors in type 2 diabetics with renal artery stenosis
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Background and aims: To estimate renal interstitial fibrosis factors in type 2 diabetics with renal artery stenosis (RAS) and without RAS.
Materials and methods: We studied 30 diabetic type 2 patients with RAS and 22 diabetic type 2 patients without RAS. All patients were invited to undergo multispiral computer tomography or selective angiography of renal arteries to define the presence of RAS (renal artery stenosis more than 60%). Transforming growth factor (TGF - β-1), vascular endothelial growth factor (VEGF), markers of endothelial dysfunctions (sICAM, VCAM), nonspecific atherosclerotic markers (C-reactive protein, homocystein), matrix metalloproteinase 9 (MMP 9), interleukin 6, angiotensin II were measured. The control group included normotensive persons of more than 45 years without diabetes (n=20). Glomerular filtration rate (GFR) was calculated by the MDRD equation.
Results: GFR was significantly lower in diabetics with RAS than without RAS [73,1 (63,3; 96,0) vs.92,5 (78,5; 114,0) ml/mines/1,73m^2, p<0,05]. TGF - β-1 level in the RAS-positive group was significantly higher than the one in the RAS-negative group [90,9 (52,2; 119,4) vs. 51,6 (25,2; 78,5) ng/ml, p<0,02]. TGF - β-1 positively correlated with MMP 9 (r=0,23; p<0,04) and VEGF (r=0,26; p <0,01). MMP 9 in a cohort of patients without RAS appeared to be paradoxically higher in comparison with persons with RAS [138,0 (109,0; 173,0) vs. 96,5 (68,0; 131,0) ng/ml, p<0,01]. Other indicators didn't differ significantly between the researched groups.
Conclusion: RAS in diabetic type 2 patients was associated with significant increase of TGF - β1, which is known to have a predictive role in the development of the tubulointerstitial expansion.

PS 98 Hypertension

1093

Effects of telmisartan on glucose metabolism, lipid profile and adiponectin in patients with type 2 diabetes complicated with hypertension
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Background and aims: Hypertension complicated with type 2 diabetes is a strong risk factor for cardiovascular disease. Telmisartan, a new angiotensin IItype 1 receptor blocker (ARB), is a more powerful stimulator of peroxisome proliferator activated receptor-γ (PPARγ) than the others, and is expected by in vitro studies to provide a better improvement of insulin sensitivity than other ARBs. The present study was performed to assess the effects of telmisartan at doses of 40 and 80 mg/day on glucose metabolism, lipid profile and serum adipokine levels in type 2 diabetic and hypertension.
Materials and methods: <Study 1> Fifty for patients (30 men and 24 women, mean age 64.6±10.8 years) with type 2 diabetes complicated with hypertension(systolic blood pressure ≥130 or diastolic ≥80 mmHg) were treated with valsartan (80 mg, n=20) or candesartan (8 mg, n=24) or losartan (50 mg, n=10). Then, telmisartan at 40 mg/day was administered instead of the other ARBs for 12 months. Physical examinations and blood tests were conducted at 0, 6 and 12 months after treatment in each patients. <Study 2> In a double-blind, randomized study, patients with type 2 diabetes complicated with hypertension (systolic blood pressure ≥120 or diastolic ≥70 mmHg) despite the treatment with telmisartan at 40 mg/day were switched to telmisartan 80 mg/day (n=32) or telmisartan 40 mg/day + amlodipin 5 mg/day (n=32) for 3 months. Physical examinations and blood tests were conducted at baseline and end of treatment in each patient.
Results: <Study 1> Telmisartan at 40 mg/day resulted in a significant decrease in systolic and diastolic blood pressures. Neither HbA1c nor fasting plasm glucose were significantly changed. Telmisartan tended to decrease fasting levels of serum insulin (9.6±7.2 to 8.7±6.1 mU/l) and HOMA-R (3.3±1.7 to 2.9±2.3). Serum levels of triglyceride were significantly reduced from 138.8±63.3 to 124.3±54.9 mg/dl (P<0.05) and HDL cholesterol was improved from 50.7±12.6 to 52.8±13.7 mg/dl(P<0.05) by the treatment, whereas total cholesterol and LDL cholesterol did not change. Serum adiponectin did not change, either. <Study 2> Telmisartan 80 mg, but not telmisartan 40 mg + amlodipin 5mg, significantly (P<0.05) reduced fasting levels of plasma glucose and insulin and HOMA-R. Ths change was marked (P<0.01) in patients with HOMA-R ≥2.0. In patients with total cholesterol ≥220 mg/dl, telmisartan 80 mg significantly (P<0.01) reduced total cholesterol, in patients with triglyceride ≥150 mg/dl, telmisartan 80 mg reduced triglyceride (P<0.05), in patients with LDL cholesterol ≥120 mg/dl, telmisartan 80 mg reduced LDL cholesterol (P<0.05), in patients with HDL cholesterol ≤50 mg/dl, telmisartan 80 mg increased HDL cholesterol (P<0.05) and in patients with adiponectin 4.0 ≤μg/ml, telmisartan 80 mg increased adiponectin (P<0.05).
Conclusion: Thease results suggests that telmisartan at 80 mg favorably affects glucose metabolism and lipid profile through stimulating PPARγ in patients with type 2 diabetes complicated hypertension despite the treatment with telmisartan at 40 mg.

1094

Lipidomic profiling reveals a deficiency of ether lipids in blood plasma of men with hypertension
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Background and aims: Dyslipoproteinemia, obesity and insulin resistance are integrative constituents of the metabolic syndrome and major risk factors for hypertension. The objective of this study was to determine whether hypertension specifically affects the plasma lipidome independently and differently from the effects induced by obesity and insulin resistance.
Materials and methods: Plasma lipidomic screens of 19 men with hypertension and 51 normotensive male controls, covering 95 lipid species of 9 major lipid classes were analyzed by top-down shotgun profiling on a LTQ Orbitrap hybrid mass spectrometer.

Results: Obesity resulted in generally higher lipid load in blood plasma, while the content of in tri- and diacylglycerols increased dramatically. Insulin resistance, defined by HOMA-IR > 3.5 and controlled for BMI, was exclusively accompanied by a significant decrease of a single phosphatidylethanolamine metabolite. Specific alterations of the plasma lipidome in hypertensive individuals evaluated by BMI- and HOMA-IR-adjusted values indicated overall decreases in ether lipids, in particular in ether phosphatidylcholines, that comprise arachidonic (20:4) and docosapentaenoic (22:5) fatty acid moieties, as well as in free cholesterol. At the same time, conventional clinical lipid homeostasis indices (total cholesterol, LDL-, HDL-cholesterol, triglycerides) remained unaffected.
Conclusion: Top-down shotgun lipidomics demonstrated that hypertension is accompanied by specific reductions of ether lipids and free cholesterol that occur independently of lipidomic alterations induced by obesity and insulin resistance. These results may form the basis for novel preventive and dietary strategies alleviating the severity of hypertension.
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1095

Inverse relation between hypertension and FASN expression in adipose tissue

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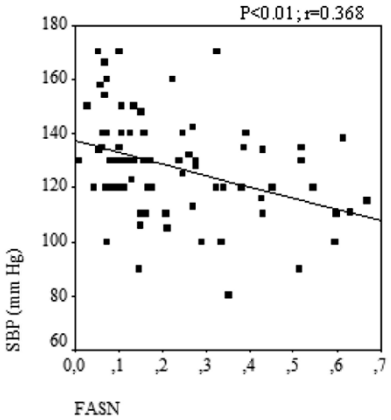
Background and aims: There are a lot of studies that relate fatty acid synthesis enzymes, FASN (Fatty Acid Synthase) and ACC (Acetyl-CoA Carboxilase) to metabolic alterations such as obesity, insulin resistance or dyslipemia. Due to the fact that there is no literature that relates these enzymes with hypertension, the aim of this study is investigate how, in adipose tissue, FASN and ACC are related to hypertension.
Materials and methods: The study included 87 patients, with a wide range of body mass index (BMI), in order to study in their visceral adipose tissue the gene expression of FASN y ACC by RT-PCR. Anthropometric and biochemical variables (glucose, glycated hemoglobin, HOMA-IR, cholesterol, c-LDL, c-HDL, triglycerides, PCR, adiponectin, leptin, ...) were measured after getting a blood sample of the patient after an overnight fast and before the laparoscopic surgery. Patients were classified according to hypertension levels into normotensive and hypertensive groups. Hypertensive individuals were defined as those with systolic blood pressure (SBP) ≥ 130mm Hg or diastolic blood pressure (DBP) ≥ 85 nm Hg or that were on medication for this condition. Subjects were classified according to BMI, as non obese (BMI<30) and obese (BMI≥30).
Results: The main result of this work is a significant decrease of FASN expression in adipose tissue of hypertensive *vs* normotensive patients. There was an inverse correlation between FASN expression and SBP values by multiple regression analysis and there was also an inverse correlation between FASN expression and SBP. The statistical analyses and graphics were performed using the SPSS software program and values were considered statiscally significant when P≤0.05.
Conclusion: We can say that for the first time, it has been proven that a decrease of FASN expression in hypertensive individuals and the clinical significance of this work, represents an exciting challenge because it would need to be clarified if the reduced values of FASN expression led to hypertension or if this is an adaptive mechanism to the presence of hypertension. Once this aspect is clarified, it could be a new target for the treatment of hypertension.

Figure 1. Linear relationship between FASN expression and SBP was determined by Pearson's correlation coefficient test.



Supported by: CIBEROBN (CB06/03/010) & SAF 2006/12894

1096

Glucose intolerance strongly correlates with high blood pressure in normocholesterolaemic morbidly obese women

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Background and aims: We studied the lipid profile, glucose tolerance and the prevalence of High Blood Pressure (HBP) in morbidly obese women with body mass index (BMI) in the range 40 - 70 kg/m^2.
Materials and methods: A total of 203 morbidly obese female patients in the range from 25 to 63 years old, with normal thyroid and renal function were investigated before gastric restrictive surgery. Conventional lipid profiles, TSH, FT4, T3, fasting blood glucose (FBG) and postprandial glucose, BUN, creatinine, ALT, serum albumin and globulins, coagulation parameters, ultrasensitive PCR, bilirrubins, lipase, RBC, WBC, hematocrit, CK and hemoglobin were measured. Careful physical exam was performed and blood pressure was measured at least in three different occasions. Glucose tolerance and lipid profiles were interpreted according to ADA Clinical Practice Recommendations 2009.
Results: 37,2% of the 163 patients with normal glucose tolerance were hypertensive with an average BMI of 44,5 kg/ m^2 (SD 5,8), mean FBG of 91,4 mg% (SD 3,8) mean total cholesterol of 191,4 mg% (SD 31,2), mean LDL-cholesterol 110,2 mg%(SD 34,3), mean HDL-cholesterol 51,4 mg% (SD 16,1) and mean triglyceride 142 mg% (SD 42,3).
Fasting glucose intolerance was found in 40 patients with a mean FBG of 108,6 mg% (SD 4,1) with a prevalence of 61% of HBP and mean BMI of 47,1 (SD 6,7). LDL-cholesterol was 121,2 mg% (SD 38,5), mean HDL-cholesterol of 49,7 mg% (SD 19,5) and mean triglyceride was 165,6 mg% (SD 58,3). No patient was diagnosed as diabetic according to the OGTT. The group of patients with fasting glucose intolerance showed a significant increase in the prevalence of HBP and higher triglyceride levels.
Conclusion: Glucose intolerance is a strong predictor of high blood pressure in morbidly obese women. There is a disparity between the conventional lipid measures, higher BMI and metabolic syndrome risk factors in severely obese women, except for those with glucose intolerance that showed elevated triglycerides levels suggesting insulin resistance.
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1097

Pattern of expression of inflammatory markers in adipose tissue of untreated patients with essential hypertension

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Background and aims: Subcutaneous (SAT) and, especially, visceral adipose tissue (VAT) may contribute to hypertension by promoting endothelial dys-

function and impaired insulin sensitivity. In hypertensive patients (HT), the inflammatory network and the metallo-proteinase/tissue metallo-proteinase inhibitors (MMP/TIMP) system are important modulators of vascular structure/function.
Materials and methods: In mature adipocytes, isolated from paired samples of VAT and SAT from 30 non-diabetic, lean, never-treated HT and 20 age- and sex-matched normotensive controls (C), we measured TNFα, TGFß, IL-6, PAI-1, MMP-2, MMP-9, TIMP-1 and TIMP-2 expression (by realtime PCR, expressed as targeted/reference ratio), MMP-2 and MMP-9 activity (by zymography) and TIMP-1 and TIMP-2 protein expression (by Western blot analysis). Plasma IL-6, TGFß and PAI-1 levels were also determined.
Results: SAT MMP-9 was similarly expressed in HT and C (0.4±0.04 *vs* 0.5±0.03 in VAT and 0.9±0.05 *vs* 1.0±0.05 in SAT). In contrast, MMP-2 expression was strongly reduced in SAT of HT (2.4±0.2 *vs* 0.9±0.03, p<0.0001), but not in VAT (0.9±0.05 *vs* 0.8±0.04, p=ns). VAT TIMP-1 was similar in C and HT (1.0±0.03 *vs* 1.0±0.06), while it was reduced in SAT of HT (0.5±0.03 *vs* 0.4±0.03; p=0.0005). TIMP-2, more abundant in SAT than in VAT, was reduced in HT VAT and SAT (2.0±0.06 *vs* 1.0±0.03 in VAT, and 3.1±0.1 *vs* 1.2±0.04 in SAT, both p<0.0001). These results were confirmed at the protein level by Western blot. MMP-2 activity was reduced in HT in both VAT and SAT (4674±209 *vs* 3889±87 and 5329±113 *vs* 3965±64 AU, p=0.0008 and p<0.0001 respectively). Despite similar expression in C and HT, MMP-9 activity was markedly reduced in HT (13408±552 *vs* 1021±32 in VAT and 3398±98 *vs* 633±11 AU in SAT; both p<0.0001). IL-6 expression, abundant in both VAT and SAT, was significantly higher in HT (15.3±2.4 *vs* 186.8±21.5 in VAT and 9.8±0.8 *vs* 38.4±3.2 in SAT, both p<0.0001). TNFα was similarly expressed in VAT and SAT of C (1.2±0.04 *vs* 1.1±0.05), while its presence in VAT of HT was markedly increased (3.9±0.3 *vs* 1.4±0.2, p<0.0001). TGFß was significantly lower in VAT (1.7±0.04 *vs* 1.2±0.05) and SAT (1.4±0.05 *vs* 1.2±0.09, both p<0.0001) of HT *vs* C. Lastly, PAI-1, more abundant in VAT than in SAT in both groups, was 18-fold more represented in VAT (2.4±0.02 *vs* 35.0±4.3, p<0.0001), and 9-fold higher in SAT (2.3±0.03 *vs* 19.0±2.4, p<0.0001) of HT than C. Circulating IL-6 concentrations were 2-fold higher in HT (2.5±0.2 *vs* 5.4±0.5 pg/ml, p<0.0001), while TGFß levels did not differ between the two groups (19.0±1.3 *vs* 20.7±1.4 ng/ml). Plasma PAI-1 levels were much higher in HT than C (27.7±1.8 *vs* 49.0±1.4 ng/ml, p<0.0001). In the whole dataset, IL-6 in VAT was directly correlated with systolic and diastolic blood pressure values. Also, significant inverse relationships were observed between blood pressure levels and MMP-2 and MMP-9 activity in VAT and SAT.
Conclusion: Untreated hypertensive patients have a peculiar pattern of expression of MMP/TIMP and inflammatory molecules in isolated mature adipocytes: MMP-2, TIMP-1, TIMP-2 and TGFß expression and activity are markedly reduced, while PAI-1 and IL-6 expression is increased, as reflected in higher circulating levels. These results support an important role for adipose tissue in the pro-inflammatory and pro-thrombotic state in lean hypertensive subjects.

1098

On the relation of visceral obesity, insulin resistance, CRP and blood pressure in a general population: the Rijswijk study
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Background and aims: Visceral obesity, insulin resistance, and low-grade inflammation are regarded as underlying pathophysiologic factors of the metabolic syndrome. The purpose of this study was to investigate blood pressure in relation to visceral obesity, insulin resistance and elevated CRP levels.
Materials and methods: In a primary health care setting, this cross-sectional study was performed in a general population. All subjects registered in the practices as patients (40-70 years) without diabetes mellitus were invited for an cardometabolic analysis. 1984 patients (response rate approx 70%) have been assessed. Laboratory assessments were performed in a representative subgroup of 1350 subjects. Access to medical records including medication use was available. Blood pressure, waist circumference, plasma glucose, insulin and hsCRP levels were measured. We used the IDF-criteria for visceral obesity (VO), HOMA-IR >2 for insulin resistance (IR) and hsCRP >2 as a marker of low grade inflammation to classify subjects.
Results: Only 20% of the subjects (CO group)were apparently healthy as defined by absence of VO, IR and normal hsCRP levels. VO was highly prevalent (73.5%). Insulin resistance was nearly always associated with VO (98.8%) and the presence of elevated hsCRP was often accompanied by visceral obesity as well (84%). In isolated form IR was observed in only 1 subject and in combination with elevated CRP in 3 subjects. These two groups were not further analysed in the present study. Blood pressure correlated with visceral obesity (r= 0.354, p= 0. 001), insulin resistance (r= 0.105, p=0.001) and slightly with CRP (r= 0.066, p= 0.013). Systolic blood pressure significantly differed between the groups (see figure for ANOVA comparisons between groups). Blood pressure increased in following order: CO, CRP+; VO+ (RR 130(19)/82(12) mmHg); VO+CRP+ (RR 133(20)/83(11) mmHg); VO+IR+ (RR 140(18)/89(9) mmHg); VO+IR+CRP+ (RR 144(23)/89(12) mmHg). For diastolic blood pressure similar results were obtained. Multiple regression analysis confirmed the independent effect of IR and VO (but not CRP levels) on blood pressure level after adjustment for age, gender and smoking. For each 10cm waist circumference respectively one unit HOMA-IR; blood pressure increased with 3,4 mmHg respectively 18,2 mmHg. Repeating analysis to subjects without blood pressure lowering medications revealed similar results.
Conclusion: Visceral obesity, insulin resistance and inflammation directly relate to blood pressure levels in a general population. Visceral obesity and insulin resistance are independent driving forces for hypertension, this was not observed for systemic inflammation measured as elevated C-reactive protein levels.

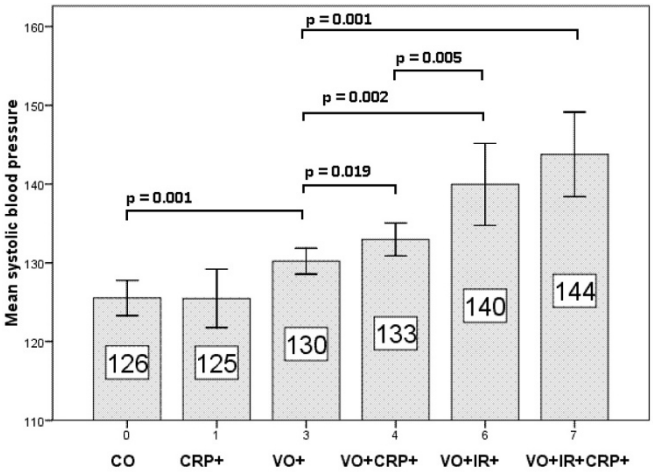

Figure: comparisons of SBP among classified groups. Vertical bars indicate mean ± s.d. Date were analysed using ANOVA. The P for trend was < 0.001. LSD P values for post hoc analysis shown.

1099

Short sleep duration is associated with blood pressure non dipping pattern in type 1 diabetes: the DIAPASOM study
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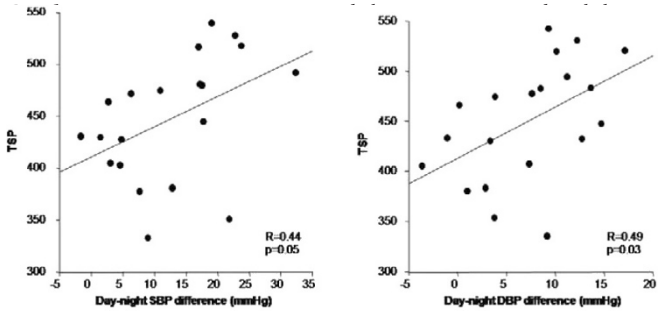
Non-dipping blood pressure status is associated with an increased cardiovascular risk in type 1 diabetes. Sleep quality or duration changes may affect glucose control and perception of sleep-related hypoglycaemias.
Objective: To assess whether blood pressure dipping status in type 1 diabetes is correlated with specific sleep characteristics and differences in continuous glucose monitoring profiles.
Research design and methods: 20 type 1 diabetic patients (age 47±12 yrs, BMI 26.7±3.2 kg/m^2) underwent sleep explorations with simultaneous 24h Ambulatory Blood Pressure Monitoring and continuous nocturnal glucose monitoring.
Results: Fifty five percent of the patients exhibited blunted blood pressure dipping and did not differ from dippers for age, BMI, 24 hour systolic (SDP)/diastolic blood pressure (DBP). Total Sleep Period (TSP) (407±44 vs. 497±30 min for non dippers and dippers, respectively, p<0.001) and Total Sleep Time (TST) (356±72 vs. 425±82 min, p=0.03) were shorter in the non-dipper group. SBP and DBP day-night differences and TSP were correlated (R=0.44, p=0.05 and R=0.49, p=0.03, respectively). Subjects with the shorter TST had a higher risk to be non dipper (OR = 9.3 [1.19-7.3], p = 0.03). Periods of nocturnal hypoglycaemia (i.e. % of TSP with glycaemia<70mg/dL) were longer in dipper subjects (0.1±0.4% vs. 8.1±10.7 for non dippers and dippers, respectively, p=0.02) whereas mean nocturnal glycaemia and duration of nocturnal hyperglycaemia were similar.



Positive correlation between Total Sleep Period (TSP) and day-night Systolic and Diastolic Blood Pressure differences (SBP and DBP, respectively).

Supported by: Direction de la Recherche Clinique Grenoble, France

1100

Blood pressure levels and dipping pattern are determined by insulin resistance independently from fat mass and adiponectin interaction in premenopausal obese women

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Background and aims: Adiponectin is an adipokine which is assumed to confer cardiovascular protection. Obesity, high blood pressure (BP), non-dipper pattern of BP variation and insulin resistance (IR) are all considered cardiovascular risk factors. The aim of our work was to evaluate the association of BP levels (mean 24 hours, daytime and nighttime levels) and its pattern of variation throughout the day with adiponectin levels and with the degree of insulin resistance, in premenopausal obese women without known hypertension.
Materials and methods: We studied 74 Caucasian premenopausal obese females not under any drug treatment (except oral contraceptives). They were characterized for BMI, waist circumference, waist:hip ratio (WHR) and a fasting blood sample was collected for adiponectin, insulin, glucose and triglycerides assessments. IR was assessed by 3 indexes: homeostatic model assessment (HOMA-IR), quantitative insulin resistance check index (QUICKI) and McAuley formula. They were submitted to a 24h ambulatory blood pressure monitoring. Nighttime was considered from 23:00 to 07:00 and non-dipper pattern was defined by less than 10% nocturnal decrease in the median BP. Statistical analysis was performed with the SPSS program, version 16.0. The established limit for statistical significance (p) was 0.05.
Results: Women were characterized by mean age=34±8.1 years, BMI=42.9±8.1 Kg/m^2, waist=117.1±14.7 cm, WHR=0.88±0.07, adiponectin=6.52±2.88 μg/ml, HOMA-IR=3.79±2.2, QUICKI=0.14±0.01 and McAuley=6.36±1.37. The 24h systolic BP=120.2±9.3 mmHg, diastolic BP=72.6±6.9 mmHg and mean nocturnal decrease=13.5±10.2 mmHg; hypertension was present is 18.9% and non-dipper pattern was present in 36.5% of patients. Dipper patients presented higher HOMA-IR (p=0.003) and lower QUICKI (p=0.001) and McAuley (p=0.032). Adiponectin correlated directly with QUICKI (p<0.001; r=0.419) and McAuley (p<0.001; r=0.468), independently from anthropometry. We also found an independent association of indexes of IR (but not adiponectin) with BP levels: daytime systolic BP with HOMA-IR (p=0.002; r=0.354), QUICKI (p=0.008; r=-0.308) and McAuley (p=0.016; r=-0.279); nighttime systolic BP with HOMA-IR (p<0.001; r=0.488) and QUICKI (p<0.001; r=-0.49); nighttime diastolic BP with HOMA-IR (p=0.001; r=0.378), QUICKI (p=0.001; r=-0.383) and McAuley (p=0.009; r=-0.304); 24h systolic BP with HOMA-IR (p<0.001; r=0.438), QUICKI (p<0.001; r=-0.404) and McAuley (p=0.004; r=-0.334); 24h diastolic BP with HOMA-IR (p=0.027; r=0.257) and McAuley (p=0.03; r=-0.253). Independently from anthropometrics, HOMA-IR was inversely related (p=0.007; r=-0.313) and QUICKI directly related (p=0.001; r=0.368) with the nocturnal decrease in BP.
Conclusion: Non-dipping pattern of BP variation is relatively common in premenopausal obese patients. Independently from the existent amount of fat mass, IR indexes contribute themselves for BP regulation and for the degree of the nocturnal decrease observed. Although lower adiponectinemia is associated with higher abdominal fat and higher IR, adiponectin itself seems not to be implicated in BP regulation or in the dipping phenomenon.

PS 99 Hypertension - clinical

1101

Blood pressure and prediction of mortality in elderly type 2 diabetic patients (ZODIAC-14)

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Background and aims: Systolic blood pressure, diastolic blood pressure, and pulse pressure, all increase the risk of (cardiovascular) mortality. Systolic blood pressure is considered to be the strongest predictor of them. In elderly patients with type 2 diabetes mellitus (T2DM) the predictive role of the various blood pressure indices has not been assessed before. Therefore, we investigated the predictability of the various indices in elderly T2DM patients.
Materials and methods: In 1998, 881 primary care patients with T2DM aged 60 years and older participated in the ZODIAC study. The cohort was divided into two age categories: 60-75 years and older than 75 years. Life status was assessed after a median follow-up time of 5.7 years. We used a Cox proportional hazard model to investigate the relationship between the blood pressure indices and all-cause as well cardiovascular mortality in both age groups. Predictability was evaluated using Bayesian information criterion (BIC) and Harrell's C statistics. The following variables were selected as possible confounders: gender, smoking (yes or no), body mass index, duration of diabetes, serum creatinine, cholesterol-HDL ratio, macrovascular complications (yes or no), albuminuria (yes or no), the use of lipid lowering and antihypertensive drugs (yes or no) and age.
Results: All of the blood pressure indices were inversely related to all-cause mortality in the high age group (> 75 years). Predictive capability, indicated by lower BIC-values and higher Harrell's C values, was about the same for all of the indices for both all-cause and cardiovascular mortality (table 1). In the low age group (60-75 years) the associations between blood pressure and mortality were not significant.
Conclusion: Systolic, diastolic, and pulse pressures are equally strong predictors of all-cause and cardiovascular mortality in an elderly T2DM population. The predictive capability of blood pressure in the general population may not be applicable to an elderly diabetic population.

Table 1. Predictability of mortality in elderly diabetic patients

	All-cause mortality		Cardiovascular mortality	
	BIC	Harrell's C	BIC	Harrell's C
Systolic blood pressure	1934,04	0,69	965,73	0,69
Diastolic blood pressure	1942,17	0,67	965,61	0,68
Pulse pressure	1943,53	0,68	967,99	0,68

1102

Prevalence of renal artery stenosis in diabetic patients undergoing cardiac catheterization

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Background and aims: To examine the prevalence and severity of renal artery stenosis (RAS) in patients undergoing coronary angiography.
Materials and methods: A total of 30 patients with type 2 diabetes and 26 patients without diabetes were undergone cardiac catheterization and renal angiography. Mean age was 59.1±8.3 and there was no difference in the groups. Moderate to severe arterial stenosis (>50%), was noted as significant angiographic findings. Glomerular filtration rate (GFR) was calculated by the MDRD equation.
Results: Angiographically evident RAS was present in 43.3% (30% monolateral, 13.3 % bilateral) of diabetic patients and in 30.7% of nondiabetic patients. Lipid level, GFR, ejection fraction (EF) was similar in both groups. RAS-negative and RAS-positive diabetic groups were similar in duration of diabetes and arterial hypertension, age, lipid specter, GFR. Diabetic patients with RAS had lower EF (p=0.008), lower hemoglobin level (p=0.046) and

revealed significant difference in the incidence of previous myocardial infarction (p=0.004). Multivessel coronary artery disease was more frequent in diabetic patients with RAS (73.3%) as compared with those without RAS (42.3%) (p=0.017) and that was the reason why they were more often undergone coronary stenting (p=0.027) and coronary bypass surgery (p=0.02). RAS was associated with incidence of previous myocardial infarction (RR=3.81, CI 95% (1.3; 11.4), p < 0.05), coronary stenting (RR=2.5, CI 95% (1.28; 4.88), p < 0.05) and coronary bypass surgery (RR=2.82, CI 95% (1.42; 5.61), p < 0.05).
Conclusion: The prevalence of RAS was more frequent among diabetics in comparison with nondiabetics undergoing coronary catheterization. Renal angiography should be considered particularly in diabetic patients with multivessel coronary disease with even normal renal function or mild renal insufficiency.

1103

Renal complications due to white-coat hypertension in type 2 diabetes
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Background and aims: The aim of this study is to assess renal complications due to white-coat hypertension in type 2 diabetes.
Materials and methods: This study is based on the exploration of 412 patients, mean age 58.4 ± 12.7 years, followed for type 2 diabetes with mean duration 10.6 ± 9.8 years. They did not receive any cardiovascular treatment. These patients were divided into 3 groups according to the values of their clinic blood pressure (cBP) and the average of their diurnal BP (dBP), measured by ambulatory BP monitoring: patients with normal BP (cBP<140/90 mmHg and dBP<135/85 mmHg, N = 225), white-coat hypertension (WCH: cBP> dBP 140/90 mmHg and <135/85 mmHg, N = 76) and hypertension (HBP: cBP> 140/90 mmHg and dBP> 135/85 mmHg, N = 111) . The impact is assessed by renal creatinine clearance (Cockcroft formula) and the urinary albumin excretion rate (UAER, mg/24 hours).
Results: Age of patients, duration of diabetes and BMI did not significantly differ in the 3 groups. The average of diurnal BP of WCH subjects was higher than those of patients with normal BP (SBP: 124 ± 8 vs 118 ± 9 mmHg, p <0.0001 and DBP: 76 ± 6 vs 73 ± 6 mmHg, p <0.001). Creatinine clearance did not differ significantly in the 3 groups. UAER of subjects presenting WCH was higher than those with normal BP (79 ± 154 vs 40 ± 121 mg / 24 hours, p <0.05). Comparison of HBP and WCH led to the same conclusions for BP (SBP: 143 ± 11 vs 124 ± 8 mmHg, p <0.0001 and DBP: 85 ± 10 vs 76 ± 6 mmHg, p <0.0001) and for UAER (139 ± 236 vs 79 ± 154 mg / 24 hours, p <0.05). For the whole population, UAER was linked to the difference between cBP and dBP (r = 0188, p = 0.002 with SBP and r = 0145, p = 0.003 with DBP). The difference between cDBP - dDBP explained 9.1% of the UAER variance.
Conclusion: This study highlights the frequency of WCH in type 2 diabetes (18.4%) and shows that it can lead do kidney damage. Then, according to this result, patients with white-coat hypertension should be treated.

1104

Circadian blood pressure variation in patients with type 2 diabetes - relationship between dipper status and early cardiovascular organ damage
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Background and aims: The role for diurnal blood pressure pattern as cardiovascular risk assessment tool in clinical practice is unclear. The aim of this study was to explore the association between nocturnal blood pressure dipper status and measures of arterial stiffness and left ventricular mass in patients with type 2 diabetes.
Materials and methods: We analysed data from 414 patients with type 2 diabetes, aged 55-66 years, in the ongoing observational CARDIPP (Cardiovascular Risk factors in Patients with Diabetes - a Prospective study in Primary care) study. Blood samples were taken for analyses of serum lipids, serum creatinin and HbA1c. Nurses measured office blood pressure (mean values of 3 measurements in sitting position) and ambulatory blood pressure during twenty-four hours. Left ventricular mass index (LVMI) was determined with echocardiography and aortic pulse wave velocity (PWV) was measured with applanation tonometry over the carotid and femoral arteries.
Results: Dippers were defined as a ≥ 10% nocturnal reduction in systolic blood pressure. We identified 264 dippers and 150 subjects with a nocturnal non-dipping pattern. There were no differences in office systolic blood pressure (138 ± 16 vs 139 ± 16) or office diastolic blood pressure (80 ±10 vs 81 ± 11) between dippers and non-dippers, respectively. Non-dippers had higher PWV (11 ±2.2 vs 10 ± 2.2, p=0.002) and increased LVMI (127 ± 29 vs 119 ± 29, p=0.02) compared to dippers. When exploring the strength of the association between dipper status and PWV and LVMI, respectively, in a linear regression adjusted for gender, age, HbA1c, office systolic blood pressure and serum creatinin, the results remained significant for PWV (p=0.004) and LVMI (p=0.025).
Conclusion: We conclude that a non-dipping pattern in nocturnal blood pressure was associated with increased arterial stiffness and increased LVMI in middle aged patients with type 2 diabetes. The association was independent of office systolic blood pressure and serum creatinin.
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1105

Relationship of retinal vessel caliber with provoked vasodilatation under flickering light in adolescents with type 2 diabetes and hypertension
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Background and aims: The assessment of retinal vessel caliber provides prognostic information regarding diabetic retinopathy. Additional information regarding retinal abnormalities may be obtained when vessels are functional challenged (dynamic vessel analysis). In response to flickering light, used as a stimulus to test vascular reactivity, the retinal vessels dilate. Reduced response to flicker is considered as a marker of endothelial dysfunction. We investigated the retinal vessel caliber (static analysis) and flicker-induced retinal vessel dilatation (dynamic analysis) in patients with type 2 diabetes and hypertension.
Materials and methods: We studied 76 healthy controls and 171 type 2 diabetic patients with known hypertension. The diameter of retinal vessels was measured with the Dynamic Vessel Analyzer. Diabetic retinopathy was classified according to the Early Treatment Diabetic Retinopathy Study criteria. Retinal vessel calibers are expressed in µm and changes in vasodilatation as percentage change over baseline value.
Results: After adjustment for age, sex, mean arterial blood pressure and blood glucose, the central retinal artery equivalent (CRAE) was significantly higher in patients without retinopathy (p<0.028) in comparison to controls. Patients with proliferative retinopathy showed significantly reduced CRAE in comparison to any other stages of retinopathy. After adjustment for age, sex, mean arterial blood pressure, blood glucose and CRAE, there was a significant trend of decreasing arterial vasodilatation after flickering light with increasing stages of retinopathy (p<0.016). Central retinal venous equivalent (CRVE) was significantly increased in diabetic patients without retinopathy (p<0.002), in mild NPDR (p<0.007) and in moderate NPDR (p<0.001) after adjustment for age, sex, mean arterial blood pressure and blood glucose. The venous vasodilatation after flickering light decreased with increasing stages of retinopathy although the trend was statistically not significant. Linear regression analysis showed a significant correlation of CRVE with HbA1c (β = 0.211, p<0.026).
Conclusion: Initial stages of retinopathy are associated with arterial and venous vasodilatation whereas the proliferative retinopathy is associated with arterial vasoconstriction. The flicker-induced arterial vasodilatation decreased significantly with increasing stages of retinopathy independent of CRAE. Static and dynamic retinal vessel analysis represent two different pathophysiological mechanisms. The implementation of both analysis may provide additional information about abnormal retinal autoregulation in diabetic retinopathy.

1106

Glycaemia and blood pressure management improves the outcome of laser coagulation in hypertensive patients with diabetes

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Background and aims: Poorly controlled diabetes mellitus (DM) and high blood pressure (BP) are major risk factors for retinopathy development. Fifteen years from diagnosis, ~50% of people with Type 1 and 80% with Type 2 DM develop proliferative retinopathy (PDR). Laser coagulation (LC) is the most effective therapeutic approach to PDR and macular edema (MO). The aim of the present work was to show that LC performed when glycaemia and BP control is good, prevents PDR progression and decreases the rate of relapse.
Materials and methods: Patients with PDR (n=52, 88%) and MO (n=7,12%) were divided into 2 groups: Gr.1-(n=37), systolic BP(SBP) 160±15 mmHg and microalbuminuria (MA)-80±20 mg\l; Gr. 2-(n=22)-SBP 162±20 mmHg and MA 85±23 mg/l. At entry mean fasting (FBG) and postprandial (PBG) blood glucose and HbA1c levels in both groups were: FBG-40±15 mg\dl; PBG-230±30 mg\dl; HbA1c-12.4±2.5%; Glycaemia and ABP control was normalized in both groups before LC was performed: Gr. 1 intensive insulin therapy and aggressive antihypertensive treatment; Gr. 2 oral hypoglycemic agents and less tight ABP control. LC comprised ~2500 pan retinal burns for PDR and standard grid for MO.
Results: At 3 months glycaemia and ABP indices had improved: FBG-05±10 mg\dl; PBG-60±20 mg\dl; HbA1c-7.6±1.5%. In Gr. 1-SBP and MA were normalized (130±10 mmHg and 25±9 mg\l, respectively, p=0, 001); in Gr. 2-SBP and MA-142±15 mmHg and 38±12 mg\l, respectively, p=0, 001. At 3 months LC was successful in 49/52 PDR patients; in 3 (5.8%) cases from Gr. 2, where glycemia and ABP control was less strict, relapse occurred. Hard exudates cleared and vision improved in MO eyes.
Conclusion: In this study population strict glycaemia and ABP control resulted in better outcomes in patients with PDR and MO.

1107

Estimated GFR but not ADMA is independently associated with diabetic retinopathy in type 2 diabetes

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Background and aims: Patients with type 2 diabetes mellitus (T2DM) and diabetic retinopathy are at increased risk for cardiovascular disease. Asymmetric dimethylarginine (ADMA) is associated with cardiovascular events in patients with T2DM. We investigated the role of ADMA in T2DM patients with and without retinopathy adjusted for prevalent cardiovascular disease.
Materials and methods: One hundred twenty seven patients with T2DM (mean age: 63 yrs.; women: n=49) were included according to a cross-sectional study design. Sixty eight of these patients had macrovascular disease. Diabetic retinopathy was present in 67 patients.
Results: Patients with diabetic retinopathy had increased ADMA, longer diabetes duration and reduced glomerular filtration rate (GFR; calculated with the MDRD formula). ADMA correlated with GFR (R=-0.35; p<0.001), diabetes duration (R=0.19; p=0.048) and age (R=0.19; p=0.033). In a crude logistic regression analysis a 1 SD log increase in ADMA was significantly associated with the presence of diabetic retinopathy (OR 1.50; 95% CI: 1.03 - 2.17; p=0.034). After adjustment for prevalent cardiovascular disease this association remained significant (OR 1.48; 95% CI: 1.02 - 2.15; p=0.039). Further inclusion of GFR into the regression analysis abolished this significant relationship (OR 1.21; 95% CI: 0.81 - 1.83; p=0.354) and revealed an independent association between GFR and diabetic retinopathy. Diabetes duration (R=-0.27; p=0.006), HbA1c (R=0.31; p=0.001) and diastolic blood pressure (R=0.25; p=0.011) correlated with GFR. A GFR decrease of 10 ml/min/1.73 m2 was associated with an OR of 1.32 (95% CI: 1.13 - 1.54; p=0.001) for the prevalence of diabetic retinopathy. After adjustment for possible confounders GFR remained significantly related to diabetic retinopathy (OR 1.25; 95% CI: 1.04 - 1.50; p=0.019).
Conclusion: These findings indicate that the prevalence of macrovascular disease does not influence the relationship between ADMA and diabetic retinopathy in patients with T2DM. However, this association seems to be caused by reduced renal function found in subjects with diabetic retinopathy. GFR was the only parameter independently related to diabetic retinopathy in this study.

1108

Diabetic retinopathy and associated conditions - what relationship? A study in patients with type 2 diabetes

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Background and aims: Diabetic retinopathy is the leading cause of blindness in adults in Western countries. There are few studies about this microvascular complication in the Portuguese population. The aim of the present study is to establish the relationship between diabetic retinopathy, risk factors and associated conditions, in a group of patients with type 2 diabetes mellitus.
Materials and methods: We performed a descriptive, transversal and case-control study that included 874 patients - 437 with and 437 without diabetic retinopathy, respectively, seen for the first time at the Portuguese Diabetes Association. Data were collected from informatic medical records.
Results: The group with retinopathy had significantly higher values of HbA1c, systolic blood pressure and years of diagnosis, compared with the group without retinopathy (p 6,5%) but in the subgroup with retinopathy, the percentage of patients in these conditions was higher (91,3%) compared to control group (73,2%) (p <0,05). The prevalence of hypertension in the sample was 73%. It was found that the group with retinopathy had a significantly higher prevalence of hypertensive patients (79,6% versus 66,4% - p<0,05). The prevalence of nephropathy was higher in the group with retinopathy (35,6% versus 20,8% - p<0,05).
Conclusion: There is a positive correlation between retinopathy and hypertension, glycaemic control and nephropathy. Systolic blood pressure seems more important than diastolic blood pressure for this association. With this study we reinforce the importance of blood pressure control and to educate patients about the benefits of a good glycaemic control.

1109

Paraoxonase gene polymorphisms are associated with carotid arterial wall thickness in essential hypertensive subjects with metabolic syndrome

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Background and aims: Paraoxonase (PON1), a HDL-associated enzyme, is protective against LDL oxidation. Two frequent mutations of the PON1 gene, Q192R and L55M, have been associated with carotid arterial wall thickness in young adults, in type 2 diabetes, and in familial hypercholesterolemia.
Materials and methods: In this pilot study, we examined the influence of the two polymorphisms on carotid atherosclerosis in 183 newly diagnosed untreated hypertensive subjects 64 of whom (35%) had Metabolic Syndrome (MS). Distributions of the two polymorphisms were determined by PCR-RFLP analysis. High resolution B-mode ultrasound assessed carotid intima-media wall thickness (IMT). MS was defined according to the latest NCEP-ATPIII.
Results: Frequencies of the QQ and LL genotypes were 56% and 52%, respectively, with no differences between MS+ (n. 64) and MS- (n. 119). QQ and R carriers, as well as LL and M carriers did not differ for age, BMI, waist, systolic and diastolic BP (ambulatory BP monitoring), creatinine, MDRD-eGFR, total-, LDL- and HDL-cholesterol, triglycerides, apolipoprotein A1 and B, fasting- and OGTT-AUC- (Area Under the Curve) glucose, fasting- and OGTT-AUC-insulin, HbA1c. No differences by genotypes emerged after stratification by MS. Carotid IMT was higher in QQ (n. 103; 0.81±0.25 mm) than in R carriers (n. 80; 0.73±0.22 mm, p=0.02). This difference was lost in MS- (0.78±0.25 vs 0.73±0.23 mm) but persisted in MS+ (0.85±0.25 vs 0.72±0.21 mm, p=0.04). No difference in carotid IMT was observed between LL genotypes and M carriers (0.79±0.25 vs 0.76±0.23 mm) in the whole co-

hort, as well as in MS- (0.76±0.24 vs 0.76±0.25 mm) and MS+ (0.84±0.27 vs 0.77±0.20 mm). Homozygous wildtype QQ/LL (48 subjects, 26%) had the highest carotid IMT (0.85±0.27 mm) as compared to the other genotypes (combined: 0.75±0.23 mm, p=0.01). Again, this difference was present in MS+ (0.91±0.31 vs 0.77±0.20 mm, p=0.05) but not in MS- (0.81±0.25 vs 0.75±0.24 mm). Multiple regressions analysis demonstrated the QQ genotype and the QQ/LL combination of the PON1 gene to be independent risk factors for increased IMT.

Conclusion: In conclusion, our data support the view of an independent association between PON1 gene variants and carotid atherosclerosis in essential hypertensive subjects with metabolic syndrome.

PS 100 Eye complications

1110

Localized peripapillary optic nerve fibers deficiency (LPONFD) in diabetic patients without retinopathy

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Background and aims: Fundus photographs examination revealed that some diabetic patients have localized peripapillary optic nerve fibers deficiency (LPONFD) without glaucoma criteria. The aim of the study was to evaluate the functional impact of these defects.

Materials and methods: This case-control study enrolled 2 000 consecutive diabetic patients (without retinopathy or with minor retinopathy) between july 2007 and july 2008 : 150 LPONFD were detected (prevalence of 7,5%). A prevalence of 3% was observed in 170 controls subjects without diabetes. A complete ophthalmologic examination was made (intraocular pressure (IOP) measurement, visual field analysis (VF), retinal nerve fiber layer measurement (RNFL) by OCT) in 40 subjects with LPONFD, 30 subjects with normal tension glaucoma (NTG) et 56 diabetic controls.

Results:

	Controls (n=56)	LPONFD (n=40)	NTG (n=30)	p
Age (years)	53.5 +/-1.71	58.04 +/-1.89	62.9 +/-1.99	0.006
Diabetes duration (years)	12.9 +/-0.94	10.25 /- 1.38	8.33 +/- 1.27	0.2
HbA1C (%)	7.98 +/- 0.16	7.85 +/- 0.31	9.2 +/- 0.48	0.04
IOP	14.8 +/-0.86	16.6 +/-0.56	16.3 +/-0.53	0.26
OCT RNFL	108 +/-1.42	99.5 +/-2.31	79.7 +/-3.49	0.0001
VF MD	-0.62 +/-0.35	-1.59 +/-0.52	-4.26 +/-1.43	0.006
VF PSD	1.7 +/-0.09	3.66 +/-0.3	4.1 +/-0.53	0.017
small optic disk (%)	18	43	0	0.001

LPONFD was observed in diabetic subjects younger than those with NTG. There were no difference between groups in prevalence of cardiovascular risk factors and diabetic complications.

Conclusion: In this diabetic population, we reported LPONFD resulting in impairment of RNFL and in corresponding visual field defects. LPONFD seems not to be related with diabetes mellitus but might be associated with a constitutional or acquired small optic disk.

1111

Low prevalence of diabetic retinopathy in a geriatric population of type 2 diabetic patients

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Background and aims: There is just a few evidence on the prevalence of diabetic retinopathy in the geriatric population. Up to now just one study concentrated specifically on the incidence of retinopathy in patients diagnosed with diabetes mellitus after the age of 70 years. We estimated the prevalence of diabetic retinopathy in patients with type 2 diabetes mellitus diagnosed after the age of 70 years in a ambulant and hospital based German population . The prevalence of retinopathy, blood glucose and blood pressure control was examined. The incidence of retinopathy in the geriatric population was compared to the incidence of retinopathy in type 2 diabetic patients diagnosed between the age of 40 and 70 years.

Materials and methods: The study was performed on a collective of type 2 diabetic patients who were being treated in a large out- and inpatient diabetes clinic at a tertiary university hospital. A total of 136 patients with the age of diagnosis of 70 years or more (age 80y, time since diagnosis 5y, HbA1c 9%, blood pressure 146/79mmHg, BMI 28 kg/m^2) and 1551 patients with the age of diagnosis between 40 and 70 years (age 66y, time since diagnosis 13y, HbA1c 8%, blood pressure 145/82 mmHg, BMI 30 kg/m^2) were investigated. Patients with an eye examination within the last 2 years were included. Diabetic retinopathy was classified as no retinopathy (no DR), non-proliferative DR, proliferative DR and blindness.

Results: In a group of 174 patients diagnosed after the age of 70 years 13.2% had DR (8.8% non-proliferative and 3.7% proliferative retinopathy). One patient was blind. In a group of 1551 patients diagnosed with diabetes between 40 and 70 years 25.8% showed signs of DR (18.9% non-proliferative and 6.9% proliferative retinopathy). In a group of patients diagnosed at the age of 70 or more, patients with diabetic retinopathy were older (82 vs. 80 J) and had significantly longer duration of the disease (8 vs. 5J) in comparison to the patients who had no signs of retinopathy. The HbA1c, systolic blood pressure, creatinine in serum and urine albumin excretion was higher in a group with retinopathy, although the difference was not significance.
Conclusion: The prevalence of retinopathy in diabetic patients diagnosed after the age of 70 is only half as much as in diabetic patients diagnosed at the age between 40 and 70 years. The prevalence of retinopathy in the elderly group is even lower as in a similar Danish analysis (Cahill et al 1997, DR 14%) and in an American study (WESDR 1984, DR 39%). The prevalence of proliferative retinopathy in the Danish study (3,3%) was similar to our results. Therefore, older patients with late onset of the disease are less endangered to suffer from diabetic retinopathy.

1112

Results of screening for diabetic retinopathy in people with diabetes mellitus type 2 in Uzbekistan

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Background and aims: Diabetic retinopathy (DR) remains to be one of the most serious complications of diabetes mellitus (DM) resulting in partial or full loss of vision and subsequent disability. Screening for DR in PDM especially in those with DM type 2 is an effective method of DR detection at various stages of its development which enables development of step by step pathogenetic therapy.
Goal: carrying out of DR screening in PDM in regions of Uzbekistan and development of methods for prevention and early diagnostics of DR.
Materials and methods: Within the framework of the WDF 07-232 project «Prevention of blindness in people with diabetes mellitus in Uzbekistan» granted to Tashkent CPA "UMID", DR screening was performed in PDM type 2 residing in the Ferghana, Bukhara regions and the Republic of Karakalpakstan. We examined 2087 people with diabetes mellitus type 2 aged 18 to 67. A level of glycaemia (a fasting one and 2 hours after meals), glycosylated haemoglobin and cholesterol were measured. Eyes were examined with a direct ophthalmoscope (after preliminary pupil dilatation), visual acuity and an intraocular tension were measured.
Results: The analysis of the results of the screening undertaken in 2087 patients demonstrated that the DR rate in PDM type 2 made 56.9%. Of them DR grade I was revealed in 32.8%, DR grade II -18.6%, DR grade III was found in 5.5%. Retinopathy was not detected in 43% of patients. Percentage of patients in who DR was diagnosed during the screening for the first time made 52%. In comparison with the date obtained by D.M. Berdykulova (Research Institute of Endocrinology 2003) epidemiological researches undertaken in the Tashkent region the frequency of DR in DM type 2 made 57.4% (grade I - 77.7%, II - 23% and III - 6.9%). The screening studies performed correspond to the data of the epidemiological research. The prevalence rate of DR in DM type 2 was reliably higher in females than in males ($p<0.05$). Studies of DR frequency in PDM type 2 of glycosylated hemoglobin (HbA1c) revealed a clear rise in prevalence of all DR stages from the HbA1c level. Prevalence of DR stages I, II and III in faces with controlled carbohydrate exchange (HbA1c<7%) made 11.3%, 3.4% and 2.0% respectively ($p<0,001$). Prevalence of DR stages I, II and III in subjects with uncontrolled carbohydrate exchange (HbA1c>8.5%) was 36.9%, 15.8% and 8.8% respectively. Mean rates of systolic and diastolic pressure in sick with DR grades II-III made 151.0±5.1 mm Hg and 90.8±2.2 mm Hg which corresponds to AH stage I. The frequency of DR in PDM type 2 depended directly on the patient's age. In PDM type 2 with DR mean rates of total cholesterol, LDL and HDL mismatched the norm having made 5.5±0.2 mmol/L, 4.14±0.22 mmol/L and 0.94±0.03 mmol/L, respectively. It should be noted that the mean LDL was reliably higher in comparison with that in patients without DR (3.65±0.07 mmol/L).
Conclusion: According to the screening performed, the frequency of DR in patients with DM type 2 made 56.9%, where the share of DR grade I was 32.8%, DR II - 18.6% and DR III- 5.5%. Advance of DR in patients with DM type 2 depends on duration of the disease and a degree of controlling carbohydrate exchange as well as AH, hypercholesteremia and dislipidemy. Screening is quite an effective method of revealing retinal lesions in PDM and promotes quality improvement of treatment and prophylactic care at early stages of DR development.
Our acknowledgement to WDF for the great contribution to improvement of diabetic service in Uzbekistan

1113

Ethnic disparities in eye care for people with diabetes

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Background and aims: Diabetes prevalence is known to vary between ethnic groups. However, variations in diabetes microvascular complication rates between ethnic groups have been less well established, as have utilisation rates for medical services to manage these complications. The aim of this study was to compare eye care for diabetes between people of South Asian and Chinese origin versus the general Canadian population (mostly of European origin).
Materials and methods: The study used administrative data sources which provided detailed information on the health care utilisation of all residents of the province of Ontario (population=12 million). Ontario is one of Canada's most ethnically diverse provinces, with 6.6% of the population reporting South Asian origin and 4.8% reporting Chinese origin. The entire population with prevalent diabetes as of 1 July 2006 in Ontario was identified from an administrative data-derived disease registry. South Asian and Chinese ethnicity was assigned based on surname, using lists of surnames specific for each ethnic group that have been validated against self-reported ethnicity. All other people were assigned to the general population group. Through linkage with administrative data, patients who had physician claims for an eye examination, for laser photocoagulation therapy or for vitrectomy surgery in the following year were identified. Differences between groups were determined, using logistic regression to adjust for age, sex, socioeconomic status and diabetes duration.
Results: The crude prevalence of diabetes was 12.9% among people of South Asian origin, 7.2% among people of Chinese origin, and 9.1% in the general population. Compared to the general population, people of South Asian origin and of Chinese origin were less likely to have an eye examination (OR 0.86, 95% CI 0.84-0.88 and OR 0.83, 95% CI 0.81-0.85, respectively). However, people of South Asian origin were more likely to require laser photocoagulation (OR 1.46, 95% CI 1.35-1.58) whereas those of Chinese origin were less likely (OR 0.60, 95% CI 0.53-0.68). The results for vitrectomy surgery were similar, although attenuated (OR 1.12, 95% CI 0.93-1.35 and OR 0.70, 95% CI 0.55-0.90, respectively).
Conclusion: People of South Asian and Chinese origin in Ontario were less likely than the general population to receive eye examinations to screen for diabetic retinopathy. South Asians had worse clinical outcomes, with a greater need for laser photocoagulation. These findings suggest that South Asians with diabetes have an increased burden of retinopathy and/or are experiencing clinical consequences of inadequate screening.
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1114

Analysis of intraocular level of VEGF in patients with diabetic retinopathy, operated from cataract

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Background and aims: Diabetic retinopathy (DR) is the main cause of blindness among young people, suffering from diabetes mellitus (DM). DR is associated with increasing of proliferative molecules in eye, which stimulate permeability, oedema and growth of new vessels. The most established agent, leading to proliferative DR, is vascular endothelial growth factor (VEGF). The aim of our study was to determine level of VEGF in aqueous humor from patient with DM and cataract and to indentify an association between level of VEGF, glycated hemoglobin (HbA_{1C}) and visual acuity (VA) at the postoperative period.
Materials and methods: In the study were included 10 patients with DM type 1; 52 - with DM type 2 and 6 - without DM (as control group). During operation from cataract (we used ultrasound facoemulsification and following implantation the acrylic intraocular lens) the aqueous humor was obtained, the sample was prepared by prompt centrifugation (15,000 g*min) and stored at -80°C. The level of VEGF was analyzed by ELISA. All patients were examined before and after operation. The grade of DR was measured using recommendation of WHO (1999). Results of data analysis were expressed as means±SD, all tests with $p<0.05$ were considered significant. Relationship between VEGF, visual acuity and evidence of retinopathy was analyzed using multiple regression.
Results: 84 eyes (68 patients) were operated from cataract, prevalence of DR has been presented in tab.1, sings of DR were absented only in 4% of patients with DM. Laser coagulation of retina were performed on several patient from DR before operation. Level of HbA_{1C} didn't significantly different (p=0,1976) in groups of patients with DM. Corrected VA before operation in patients without DM were 0,33±0,32 and 0,26±0,23 in patients with DM, in groups of DR significant difference didn't observe. After operation VA was improve and was 0,58±0,39 in patients without DM and 0,45±0,33 in patient with DM. It progressively was worsen in patient with proliferative DR (0,25±0,26), difference was statistically significant. Level of VEGF in aqueous humor in patients without DM was 61,17±33,78 pg/ml and progressively increased in patients with DM, in patients with proliferative DR level of VEGF was 243,39±203,82 pg/ml (p=0,0175).
Conclusion: Proliferative DR was associated with worsen corrected VA after operation from cataract. Level of VEGF in aqueous humor was considerably increased in patients with proliferative DR (comparably with patients with preproliferative and nonproliferative DR). High level of VEGF may be factor, causing deterioration vision in patients with DM.

Characteristic	Non DM	DM type 1 and 2				p-value
		No DR	Nonproliferative DR	Preproliferative DR	Proliferative DR	
Eyes, n	7	3 (4%)	26 (33,7%)	31 (40,2%)	17 (22,1%)	-
HbA_{1C}, %	-	6,07±0,98	8,22±1,81	8,64±2,32	8,47±1,56	0,1976
VA before operation	0,33±0,32	0,5±0,115	0,28±0,24	0,27±0,23	0,17±0,22	0,1013
VA after operation (1 month)	0,58±0,39	0,93±0,06	0,47±0,32	0,51±0,33	0,25±0,26	0,0024
VEGF, pg/ml	61,17±33,78	68,3±25,07	99,23±92,02	181,94±167,16	243,39±203,82	0,0175

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1115

Pregnancy-induced sight threatening diabetic retinopathy in women with type 1 diabetes

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Background and aims: To evaluate the progression of diabetic retinopathy in pregnant diabetic women offered tight glycaemic and blood pressure control.
Materials and methods: Prospective study of 102 (87%) out of 117 consecutive pregnant women with type 1 diabetes for median 16 years (range 1-36) and HbA_{1c} 6.7 % (4.9-10.8) in early pregnancy. Fundus photography was performed at 10 and 28 gestational weeks. Diabetic retinopathy was classified in five stages. Macular oedema was classified as present in a mild form or as clinically significant macular oedema (CSME). Progression was defined as at least one stage of deterioration of retinopathy and/or development of macular oedema in at least one eye. Sight threatening progression was defined as loss of visual acuity ≥0.2 on Snellen's chart or laser treatment performed during pregnancy due to proliferative retinopathy or CSME.
Results: Diabetic retinopathy was present at inclusion in at least one eye in 64 (63%) women and proliferative retinopathy and macular oedema was present in nine and 16 women, respectively. Progression of retinopathy occurred in 28 (27%) women with sight threatening progression in six of whom three deteriorated in visual acuity and four required laser treatment. Sight threatening progression was associated with presence of macular oedema (p=0.007), impaired vision (p=0.03) and higher blood pressure in early pregnancy (p=0.016), but not with HbA_{1c}, decline in HbA_{1c} or prevalence of severe hypoglycaemia.
Conclusion: Pregnancy-induced sight threatening diabetic retinopathy is still a clinical problem and associated with presence of macular oedema, visual impairment and higher blood pressure in early pregnancy.

PS 101 Retinopathy - experimental

1116

Angiotension II type 1 receptor blocker decreases the nitric oxide end products and inducible nitric oxide synthase preventing the early oxidative markers of diabetic retinopathy

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Background and aims: In addition to be a very frequent disease in patients with diabetes mellitus (DM), hypertension is the major risk factor associated with the development of diabetic retinopathy (DR). Oxidative stress mechanisms, renin-angiotensin system (RAS) and nitric oxide (NO) are described to be involved into the pathogenesis of DR. This investigation sought to determine whether concomitance of diabetes and hypertension exacerbates these parameters, to evaluate the potential retinal antioxidant effects of the angiotensin II type 1 receptor blocker (ARB) on retinal tissue by oral administration of losartan in diabetic hypertensive rats and to assess the mechanisms of its beneficial effects.
Materials and methods: Four-week-old spontaneously hypertensive rats (SHR) were rendered diabetic by intravenous injection of streptozotocin. Diabetic SHR rats were randomized to receive no treatment (DM-SHR) or treatment with ARB losartan (200mg/Kg, DM-SHRLos); control rats received citrate buffer. After 20 days, the rats were euthanized and the retinas collected. The results were compared by analysis of variance (ANOVA) followed by Fisher's protected least-significant difference test.
Results: As expected, the angiotensin II biodisponibility evaluated by Western blot assay was higher in retina of treated rats (p=0.02). The oxidative status, evaluated by superoxide production and NO end products levels estimated by Nitric Oxide Analyser (p=0.03) and the antioxidative systems, estimated by reduced glutathione (p=0.04) and activity of total superoxide dismutase through a colorimetric kit (p=0.002) revealed an accentuated unbalance in favor to oxidants in DM-SHR rats. As a consequence, the tyrosine nitration assessed by immunohistochemistry for nitrotyrosine (p=0.02) and DNA damage evaluated by 8-hydroxy-2'deoxyguanosine immunohistochemistry (p=0.0003) were higher. The retinal protein expression of inducible nitric oxide synthase (iNOS) was also elevated in DM-SHR rats (p=0.01). The treatment with losartan prevented all the above alterations.
Conclusion: These data demonstrate that the therapy with ARB losartan increases the reduced glutathione and total SOD activity and reduces the amount of nitric oxide end products (NO_x^-) and iNOS expression preventing the retinal nitrosative damage in a model of diabetes and hypertension. This study provides new information on understanding of the molecular basis underlying the beneficial effect observed by AT_1 blockage in the retina of patients with diabetes.
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1117

Beta2-glycoprotein I inhibits insulin and insulin-like growth factor induced angiogenesis through its amino terminal domain

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Background and aims: Insulin treatment is epidemiologically known as an independent risk factor in the progression of diabetic retinopathy. Insulin elicits angiogenesis through the induction of autocrine VEGF, thereby leading to the exacerbation of diabetic retinopathy. Beta-2 glycoprotein I (beta(2)GPI) is a plasma glycoprotein which interacts with various proteins of the coagulation and fibrinolysis system. beta(2)GPI has recently been shown to have anti-angiogenic properties. We undertook this study to investigate the specific domain of beta(2)GPI involved in the anti-angiogenic function and its effect on downstream signaling of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF).
Materials and methods: Various preparations of beta(2)GPI were used on human retinal endothelial cells (HRECs) in the absence or presence of insulin and insulin-like growth factor (IGF). The effect on HRECs' proliferation, migration and tubule formation in Matrigel matrix was investigated. The effect of beta(2)GPI on the mRNA expression of VEGF receptors and phosphorylation of signaling molecules was also studied.
Results: beta(2)GPI is shown in this study to be an anti-angiogenic molecule in vitro by inhibiting insulin and IGF-induced proliferation, migration and papillary-like tubule formation of HRECs. This inhibition was achieved by native, proteolytically clipped and domain deletion mutants, domain I-IV (DI-IV) but not domain II-V (DII-V) of beta(2)GPI. Native beta(2)GPI was found to downregulate the expression of the VEGF receptor KDR/Flk-1 on endothelial cells and to block the phosphorylation of VEGF's downstream effector molecules in the MAPK/ERK and PI3K/Akt/GSK3beta pathways.
Conclusion: These results indicate that beta(2)GPI has anti-angiogenic functions which depend on the presence of domain I. This anti-angiogenic activity may have important implications for the therapeutic manipulation of angiogenesis in diabetic retinopathy especially during insulin treatment.
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1118

Exogenous superoxide dismutase mimetic tempol prevents the early markers of diabetic retinopathy through inhibition of poly (ADP-ribose) polymerase

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Background and aims: Hypertension is an important factor associated with development of diabetic retinopathy (DR). Oxidative pathways are proposed to explain how the hyperglycemia initiates the biochemical processes in the pathogenesis of DR. The aim of this study was to investigate the efficacy of tempol (4-hydroxy-2,2,6,6-tetramethylpiperidinyloxy) a superoxide dismutase (SOD) mimetic in preventing the early retinal changes in a model that combines hypertension and diabetes.
Materials and methods: Four-week-old spontaneously hypertensive rats (SHR) were rendered diabetic by streptozotocin (60mg/kg). Diabetic SHR rats (DM-SHR) were randomized to receive or not intraperitoneal injection of tempol (250mg/kg/day); control rats received vehicle only. After 20 days of diabetes, rats were euthanized and the retinas were collected for protein extraction and colorimetric assays or the eyes were prepared for imunohistochemistry. The results were compared by analysis of variance (ANOVA) followed by Fisher's protected least-significant difference test.
Results: The early markers of DR, glial fibrillary acidic protein (GFAP) and fibronectin (FN) evaluated by western blot assays were increased in DM-SHR compared with SHR group (p=0,0001 and p=0,02, respectively). The oxidative balance, evaluated by superoxide production (p=0,05) and nitric oxide end products levels estimated by Nitric Oxide Analyser (p=0,002), and the counterpart antioxidative defense revealed an accentuated unbalance in DM-SHR compared to CT-SHR group (p=0,01 and p=0,02 for activity and expression of SOD, respectively evaluated by colorimetric kit and western blot). As a result, the product peroxynitrite detected, by retinal immunohistochemistry for nitrotyrosine, was higher in DM-SHR rats (p=0,04). The retinal poly(ADP-ribosyl)ation (PARP) and the iNOS protein expression were found to be increased in this group (p<0,05). The treatment with tempol re-established the oxidative parameters and reduced the PARP activation thus preventing the early markers of DR.
Conclusion: Our data demonstrated that the administration of tempol inhibited the ribosylation of PARP, decreased the iNOS levels and prevented the oxidative damage in the retina of diabetic hypertensive rats. As a consequence the extracellular matrix accumulation and glial reaction, accepted as early markers of DR were prevented in tempol treated DM-SHR rats. These findings provide rational for development of PARP inhibitor/SOD mimetic compound for prevention of diabetic retinal disease.
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1119

High glucose-induced downregulation of mitochondrial connexin-43 expression triggers mitochondrial shape change and cytochrome-C release in retinal endothelial cells

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Background and aims: Retinal vascular cell loss is a prominent lesion of diabetic retinopathy. While mitochondrial dysfunction has been shown to contribute to the development of pericyte loss and acellular capillaries, it remains unclear how high glucose promotes mitochondrial damage. Since de-

pletion of mitochondrial connexin 43 (Cx43) facilitates reactive oxygen species (ROS) formation, in this study, we investigated the effect of high glucose (HG) on mitochondrial connexin43 (mt-Cx43) expression and cytochrome C (CytC) release in retinal endothelial cells. Additionally, we examined whether HG-induced mitochondrial shape change was influenced by altered mt-Cx43 channel activity by inhibiting mt-Cx43-channels.
Materials and methods: Rat retinal endothelial cells (RRECs) were grown in normal (5mM) or HG (30mM) medium for 8 days, and mt-Cx43 protein level and CytC release in the cytosolic fraction was simultaneously assessed by Western Blot (WB) analysis. In parallel, mt-Cx43 immunostaining was performed using MitoTracker Red (100nm) and FITC-labeled Cx43, and overlapping Cx43 and mitochondria signals were counted to assess the number of mt-Cx43 plaques. To determine the effect of inhibition of mt-Cx43 channels on mitochondrial shape change, RRECs were plated sparsely without cell to cell contacts and grown for two days in three groups: untreated cells (control), cells treated with β-glycerrhetinic acid (β-GA), an inhibitor of Cx-channels (50uM), and cells treated with glycyrrhizic acid GZ (50uM), a chemical analog of β-GA that does not inhibit Cx-channels. Cells were then stained with tetramethylrhodamine-ethyl-ester-perchlorate (TMRE, 8nM) and assessed for mitochondrial morphology using live confocal imaging. Analysis for mitochondrial shape change was calculated using Form Factor (FF) and Aspect Ratio (AR) values of the mitochondria.
Results: Western blot analysis indicated that mt-Cx43 protein level was significantly reduced in RRECs grown in HG media compared to those grown in N media (59 ±19% of control, p=0.027), with concomitant increase in cytosolic CytC (421 ±155% of control, p=0.005). CoCl2 used as a positive control showed similar increase in CytC release (404 ±143% of control, p=0.007). Immunofluorescence data also confirmed HG-induced downregulation of mtCx43 plaques (63 ±27% of control, p=0.030). Treatment of RRECs lacking cell contacts with β-GA significantly changed mitochondrial shape to rounded forms (FF for β-GA treated RRECs: 1.71 vs. 2.60 in normal, p=0.009; AR: 1.88 vs. 2.25 in normal, p=0.010), which coincided with decreased Cx43 protein on the mitochondria (46 ±16% of control, p=0.007). Treatment with the β-GA, an inactive analog of GZ, did not significantly inhibit Cx43 protein (74% of control, p=0.081) and did not show significant shape change or fragmentation of mitochondria (FF for GZ treated RRECs: 2.14 vs. 2.60 in normal, p=0.068, AR: 1.98 vs. 2.25 in normal, p=0.079).
Conclusion: Results indicate that HG downregulates mt-Cx43 protein and triggers concomitant release of CytC. Inhibition of mt-Cx43 channel activity resulted in mitochondrial shape change, suggesting that mt-Cx43 may be important to mitochondrial shape regulation and overall homeostasis, which, when compromised under HG condition may result in the release of CytC and apoptosis. This suggests a potential mechanism for hyperglycemia-induced apoptosis in diabetic retinopathy.
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1120

Retinal pigment epithelial cells express a functional receptor for glucagon-like peptide-1
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Background and aims: Glucagon-like peptide-1 (GLP-1) is a gut-derived incretin hormone of great interest for the management of diabetes for its glucose lowering action. Treatment with GLP-1or with dipeptidyl peptidase-4 inhibitors, the enzyme that degrades GLP-1, reduces HbA_{1c} and the levels of free fatty acids, and improves insulin sensitivity. The biological effects of GLP-1 are mediated by its specific receptor GLP1R, that is expressed in a wide range of tissues, where it is responsible of the extra-pancreatic effects of GLP-1. Recently it has been reported that treatment with a long-acting agonist of GLP1R provides protection for early experimental diabetic retinopathy, suggesting an important role of GLP-1 in preventing diabetic complication. Since the retinal pigment epithelium (RPE), that forms the outer retinal barrier, has a key role in maintaining retinal homeostasis we verify the expression of GLP1R in the RPE cell line ARPE-19 and investigate the signal transduction pathway activated by GLP-1.
Materials and methods: ARPE-19 cells were cultured in DMEM/F12 supplemented with 10%FBS. A batch of cells was lysed and total protein extract were used for Western blot analysis of GLP1R. Lysates from the pancreatic insulin secreting cell line HIT-T15 were used as positive control in immunoblot analysis with specific antibody against GLP1R. Another batch of cells was cultured in serum free medium for 24 hours and stimulated for 10, 20 and 40 minutes with 10 nmol/l GLP-1. Cells were then lysed and total protein extract were used for Western blot analysis of phospho- and total extracellular signal-regulated kinases 1 and 2 (ERK1/2); phospho- and total protein kinase B (PKB). In addition a batch of cells were stimulated with 10 nmol/l GLP-1 for 20 minutes with or without pharmacological inhibitors either of the mitogen-activated protein kinase MEK1/2 (U0126) or of Phosphatydilinositol-3kinase (PI3K) (LY294002) (10 μmol/l of U0126 or 50 μmol/l LY294002 added respectively 2 hours and 30 minutes before incubation with GLP-1). Cells were then lysed and analysed as described above.
Results: Immunoblot analysis with specific antibody against GLP1R reveals a band between 60 and 70 kDaltons in protein extracts from ARPE-19 cells analogous to that detected in HIT-T15 protein lysate. Exposure of ARPE-19 cells to GLP-1 increases the protein levels of phosphorylated ERK1/2 and PKB compared to the control cells. The increment in PKB phosphorylation reaches maximum levels after 20 minutes of exposure and remains elevated after 40 minutes; whereas phosphorylation of ERK1/2 shows a progressive increase also at 40 minutes. Interestingly, inhibition of PI3K, one of the upstream activators of PKB, completely abrogates GLP-1-induced phosphorylation of PKB and of ERK1/2. Inhibition of MEK1/2, the upstream activator of ERK1/2, suppresses GLP-1-induced ERK1/2 phosphorylation, but it has not effect on GLP-1-induced PKB phosphorylation.
Conclusion: We show, for the first time, that a functional GLP1R is expressed in RPE cells. The signal transduction pathway of GLP-1 in ARPE-19 cells involves both PKB and ERK1/2, and our results suggest that it is prevalently mediated by PI3K. GLP-1, affecting RPE cells, may have a direct role in preventing RPE cell dysfunction and, consequently, diabetic retinopathy.

1121

Fenofibrate restores the barrier function of human retinal pigment epithelial cells cultured under conditions mimicking the diabetic milieu
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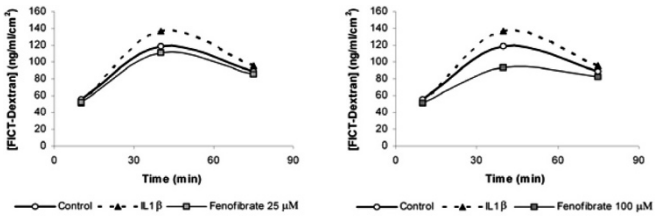
Background and aims: Diabetic retinopathy remains the leading cause of blindness and vision loss in adults under 40 years in the developed world. In the FIELD study on diabetic retinopathy, treatment with fenofibrate reduced the need for laser treatment for diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR) by 30%. Because of this notable effect of fenofibrate in preventing DME progression, the aim of this study was to explore the effect of fenofibrate on the barrier function and the expression of tight junction proteins (occludin, ZO-1 and claudin-1) in a human retinal pigment epithelial (RPE) cell line under culture conditions mimicking the diabetic milieu.
Materials and methods: ARPE-19, a spontaneously immortalized human RPE cell line, was cultured for 18 days at 37°C under 5% CO_2 in medium (DMEM/F12) supplemented with 10% fetal bovine serum in hyperglycemic conditions (25 mM D-glucose). To study the potential protective effect of fenofibrate on the barrier function of RPE cells, two concentrations of fenofibric acid (25 μM and 100 μM) were added to the standard culture medium in the last 3 days of the experiment (days 19, 20, 21) (1 application/day). Cells were also treated with IL1β (10 ng/ml) for 48 hours until the end of the experiment in order to mimick the diabetic milieu (days 20, 21). The cells were subjected to serum starvation (1% FBS) during the treatments. Barrier function of RPE (permeability) was evaluated by measuring apical-basolateral movements of FICT-dextran (40 kDa). Occludin, zonula occludens-1 (ZO-1) and claudin-1 expression were determined by western blot analysis.
Results: Treatment of ARPE-19 cells with fenofibric acid reduced the increment of permeability induced by IL1β. This protective effect on monolayer permeability was more evident in cultures treated with fenofibric acid 100 μM than in cultures treated with fenofibric acid 25 μM, thus modulating permeability in a dose-dependent manner. No significant differences were observed in occludin, ZO-1 and claudin-1 protein content between cultures treated with fenofibric acid and untreated cultures.
Conclusion: These results indicate that fenofibric acid has a protective dose-dependent effect on RPE disruption that is unrelated to changes in occcludin, zonula occludens-1 (ZO-1) and claudin-1 expression. This protective effect on the leakage of the outer blood-retinal barrier could be involved in the beneficial effects of fenofibrate on DME. However, further investigation addressed to determining the mechanisms by which fenofibrate exerts its effects in reducing permeability is needed.



Results of 40 kDa FICT-Dextran Permeability

1122

WITHDRAWN

1123

Beneficial effects of erythropoietin on retinal pigment epithelial cells cultured under conditions mimicking the diabetic milieu

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Background and aims: Visual loss in diabetic macular edema (DME) results from fluid accumulation due to the breakdown of the (inner and outer) blood retinal barriers (BRBs). We have previously reported that erythropoietin (Epo) is synthesized by the human retina and that its levels are increased in patients with diabetic macular edema (DME). In addition, there is growing evidence that Epo acts as a prosurvival and neuroprotective factor in the early stages of DR. Furthermore, it has been reported that Epo administration improves DME in diabetic patients with anemia and renal failure. However, there is no information regarding the mechanisms involved in this beneficial effect of Epo.

The aim of this study was to explore the effects of Epo on the outer BRB function by means of measuring permeability and transepithelial resistance (TER) in cultures of human RPE. In addition, the effects of Epo on the expression and distribution of three TJs proteins (occludin, ZO-1 and claudin-1) were also investigated.

Materials and methods: ARPE-19 cells (a spontaneous immortalized RPE cell line) were cultured in monolayer during 21 days in standard Ham´s-F12 DMEM medium supplemented with fetal calf serum (FCS 10%). Epo treatments were performed by adding Epo (50-1000 mU/mL) at the apical side of the monolayer in standard conditions (control) or after mimicking the diabetic milieu (by addition of IL-1β or VEGF at 10 ng/mL). To further clarify the Epo-induced effects, the experiments were repeated in the presence and absence of a neutralizing antibody or specific inhibitors (AG490, a JAK2 kinase Epo receptor associated inhibitor, LY294002, a PI3K inhibitor and GF109203X a PKC inhibitor). The outer BRB function was evaluated by measuring TER and the apical-basolateral movements of fluorescein isothiocyanate dextran (70 KDa). ZO-1, occludin and claudin-1 mRNA levels were assessed by Real-Time PCR and protein content by Western blot. Inmmunohistochemistry for ZO-1, occludin and claudin-1 was performed and fluorescent images were acquired by confocal microscopy

Results: Epo treatment (200 mU/mL) significantly increased TER *vs*. control ($p=0.0038$, after 6 h) and reduced permeability to 70 KDa dextran ($p=0.0062$). These effects were blocked by the neutralizing antibody (3-1.5 μg/mL) as well as by AG490. LY294002 and GF109203X also decreased the effect of Epo but did not reach statistical significance. Confocal microscopy images confirmed a positive staining of TJs at the intercellular outlining, necessary for barrier function. mRNA levels of occludin, claudin-1 and ZO-1 were significantly increased after 24 h of Epo treatment ($p<0.002$; $p<0.05$; 0.05; $p<0.0019$ *vs*. control). However, we did not find a significant increase in protein levels.

Conclusion: Epo administration reduces the hyperpermeability induced by the diabetic milieu in human RPE cells *in vitro*. This beneficial effect seems to be mainly mediated by the downstream signalling of JAK2 kinase Epo receptor protein. Overall our findings suggest that Epo could be proposed as new treatment for DME by targeting the outer BRB.

1124

AAV-mediated PEDF gene transfer counteracts diabetic retinopathy in IGF-I transgenic mice

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Background and aims: Diabetic retinopathy is the leading cause of loss of visual acuity and blindness in adulthood. Current therapeutic approaches for diabetic retinopathy, such as laser photocoagulation and vitrectomy, are invasive and merely palliative and repetitive local delivery of drugs is not desirable in a chronic disease. Transgenic mice overexpressing Insulin-like Growth Factor (IGF-I) in the retina have retinal alterations characteristic of non-proliferative retinopathy and, with age, mice develop alterations that mimic the proliferative stage of diabetic retinopathy such as neovascularization in the vitreous cavity and retinal neovascularization. The aim of this study was to assay new therapeutic approaches for diabetic retinopathy in IGF-I transgenic mice.

Materials and methods: AAV2 vectors expressing Green Fluorescent Protein (GFP) or Pigmented Epithelium Derived Factor (PEDF) were injected in the vitreous cavity of transgenic mice. Inmunohistochemical studies were performed to check the transgene expression. After, dextran-conjugated fluorescein injection through the mice tail vein, retinas were dissected and fixed and the vasculature was analyzed using morphometric techniques. The expression of key angiogenic factors was checked by RT-PCR or Western blot in non-treated and PEDF-treated mice.

Results: With the aim of overcoming the angiogenic process present in the transgenic mice retina, the antiangiogenic factor PEDF was overexpressed in the mice retinas using an AAV2 viral vector. PEDF is one of the most potent anti angiogenic factors and also has neuroprotective properties, making it an excellent candidate for gene transfer in diabetic retinopathy. Our studies show that AAV2 vectors have a high efficiency in the transduction of cells of the ganglionar layer and also cells in the inner nuclear layer. PEDF was detected in retinas of mice six months after intravitreal injection of AAV2 PEDF. These animals had a significant reduction of neovascularization in the retina, as measured by fluorescein angiography morphometry and also showed a decrease in retinal inflammatory molecule levels, such as Intercellular Adhesion Molecule 1 (ICAM-1).

Conclusion: PEDF overexpression was able to counteract neovascularization and reduce inflammation in TgIGF-I retinas, indicating that AAV-PEDF treatment could be a feasible therapy for diabetic retinopathy.

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PS 102 Retinopathy

1125

Prevalence of diabetic retinopathy through the years 1994 to 2007

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Background and aims: To assess the prevalence of diabetic retinopathy in patients with type 2 DM and duration of diabetes 5-10 years from 1994 to 2007.
Materials and methods: This study includes 303 patients (F/M 160/143) with type 2 DM with duration of DM 5-10 years. The fundus findings at their first visit to the Diabetes Outpatient Clinic were considered. Patients were grouped regarding the year of their application to the Clinic as follows: between the years 1994 and 1999 (Group 1, n: 128) and between the years 2000 and 2006 (Group 2, n:175).
Results: The mean duration of DM was 7.3±1.7 years in Group 1 and 7.7±1.8 years in Group 2 (p>0,05). The mean chronological age was 55.6±9.7 years in Group 1 and 57.5±10.0 years in Group 2 (p>0,05). In Group 1 73.4% and in Group 2 77.9% of patients had diabetic retinopathy. Nonproliferative diabetic retinopathy (NPDR) was present in 24.1% and proliferative diabetic retinopathy (PDR) in 2.5% of patients in Group 1; 21.2% and 0.9%, respectively in Group 2. Although the number of patients with no diabetic retinopathy were less this did not reach statistically significant levels. Regarding the prevalence of NPDR and PDR, there was no statistically significant difference in the severity of diabetic retinopathy between the Group 1 and Group 2.
Conclusion: Despite efforts for increasing awareness for diabetes and its complications, availability of wider range of technologies and drugs for better diabetes management in Turkey the prevalence of diabetic retinopathy seems not to be dramatically declined from 1994 to 2007.

1126

Glycated haemoglobin as a surrogate marker for the appearance and progression of retinopathy in type 1 diabetes mellitus: systematic review and meta-analysis

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Background and aims: Glycated haemoglobin (HbA1c) is commonly employed in clinical trials as a surrogate marker of diabetes control and the risk for diabetic complications in type 1 diabetes mellitus (T1DM). The efficacy of many glucose-controlling agents has been characterised with respect to HbA1c, without their effects on clinically important endpoints being demonstrated. To date, several trials have examined the relationship between HbA1c level and microvascular complications in T1DM, but no systematic review has been published. We performed a systematic review and meta-analysis to examine the association between HbA1c and the appearance and progression of diabetic retinopathy (DR) in T1DM.
Materials and methods: We conducted a systematic literature search using two electronic medical databases (MEDLINE and The Cochrane Library). Observational studies and randomised, controlled trials (RCTs) of retinopathy in T1DM patients that reported HbA1c level were included in the analysis. Our chosen search strategy was highly sensitive, including over 100 terms grouped into three categories: population, e.g. "diabetes mellitus", "insulin-dependent diabetes mellitus", "IDDM"; surrogate, e.g. "glycosylated haemoglobin", "HbA1c"; and clinically important outcomes, e.g. "retinopathy". Estimates were made of the adjusted relative risk (RR) of complications for an increase in HbA1c of 1%. If there were insufficient data to calculate RR, the odds ratio (OR) was estimated instead. Weighted mean differences (WMD) in HbA1c level (mean value during the observation period) between the case group (with DR) and the control group (without DR) were also calculated.
Results: We identified 16 trials that fulfilled the inclusion criteria, involving a total of 4176 patients. Based on four RCTs (n=1597), pooled RR for progression of DR was calculated as 1.24 (confidence interval [CI]: 1.01-1.52; p=0.039) for an increase in HbA1c of 1%. Pooled data from four observational studies (n=910) showed that RR of the incidence of DR was 1.59 (CI: 1.34-1.89; p<0.0001) for an HbA1c increase of 1%. A meta-analysis of eight observational studies (n=1171) demonstrated a lower mean HbA1c level in patients without DR compared with patients with DR (WMD=0.82 [CI: 0.69-0.96]; p<0.0001). In addition, a meta-analysis of five observational studies revealed that mean HbA1c values were significantly lower in the group without progression of DR relative to the group with DR (WMD=1.05 [CI: 0.37-1.72]; p<0.001). One RCT included data on visual deterioration and macular oedema; analysis demonstrated that an increase in HbA1c level of 1% increased the risk of both macular oedema (RR=1.81 [CI: 1.17-2.81]; p=0.008) and visual deterioration (OR=2.2 [CI: 1.2-3.9]; p=0.008).
Conclusion: The results of our systematic review indicate a strong correlation between HbA1c level and appearance and progression of DR in T1DM. Thus, HbA1c may be considered an excellent surrogate endpoint for DR in T1DM.
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1127

Increased clonogenic capacity of endothelial progenitor cells (EPCs) in type 1 diabetic patients with early non proliferative diabetic retinopathy

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Background and aims: It has been proposed that the formation of abnormal new vessels in proliferative diabetic retinopathy (PDR) occurs through both the self-expansion of vascular endothelium and the contribution of bone marrow-derived endothelial progenitor cells (EPC). Indeed, we observed in previous work that circulating EPC from patients with type 1 diabetes and untreated PDR had a greater clonogenic capacity than EPC from patients with no signs of retinopathy. We wished to ascertain whether the EPC number and or characteristics manifest changes before the onset of PDR, and even at early stages of retinopathy.
Materials and methods: We studied type 1 diabetic patients with 25 years of diabetes and no retinopathy (n=21), and age-and gender-matched nondiabetic controls (n=18). The patients were free of any other complication of diabetes and took no medications except insulin. We measured the number of circulating EPCs (CD45dim, CD34+,VEGFR-2+) by flow cytometry; their in vitro clonogenic capacity by the Hill's assay; and plasma concentrations of VEGF and SDF-1, the two cytokines known to mobilize EPC, by ELISA. All measurements were performed when plasma glucose levels were between 70 and 200 mg/dl.
Results: The clonogenic capacity of EPCs was increased in patients with non-proliferative retinopathy when compared to those without retinopathy (p<0.002) and to nondiabetic controls (p<0.01). In contrast, the number of circulating EPCs and the plasma levels of cytokines were similar in the three groups. The difference in clonogenic capacity between the diabetic groups was not explained by different glycemic control (blood glucose and HbA1c), and no differences in clonogenic capacity were noted between patients with minimal (ETDRS 20) as compared to mild (ETDRS 35) degree of retinopathy.
Conclusion: Increased clonogenic capacity of EPCs occurs in a portion of patients with type 1 diabetes and no other complications but early non-proliferative diabetic retinopathy. This finding indicates that some type of EPCs "activation" precedes the onset of PDR, and suggests that even early stages of retinopathy signal to EPC. Longitudinal studies may help us determine whether the early EPC activation has a protective or accelerating role on the course of retinopathy.
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1128

Retinopathy screening in patients with type 1 diabetes diagnosed at a young age

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Background and aims: Up to 60% of patients with type 1 diabetes mellitus (T1DM) for more than 15 years develop diabetic retinopathy (DR). Guidelines for DR screening have been formulated, to detect DR at an early stage.
Methods: We estimated the prevalence of DR in our patients with T1DM using a Nonmydriatic Digital Stereoscopic Retinal Imaging (NMDSRI), and evaluated the role of socio-demographic and clinical variables associated with the presence of DR, using a multivariate logistic regression analysis. We

enrolled 247 patients with T1DM who underwent to fundus photography during the biennium 2006-2007. At diabetes diagnosis we considered gender, age, pubertal stage, presence of diabetic ketoacidosis, autoimmunity and HLA-DQ heterodimers of susceptibility for T1DM. At NMDSRI evaluation, we collected data for age, disease duration, pubertal stage, body mass index (BMI-SDS), insulin requirement, degree of metabolic control (mean Hb1Ac), coexisting autoimmune diseases, and other diabetic complications.
Results: Retinopathy was found in 26/247 (10.5%) patients: 25 presented background retinopathy, while only one a macular oedema and needed a laser therapy. At the bivariate analysis, we found a significant relationship between retinopathy and female gender (p=0.03), age at study visit (p<0.0001), pubertal stage at last retinal photography (p=<0.0001), age at onset ≥ 5 - 9.9 years or ≥ 10 years (p=0.037), duration of disease ≥15 years (p<0.0001), presence of microalbuminuria (p=0.036), and mean HbA1c ≥ 7.5% or >9% (p=0.015). Using a multivariate analysis, we confirmed a significant association between DR and disease duration ≥ 15 years, mean HbA1c>9%, female gender and the presence of microalbuminuria.
Conclusion: Metabolic control is the most important modifiable factor and therefore the promotion of continuous educational process in order to reach a good metabolic control is a corner stone to prevent microangiopathic complications. DR is the first cause of blindness in industrialized countries. Symptoms appear when DR is already developed; a screening program with an early diagnosis is mandatory to prevent an irreversible damage.

1129

Prevalence of diabetic retinopathy and related risk factors in diabetic patients. Results of Tirana district/Albania

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Background and aims: Diabetic Retinopathy (DR) is a serious and frequent complication of diabetes. Often it is present since the diagnosis of type 2 diabetes. It remains largely preventable with proper screening, followed when required by proper interventions' therapy. The aims of our study were: 1) To define the prevalence of diabetic retinopathy in Tirana district. 2) To analyze the clinical features associated with this complication in the examined group.
Materials and methods: As part of ALBDIAB Project, we examined all the medical records of diabetics updated during the last year. In total, we included 7226 diabetics. Male 3530 (48.8 %). 740 (10.2%) type 1 Diabetes and 6480 (89.3%) type 2. Type 1 Diabetes duration 9.7±7.8 years and T2 Diabetes duration 4.6±4.9 years. In all patients clinical and metabolical profiles were determined. Trained ophthalmologists defined the stages of retinopathy by ophthalmoscope after papillary dilatation.
Results: Diabetic retinopathy of different stages was present in 43% of T1DM and 22% of T2DM. The multivariate analyses revealed that significant predictors for diabetic retinopathy were: diabetes duration (p= 0.0001 OR= 4.36), poor metabolic control as HbA1c >8% (p= 0.0015 OR= 3.02), insulin treatment (OR=4.24, HTA >150/95mm Hg (p= 0.002 OR= 2.45) and smoking status more than 10 years (p= 0.0023 OR= 1.34) although for smoking 6-10 years OR= 0.57. We found a good correlation between retinopathy and nephropathy. The other features such as BMI, coronary artery disease and diabetes or HTA treatment were univariate predictors of diabetic retinopathy, but they lost significance in multivariate analyses.
Conclusion: Our study showed that DR remains one of the most frequent complication of Diabetes. We were able to confirm that type 2 diabetes with poor metabolic control, longer diabetes duration, nephropathy, uncontrolled HTA, and on insulin treatment are more prone to develop DR. The smoking status remains of a controversial feature.

1130

Incidence of diabetic retinopathy in type 2 diabetic subjects in a tertiary care hospital in Bangladesh

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Background and aims: Diabetic Retinopathy (DR) is one of the major micro vascular complications of diabetes that imposes a huge economic burden on diabetic subjects in developing countries like Bangladesh. It is a vascular disorder affecting the microvasculature of the retina and the major cause for new cases of blindness in people with in the aged of 25-74. Therefore, it is interest to estimate the number of new cases of diabetic retinopathy in developing countries. The aim of the present study was to investigate the 5 year, 10 year and 15 year incidence rate of DR in Type 2 Diabetic Subjects (T2DM) in Bangladesh.
Materials and methods: It was a cohort study. 977 T2DM were taken randomly from the patient registry of 1993 and came for the follow up in 2008 and in between in the Out Patient Department, BIRDEM. DR was graded using the Early Treatment Diabetic Retinopathy Study. Ophthalmologist performed comprehensive eye examinations, which were reconfirmed by senior ophthalmologist.
Results: Of the 977 subjects observed, 468 were male and 509 were female (mean±SD, age 56±8 years). According to the age distribution of the study subjects the age groups were <45, 45-60 and >60 years. The number of the study subjects according to the age groups were (115/12%), (617/63%) and (245/25%) respectively. Five, ten and fifteen years after diagnosis, retinopathy was found in 115 (11.8%), 151 (17.5%) and 229 patients (32.2%). Among the study subjects 115 patients (11.8%) had mild nonproliferative DR (NPDR) in first five years from the initial stage (1993). Ten years after diagnosis the retinopathy was mild in 136 (13.9%), moderate in 14 (1.4), 1 (0.1%) patients had severe NPDR. Fifteen years after diagnosis the retinopathy was mild in 111 (11.4%), moderate in 77 (7.9), severe in 33 (3.4%), whereas 5 (0.5%) patients had very severe NPDR and 3 (0.3%) had proliferative DR (PDR). Five, ten and fifteen years the incidence of DR (Incidence per 1000 person years with 95% Confidence Interval) were 23.54 (19.61-28.26), 17.52 (14.93-20.55) and 21.47 (18.86-24.44). Compared to other age group the incidence was higher in the age group >60 years in three different time period. It was slightly higher in males 23.64 (19.80-28.23) than females 19.44 (16.08-23.49). Comparatively it was also higher 22.17 (18.94-25.96) in the 45-60 year age group in fifteen years.
Conclusion: Working age group (45-60 years) had higher incidence for the development of DR. The overall incidence of DR increased as the duration of DM and age increases. Strategies aimed at early detection and prevention of DR will reduce the incidence rate.

PS 103 Autonomic neuropathy

1131

Plasma asymmetric dimethyarginine (ADMA) is associated with autonomic neuropathy in long standing type 1 diabetes

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Background and aims: Decreased availability of nitric oxide (NO), which contributes to the development of diabetes vascular complications, is partially related to excessive accumulation of asymmetric dimethylarginine (ADMA), an endogenous NO synthase inhibitor. Abnormalities of the L-arginine-NO pathway have been demonstrated in type 1 diabetics without clinical evidence of vascular disease. Increased circulating ADMA had previously been associated with propensity to atherosclerosis and its complications in non-diabetic populations and with cardiovascular morbidity and early nephropathy in type 1 diabetes. Plasma ADMA has recently been identified as independent predictors of cardiovascular events and progressive renal deterioration in patients with type 1 diabetic nephropathy.
Our objective was to evaluate the relationship between plasma ADMA and diabetic autonomic neuropathy in type 1 diabetes.
Materials and methods: We studied seventy eight patients with type 1 DM without clinical evidence of macrovascular complications, defined as a positive medical history for myocardial infarction, angina, coronary artery bypass gaft and stroke, and ischemic changes in ecg. To evaluate the pattern of cardiovascular autonomic balance a standard battery of cardiovascular reflex tests and a short-term heart rate variability (HRV) analysis were performed. HRV analysis included absolute and relative (%) spectral HRV power at rest in 3 standard frequency bands: VLF: 0.01-0.05 Hz); LF: 0.05-0.15 Hz; HF: 0.15-0.5 Hz.
Results: Using computer assisted method (ProSciCard), autonomic neuropathy was diagnosed in 31 diabetics (N1), being absent in 47 patients (N0). Height, weight, body mass index (BMI), blood pressure, ADMA, fasting glucose, HbA1c, serum creatinine, CRP, total chol, HDL-chol, LDL-chol and triglycerides (Tg), were measured in all subjects. The subjects with neuropathy were significantly older than the remainder (N1 vs N0: 36.2 ± 10.3 vs 43.7 ± 9.2 years ; N1 vs N0, p=0.002). No differences were found between respective groups group with regard to diabetes duration (21.8 ± 9.2 vs 23.4 ± 9.9), total chol (4.76 ± 0.90 vs 4.85 ± 1.00 mmol/l), LDL-chol (2.44 ± 0.55 vs 2.47 ± 0.84 mmol/l), HDL-chol (1.96 ± 0.77 vs 4.85 ± 1.25 mmol/l), triglycerides (0.84 ± 0.55 vs 0.92 ± 0.41 mmol/l), CRP (1.81 ± 2.38 vs 1.69 ± 2.21 mg/l). Diabetics with neuropathy exhibited elevated plasma ADMA (N1 vs N0: 0.90 ± 0.28 vs 0.69 ± 0.39 μmol/l; p=0.01).
Conclusion: This is the first report suggesting a coincidence between elevated circulating ADMA levels and autonomic neuropathy in type 1 diabetes. Prospective studies are required to validate the concept of a cause-and-effect relationship between altered NO activity and risk of diabetic neuropathy in type 1 diabetes. The potential limitation of this study is that ADMA was measured at a single time point. Thus, it did not answer the question whether elevated ADMA levels stimulate the development of diabetic neuropathy or merely constitute its marker. Prospective studies are necessary to clarify this.
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1132

Corneal confocal microscopy: a novel surrogate marker for diabetic autonomic neuropathy

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Background and aims: The accurate quantification of the severity of diabetic autonomic neuropathy (DAN) can be time consuming and challenging. At present, most clinicians undertake cardiac autonomic function tests to use as a surrogate of autonomic neuropathy of the GI tract. We undertook a range of tests of small fibre and autonomic neuropathy including the novel technique of corneal confocal microscopy (CCM) to define how they may detect and quantify the severity of DAN in patients with gastroenteropathy.
Materials and methods: 20 subjects with diabetic gastroenteropathy and 14 control subjects underwent cardiovascular and peripheral autonomic function testing to establish the Composite Autonomic Severity Score (CASS). Quantitative Sensory testing (QST) was performed to assess the severity of somatic neuropathy and corneal sensitivity and CCM were performed to quantify c-fibre dysfunction and damage.
Results: 12 patients (60%) had moderate-severe DAN (CASS 4.0 ± 2.2). Corneal Nerve Fibre Density (NFD) 17.8 ± 13.3 (95% CI 11.6-23.9) vs 48.3 ± 12.4 (95% CI 41.1-55.4), Nerve Branch Density (NBD) 8.9 ± 7.9 (95% CI 5.2-12.6) vs 30.1 ± 5.3 (95% CI 27.0-33.2) and Nerve Fibre Length (NFL) 3.6 ± 3.1 (95% CI 2.1-5.2) vs 9.7 ± 2.5 (95% CI 8.3-11.2) were all significantly reduced in DAN vs controls (p<0.0001) and corneal sensation threshold was significantly elevated 1.6 ± 0.9 (95% CI 1.2-2.1) vs 0.7 ± 0.2 (95% CI 0.6-0.8); (p <0.0004). Corneal abnormalities correlated highly significantly with the severity of DAN (CASS vs NFD [r = -0.713], NBD [r = -0.726], NFL [r = -0.786] and CS [r = 0.659]; p<0.0001).
Conclusion: Patients with DAN had significant corneal nerve abnormalities compared to healthy controls and the severity of nerve damage was very strongly associated with the severity of autonomic neuropathy. Thus CCM may act as a simple non-invasive surrogate marker for DAN in patients with diabetic gastroenteropathy.
Supported by: Core-Diabetes UK

1133

Effects of aldose reductase inhibitor on peripheral muscle sympathetic nerve activity in streptozotocin-induced diabetic rats

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Background and aims: Peripheral neuropathy is one of the most common complications of diabetes mellitus. Excessive aldose reducatase activation in the polyol pathway due to high blood glucose is one of the main factors inducing the neuropathy. Therefore, aldose reductase inhibitor (ARI) was expected to prevent the neuropathy, and it has been used in clinical cases in several countries. However, the effects of ARI on small peripheral, unmyelinated nerve fibers, such as muscle sympathetic nerve ones, have not been documented well. The purpose of this study is to determine the effects of ARI on peripheral muscle sympathetic nerve activity directly measured with microneurography in streptozotocin (STZ)-induced type 1 diabetes model rats.
Materials and methods: Wistar rats were separated into three groups: normal, STZ and ARI. STZ (80 mg/kg) was administered ip to each rat at 8 weeks of age in the STZ and ARI groups. ARI (150 mg/kg) was orally given every day to rats in ARI group for 3 weeks after the STZ administration. Microneurographic technique was used for the direct measurement of the compound sympathetic nerve signal in the sciatic nerve of anesthetized, 11-week rats in the 3 groups. In the supine position, a tungsten microelectrode was inserted several times into the nerve bundle until the specific burst signal of sympathetic flow was observed. Glucose solution (50 %, 0.25 mL) was administered via the right jugular vein, and the sympathetic signal and blood pressure (BP) were recorded on DAT tape. The data were fed offline into PC after each experiment, and analyzed using Matlab. After noise reduction procedures, action potentials (APs) were manually detected and the firing rate was evaluated.
Results: The blood glucose level was transiently increased after glucose administration by about 100 mg/dL from about 60 , 400 and 400 mg/dL in the normal, STZ and ARI rats, respectively. Before glucose administration, AP firing rate in STZ rats was lower than that in normal rats (212±117 vs. 408±177 spikes/min) although the difference was not statistically significant. The rate of ARI rats (358±166 spikes/min) was higher than that of STZ rats and close to that of normal rats. In each group, the glucose administration caused little change in BP. After the glucose administration, the firing rate increased in normal rats (408±177 and 628±359 spikes/min, before and 60 min after the administration, respectively), which may imply that the sympathetic system plays some role in the glucose metabolism in peripheral tissues. In contrast, the rate in STZ and ARI rats increased little (from 212±117 to 220±73 and from 358±166 to 395±169 spikes/min in STZ and ARI rats, respectively), which may show diminished sympathetic response to the glucose administration. The results may suggest that the sympathetic functional deterioration takes place within 3 weeks under high blood glucose and low plasma insulin condition in STZ rats, and that ARI prevents the sympathetic function at least from reduction of basal activity. Further study is necessary to

evaluate the effects on dynamic response of sympathetic system because it is necessary to evaluate the condition of afferent system as well in the feedback response.
Conclusion: The sympathetic functional deterioration may bring about within 3 weeks in STZ rats, and ARI administration may be useful to prevent the sympathetic function at least from reduction of basal activity.
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1134

Blunted postprandial parasympathetic drive and its relation to blood pressure control in patients with diabetes mellitus
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Background and aims: The autonomic nervous system plays an important role in the regulation of metabolic and cardiovascular events following food ingestion. Postprandial hypotension, a hemodynamic abnormality in diabetes mellitus (DM), indicates a lack of cardiovascular compensatory changes to offset the fall in splanchnic vascular resistance. But data showing the relation between postprandial hypotension and the autonomic control of the heart in patients with diabetes is contradictory. Several studies have suggested blunted autonomic postprandial regulation in subjects with diabetes. The aim of our study was therefore to assess the postprandial regulation of the autonomic nervous system in relation to blood pressure control in subjects with DM without further complications.
Materials and methods: We have investigated 27 patients with type 2 DM (age: 55.7±7.7 years, diabetes duration: 7.8±4.8 years, HbA_{1c}: 7.4±1.0 % and BMI: 30.6±4.6 kg/m^2). Patients were investigated in the morning, after an overnight fast and 4 hours after the ingestion of a breakfast (590 kcal). At any time point, HRV was measured in the supine position over a period of 5 minutes, using the SUEmpathy 100 System along with measurements of systolic (sBP) and diastolic (dBP) blood pressure. The following HRV parameters were analyzed: average length of the RR interval (RRi), variance, the square root of the mean squared differences of successive RR intervals (RMSSD) as indexes of the parasympathetic drive and the LF/HF ratio as index of the sympatho-vagal balance. All results are expressed as mean ± SEM and presented under fasting conditions vs. postprandial.
Results: There was a significant decrease of the RRi (867.4±25.3 ms vs. 836.9±22.5[3] ms, [3]p<0.01) and a mild but significant decrease of both systolic and diastolic blood pressure at 4 hours postprandially (sBP: 128.0±2.4 mmHg vs. 120.8±2.8[3] mmHg; dBP: 82.2±1.8 mmHg vs. 78.0±2.0* mmHg; [3]p< 0.01 and [*]p< 0.05). We also noticed a non-significant decrease in variance (4.5±0.8 % vs. 3.2±0.3 %, p=0.09), a significant decrease in RMSSD (46.0±12.6 ms vs. 14.8±4.9* ms, [*]p< 0.05) and a significant increase of the LF/HF ratio (4.4±0.7 vs. 7.3±2.0*, [*]p< 0.05).
Conclusion: Our data suggest that:
1) patients with diabetes show a mild but prolonged postprandial fall in blood pressure, both systolic and diastolic;
2) the blood pressure fall occurs despite a statistically significant increase in heart rate;
3) since parameters reflecting the parasympathetic drive significantly decrease postprandially, the marked increase in the sympatho-vagal balance is due to a parasympathetic withdrawal;
4) overall our data suggest that in DM subjects without other complications, postprandial autonomic nervous regulation is not preserved.

1135

Increased left ventricular torsion rate in subjects with type 1 diabetes correlates with cardiac autonomic neuropathy: a pilot study
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Background and aims: We have previously demonstrated that left ventricular (LV) torsion is increased in patients with Type 1 Diabetes Mellitus (T1DM) without known coronary artery disease (CAD) or heart failure. Increased LV torsion may be one of the earliest features of diabetic cardiomyopathy. Since we have previously demonstrated altered sympathetic function in these subjects, our aim was to determine the role of cardiac autonomic neuropathy (CAN) in the development of increased torsion in these individuals.
Materials and methods: A pilot cross-sectional study is being conducted in subjects with T1DM recruited from two diabetes centres in Birmingham, UK. LV function was assessed by echocardiography. LV rotation and strain was measured using a commercially available speckle tracking system in an ECHOPAC (version 4.2.0) workstation. LV torsion, torsion rate and untwist rates, were determined from the apical and basal rotation measurements. CAN was assessed using ANX-3.0 (Ansar, Inc., Philadelphia, PA). CAN was assessed by heart rate variability (HRV) and blood pressure changes during four stages: at rest, deep breathing, valsalva manoeuvre and during the stand response. We used spectral and time domain analysis. Correlation coefficient (r) was calculated using Pearson's method of correlation.
Results: So far 16 subjects with T1DM of 17±9 years duration (mean±1SD), aged 35±7 years, 63% male, HbA1c of 8±1.2% have been included. With root mean square successive difference (rmsSD) during valsalva manoeuvre, there was a significant negative correlation with LV torsion rate during systole (r= -0.52, p=0.044), and a significant positive correlation with LV untwist during early diastole (r= 0.57, p=0.025). The ratio of sympathetic to parasympathetic response (LFa/RFa) in the stand response was significantly correlated positively with intraventricular relaxation time (IVRT) (r= 0.77, p=0.001) and also negatively with inferior septum contraction rate (r= -0.57, p=0.03), both markers of diastolic dysfunction. IVRT was also significantly positively correlated with Expiration Inspiration (E/I) ratio (r= 0.56, p=0.02) and with valsalva ratio (r= 0.52, p=0.04). Radial strain of the LV was significantly negatively correlated with E/I ratio (r= -0.57, p=0.02) and valsalva ratio (r= -0.54, p=0.03).
Conclusion: Our pilot study suggests that LV torsion is related to sympathetic dysfunction in subjects with T1DM without CAD or heart failure. This suggests that CAN is possibly implicated early in the development of cardiomyopathy in patients with T1DM. Larger scale studies exploring the relation between CAN and early diabetic cardiomyopathy are ongoing.
Supported by: The British Heart Foundation

1136

The presence of sympathovagal abnormalities in patients with subclinical diabetic autonomic neuropathy
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Background: Diabetic autonomic neuropathy (DAN) is associated with a five-fold increase in cardiovascular mortality. Recognised causes of death include silent myocardial Infarction (MI) and sudden cardiac death (SCD) due to malignant arrythmias. This study involved analysis of heart rate variability (SHRV) as an early, non-invasive, test useful for detecting the presence of sympathovagal abnormalities in a group of diabetic patients with sub-clinical DAN.It is well recognised that in patients who die of sudden cardiac death, HRV abnormalities are demonstrable.These represent sympathovagal imbalance.They are seen in the five minute period preceding the event and are well characterised using a time domain parameter, the standard deviation of all normal RR interval variation (SDNN).
Aim: To assess the usefulness of HRV in demonstrating sympathovagal abnormalities associated with sudden cardiac death and myocardial infarction.
Methods: Sixteen age and sex matched, healthy volunteers (HV) and 48 patients with diabetes (type-1, n=16; type-2, n=32) underwent standard cardiovascular autonomic testing (CAT) using the O'Brien protocol, and specialised autonomic tests (baroreceptor sensitivity [BRS], and HRV). Subjects were classified as no-DAN (n=14, normal CAT and BRS), subclinical-DAN (sub-DAN, n=17,normal CAT, abnormal BRS) and established DAN (est-DAN, n=17, BRS and CAT abnormal).
Results: Analysis of HRV showed subjects with sub-DAN (12.3±5.2ms) and est-DAN (17.0±7.1ms) had significantly lower SDNN than HV (46.9±9.6ms) and no-DAN (39.9±4.5ms); ANOVA p< 0.001. Subjects in the sub-DAN had the lowest RR interval variation (677.2±73.2ms) and this was significantly lower than in HV (937.0±114.1ms) and no-DAN (880.9±138.8ms);ANOVA p<0.05.The lower RR interval variation for sub-DAN was not significant when compared to est-DAN (830.9±120.7).
Conclusion: This study suggests that SHRV can detect autonomic dysfunction in asymptomatic diabetic subjects with normal CATs and that these individuals have abnormal SDNN,which represents sympathovagal imbalance. Such imbalance, even when transient, have been shown to predispose to sudden cardiac death.Prospective studies are required to see if these patients are at risk of increased cardiovascular mortality due to sudden cardiac death.
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1137

Severe symptomatic diabetic gastroparesis and cardiovascular tests exhibit a close relationship

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Background and aims: Diabetic gastroparesis is associated with autonomic dysfunction, but the nature of this relationship remains elusive. This study presents detailed clinical data. Aim: To describe Type 1 diabetes patients suffering severe symptomatic gastroparesis.
Materials and methods: 19 consecutively recruited type 1 diabetes patients (6 males and 13 females) with delayed gastric emptying and correlating symptom scores. Age 43 years (+/- 14 years). Diabetes duration 21 years (+/- 8 years). Body mass index 26 (+/- 5). All demonstrated autonomic symptoms from more than one organ system. Cardiovascular autonomic neuropathy (CAN) was assessed by heart rate variability resting, expiration:inspiration and heart rate variability from lying to standing. A ^{13}C-octanoic breath test determined rate of gastric emptying and symptom scores by a validated questionnaire. All patients underwent through physical, paraclinical and clinical examinations including endoscopies.
Results: Gastric emptying rates were all significantly increased ($T^1/_2$ = 152 minutes (+/- 33 minutes). All patients but two were diagnosed with CAN. Of the 17 patients exhibiting CAN 7 were at an early stage of progression whereas 10 were at an endstage of CAN. Blood pressure lying down 140/82 mmHg (systolic +/- 29 mmHg and diastolic +/- 14 mmHg) and blood pressure standing 126/83 mmHg (systolic +/- 32 mmHg and diastolic +/- 15 mmHg) signifies postural hypotension. On validated questionnaires all patients demonstrated symptoms pathognomic for gastroparesis and severely so.Examination for CAN may support a gastroparesis diagnosis in Type 1 diabetes patients suffering upper gastrointestinal symptoms. The finding of CAN in a Type 1 diabetes patient may warrant a need for further investigations regarding gastric motility.
Conclusion: All consecutively recruited patients suffered symptomatic gastroparesis and all but two patients demonstrated cardiovascular autonomic dysfunction. There exists a close relationship between severe gastric motility and cardiovascular autonomic neuropathy.
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1138

Endothelial impairment and bone marrow-derived CD34$^+$/133$^+$ cells in diabetic subjects with erectile dysfunction

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Background and aims: The present study was undertaken to determine endothelial impairment and erectile dysfunction (ED) in subjects with type 2 diabetes mellitus.
Materials and methods: One hundred sixteen type 2 diabetic men were enrolled in the present study. Endothelial function and exercise capacity were evaluated by flow-mediated vasodilation (FMD) and cardiopulmonary exercise test (CPX). Also, endothelial progenitor cells were determined by FACS.
Results: In the ED diabetic subjects FMD and anaerobic threshold (AT) after exercise tolerance were significantly less than those in the subjects with normal erectile function (FMD: 2.8 vs. 3.8%, p=0.02, and AT: 11.1 vs. 12.8 ml/kg/min, p=0.01). Basal levels of bone marrow-derived CD34$^+$/133$^+$ cells were comparable in both the diabetic subjects with and without ED, but exercise tolerance significantly increased the number of CD34$^+$/133$^+$ cells in both groups (77 to 90 cells/100μl, p=0.006, and 73 to 101 cells/100μl, p<0.001). The diabetic subjects were subgrouped according to the development of autonomic neuropathy. In the subjects having a CVRR more than 2%, FMD was significantly reduced in the ED subjects than those without ED (2.5 vs. 3.9%, p=0.021). In response to exercise tolerance the number of CD34$^+$/133$^+$ cells increased in both the diabetic subjects with ED (78 to 93 cells/100μl, p=0.023) and without ED (99 to 134 cells/100μl, p=0.015). On the contrary, in the diabetic subjects with autonomic neuropathy (CVRR <2.0%), FMD was reduced and there was no difference in FMD between the subjects with and without ED. The exercise tolerance increased the number of CD34$^+$/133$^+$ cells in the subjects without ED (45 to 60 cells/100μl, p=0.023), but it disappeared in those with ED (76 to 88 cells/100μl, ns). Similar results were obtained with the ED diabetic subjects according to the progression of nephropathy.
Conclusion: These findings may indicate that ED diabetic subjects have endothelial impairment during the early period of diabetic complications, who concomitantly repair endothelial function by promoting bone marrow-derived endothelial progenitor cells.

PS 104 Neuropathy and wound healing - experimental

1139

Deletion of the redox enzyme p66shc promotes diabetic wound healing in mice

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Background and aims: Diabetic wounds challenge patients and clinicians. Limb ischemia is the most important risk factor for the development of diabetic wounds, but the mechanisms that delay wound healing in diabetes are poorly understood. Deletion of the lifespan determinant redox enzyme p66shc protects against experimental diabetic nephropathy and endothelial dysfunction. We aim to study the effects of p66shc knock-out on skin wound healing in the presence/absence of experimental diabetes and hind limb ischemia.
Materials and methods: Excisional skin wounds were created on the dorsal surface of hind limbs in diabetic and non-diabetic wild type (w/t) and p66shc-/- mice. Diabetes was induced through intraperitoneal streptozotocin. Wound size was monitored with digital imaging. Skin concentrations of pentosidine and nytrotyrosine were also assessed. In separate sets of experiments, hind limb ischemia was induced by femoral artery excision, and wounds were created on the ischemic skin 2 weeks later. Post-ischemic skin microcirculatory blood flow was monitored with laser Doppler imaging. Sections of adductor muscles were assessed for necrosis, apoptosis and capillary density 2 weeks after ischemia. Mobilization of CD34+/Flk1+ endothelial progenitor cells (EPCs) was assessed 4 days after ischemia using flow cytometry. Dermal fibroblasts were isolated from w/t and p66shc-/- mice and used for an in vitro scratch-wound assay under 5 or 25 mM glucose and under normoxic or hypoxic conditions.
Results: There were no differences in wound healing between non diabetic w/t and p66shc-/- mice. The presence of diabetes delayed wound healing by about 34% in w/t mice but not in p66shc-/- mice. Skin pentosidine and nytrotyrosine concentrations were increased by 40% and 35% respesctively in diabetic w/t as compared to non-diabetic w/t mice, but not in diabetic versus non-diabetic p66shc-/- mice. The co-existence of hind limb ischemia and diabetes almost doubled healing time in w/t mice, while p66shc-/- mice still showed faster healing. Post-ischemic skeletal muscle damage was reduced in p66shc-/- mice as compared to w/t mice in the presence or absence of diabetes. Post-ischemic skin perfusion and muscle capillary density were significantly improved in diabetic p66shc-/- mice as compared to diabetic w/t mice (0.41±0.03 vs 0.29±0.04 units and 1.15±0.20 vs 0.87±0.1 capillaries/fiber), while there was no difference between non-diabetic w/t and p66shc-/- mice. Mobilization of CD34+/Fkl1+ EPCs was impaired in diabetic versus non-diabetic w/t mice, while p66shc-/- mice did not mobilize EPCs, independently of the presence of diabetes. Dermal fibroblasts from p66shc-/- mice showed almost complete resistance to oxidative stress induced by hydrogen peroxide (6% vs 85% cell death) and improved migration under high glucose or hypoxia, as compared to w/t fibroblasts.
Conclusion: Deletion of p66shc promotes diabetic wound healing, especially in the setting of ischemia. The mechanisms include reduced oxidative stress and accumulation of glycation end-products, improved cell migration and post-ischemic angiogenesis, which was not associated with improved EPC mobilization.
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1140

Modified epidermal growth factor(FRM-EGF) gene therapy markedly improved histologic findings of diabetic mice skin wound

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Background and aims: Skin ulcers of diabetes mellitus are notorious for delayed healing. The clinical role of topical growth factor treatment might have been limited because of its high cost, low efficacy and short half-life of applied factor due to over-expressed non-specific proteases in the wound bed. Growth factor gene therapy into the wound tissue might be a fascinating alternative to overcome these limitations. We already have shown that human $VEGF^{165}$ gene therapy improved the healing of diabetic mice skin wound. This study was performed to see that the sonoporation gene therapy using modified human EGF DNA could improve the histologic quality of diabetic skin wound healing.
Materials and methods: Novel plasmid DNA containing FRM(furin recognition motif)-EGF for facilitating the secretion of produced EGF outside the cell driven by the ubiquitous strong chicken β-actin promoter was cloned. The competency of cloned plasmid DNA was verified using cultured HEK-293 cells. Ultrasound micro-bubble destruction method was employed to deliver plasmid DNA into the 6mm circular skin wound of streptozotocin induced diabetic C57BL/6J mice. The quality of wound healing was monitored via H&E and Mason-Trichrome stain along with the measurement of the expressed EGF in the skin tissue.
Results: Insertion of FRM prior to the EGF DNA sequence dramatically enhanced the secretion of produced EGF outside the transfected HEK293 cells. Sonoporation of EGF plasmid DNA into the skin wound of diabetic mice showed marked improvement of the histologic findings. Rapid epithelization and deposition of large amount of collagen in the healing skin tissue was nearly similar with the healing process of normal control mice skin wound. And the histologic improvements coincide with the expression pattern of EGF in the sonoporated skin.
Conclusion: In conclusion, sonoporation of modified EGF plasmid DNA into the skin wound of diabetic mice not only hasten the healing, but also normalized the microarchitectures and tissue collagen deposition.

1141

Estrogen receptor beta modulates wound healing in diabetes

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Background and aims: The impaired wound healing in diabetes represents a major medical, social and economic problem. It is therefore a high need to find new therapeutical approaches. The effects of estrogens on cutaneous wound healing are well established and explain the defective wound healing in elderly. Estrogen receptors beta (ERβ) have been linked to venous ulcers. However the effect of estrogens on diabetic wounds is still unexplored. The present study analyzed the contribution of the estrogen receptor beta (ERβ) for wound healing in diabetic mice.
Materials and methods: We studied the effect of diabetes (induced by streptozotocin) on wound healing rate in estrogen receptor-beta (BERKO) knockout mice and in wild type mice (C57BL/6). The wound model consists in full-thickness wounds made on the dorsum of the animals. The wounds were followed every other day using a digital camera. Wound granulation, dermal and epidermal regeneration were evaluated by hematoxylin and eosin staining and angiogenesis by GS-1 isolectin staining. Markers for inflammation, endothelial precursor's cell recruitment, cell migration were analyzed by qRT-PCR.
Results: Diabetic BERKO mice (50% and 90% wound closure at 3.4+/-0.3 days respectively 8.7+/- 0.2 days) have a faster wound healing rate compared with diabetic wild mice (50% and 90% wound closure at 4.7 days+/- 0.5 days respectively 10+/-0.3 days).
Conclusion: Estrogen receptor-beta modulates the wound healing rate in diabetes and suggests the need of specific ER agonists for therapeutical trials.
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1142

Effects of PKF275-055 in peripheral diabetic neuropathy

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Background and aims: Dipeptidyl peptidase IV (DPP IV) inhibitors exert antidiabetic effects of by potentiating the biological activity of incretin hormones like glucagon-like peptide (GLP)-1. PKF275-055, a vildagliptin analog, is a novel, selective, potent, orally bioavailable, long-acting DPP IV inhibitor. We studied the effect of chronic dosing with PKF275-055 (1-2 months) in two experimental settings.
Materials and methods: The first was aimed at testing the efficacy of PKF275-055 in preventing and/or protecting (prevention and protection schedules, respectively) peripheral diabetic neuropathy in the streptozotocin-induced (STZ) diabetes model in rats; the second at investigating therapeutic efficacy in established peripheral diabetic neuropathy in the same model (therapeutic schedule). In addition to general observations (body and muscle weight, blood glucose) several other parameters were examined including nerve conduction velocity (NCV), Na+,K+-ATPase activity, thermal and mechanical thresholds and oral glucose tolerance.
Results: Chronic PKF275-055 besides improving anabolic function, as indicated by body and muscle weight gain and supported by oral glucose tolerance test (OGTT) data, counteracted the impairment of NCV, nociceptive thresholds and Na+,K+-ATPase activity. OGTT after drug washout showed marked improvement in glucose tolerance in all the treatment schedules. In the experimental therapeutic setting, compared to non-diabetic rats NCV was 36% lower ($P<0.001$) at ten weeks in the STZ-untreated group, but only 17% lower in PKF275-055 treated animals ($P<0.01$ vs. diabetic). As expected, Na+,K+-ATPase activity was significantly reduced in diabetic nerves (by 40%) and PKF275-055 halved this decrease with a significant difference from the untreated diabetic group ($P<0.05$). Thermal and mechanical allodynia nociceptive thresholds were checked every 15 days throughout the study. Rats develop mechanical hypoalgesia within two weeks after STZ injection. PKF275-055 therapeutic schedules partially protected diabetic rats (about 50%), but had no such effect in non-diabetic controls. Hind paw thermal response latencies were significantly longer ($P<0.01$) in untreated diabetic rats than in untreated controls. Treatment had no effect on these latencies in controls, but progressively prevented the increase in diabetic rats.
Conclusion: Although further studies are needed the present data indicate PKF275-055's efficacy and highlight its potential for treating established diabetic neuropathies.
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1143

The functions of bone marrow-derived mononuclear cells and their therapeutic efficacy on diabetic polyneuropathy are affected by their age

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Background and aims: Recent studies have shown that cell transplantation therapy such as endothelial precursor cells (EPCs), mononuclear cells (MNCs) and mesenchymal stem cells (MSCs), is effective on diabetic polyneuropathy (DPN) through ameliorating impaired nerve blood flow in diabetic rats. Among them, bone marrow (BM) MNCs are isolated relatively easy and collected in large quantities at one time. Moreover, it has been reported that the functions of EPCs are impaired under the diabetic condition and the number of MSCs in BM decreases with age. Although these indicate that BM-MNCs are the most suitable for clinical application in DPN, their functions in diabetes or with age has not been reported. Here, we investigated the effects of BM-MNCs transplantation on DPN by using BM-MNCs from 16-week-old 8-week-diabetic (AD), 16-week-old normal (AN) and 6-week-old normal (YN) rats.
Materials and methods: 1) Diabetes was induced by intraperitoneal injection of STZ to 8-week-old male Spraugue-Dawley rats. 2) After 8 weeks, MNCs were isolated from BM of AD, AN and YN rats. 3) bFGF, VEGF, NGF and

NT3 mRNA levels in each group of MNCs were measured. 4) Then, MNCs with saline or saline alone were injected into the right and left hindlimb muscles of normal (N) and 8-week STZ diabetic (D) rats, respectively. 5) Four weeks later, plantar test (PT), nerve conduction velocity (NCV) and nerve blood flow of sciatic nerves (SNBF) were evaluated.
Results: 1) bFGF mRNA levels in MNCs of YN rats were significantly increased compared with those of AD and AN rats. There were no significant differences between AD and AN rats. 2) Impaired thermal sensation (N: 17.0±1.4s, D: 22.4±3.9), decreased SNBF (N: 17.2±3.9 ml/min/100g, D: 8.0±2.5) and delayed NCV (N: 53.9±3.9 m/s, D: 41.8±3.7) in 8-week diabetic rats were significantly ameliorated by YN rat-derived MNCs (PT: 17.3±2.3s, SNBF: 13.4±4.1 ml/min/100g, NCV: 50.7±5.1m/s). Whereas MNCs from AD or AN rats did not show any effects on these functional tests.
Conclusion: These results indicate that cytokine production abilities of MNCs would highly depend on their age, and that this difference would explain the disparity of the therapeutic efficacy on DPN.

1144

The efficacy of methylcobalaminamin was influenced by diabetic conditions on experimental peripheral neuropathy via IGF-1
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Background and aims: Neurotrophic substances have been implicated in the pathogenesis of diabetic neuropathy,a degenerative defect ameliorated by exogenously administered IGF-I. Continuous treatment with methylcobalamin, one of the coenzyme forms of vitamin B(12),also has an ameliorative effect on this lesions in a neurotrophic pattern. However, little is known about potential association of methylcobalamin with IGF-1 on neurotrophic mechanism. On the basis of previous studies,we explored the link by observing dynamic changes of serum IGF-1 and it's main contributor-liver IGF-1 mRNA following exogenous methylcobalamin administration,and furthermore suggested an effective way of improving methylcobalamin effect on diabetic neuropathy.
Materials and methods: We included ninety adult male Sprague-Dawley rats in this study. Diabetes was induced in rats with STZ. Thirty rats were in normal control group(NG), thirty in a relatively good -controlled diabetic grou p(GG)(FPG:10.94±1.08mmol/L), and thirty in a relatively poor -controlled diabetic group(PG)(FPG:18.34±1.03mmol/L). Each rat of treatment groups was given methylcobalamin(500ug/kg/d, im). We measured liver IGF-1 cDNA to β-actin cDNA band density ratio and IGF-1 peptide to total protein ratio(ng/ mg)by using RT-PCR or ELISA, sciatic sensory and motor nerve conduction velocity [SNCV, MNCV(m/s)]by using evoked electromyograph, respectively.
Results: At week 2 after onset of diabetes, untreated GG and PG showed a reduced serum IGF-1 peptide and liver IGF-1 mRNA (371.0±12.5, 223.2±9.39 vs 511.2±24.7, P<0.0025,P<0.001 for serum IGF-1; 0.79±0.048, 0.53±0.023 vs1.15±0.09 ,P<0.0025,P<0.001 for liver IGF-1 mRNA). IGF-1s in methylcobalamin treated GG and PG were greater than untreated GG and PG (423.4±13.41 vs 371.0±12.5, 240.0±7.8 vs 223.2±9.39, P<0.01,P<0.025 for serum IGF-1; 0.93±0.049 vs 0.79±0.048, 0.69±0.031vs 0.53±0.023 P<0.01,P<0.05 for liver IGF-1 mRNA). Physioelectroparameters did not differ between the treated GG, PG and NG till week 8, when it showed impaired SNCV(25.70±1.59 vs 26.32±1.51 P<0.05) and MNCV(18.61±1.86 vs 20.11±1.76 P<0.05) as compared to NG. The IGF-1s and physioelectroparameters downturn progressed through week 12(end of experiment), when the IGF-1s and physioelectroparameters of the treated PG gradually dropped to that of the untreated PG, though they were more delayed to decrease in treated GG than in untreated GG (345.16±18.7, 199.25±11.41 vs 525±30.2, P<0.01,P<0.001 for serum IGF-1; 0.74±0.041, 0.48±0.017 vs 1.12±0.056 P<0.005,P<0.0001 for liver IGF-1 mRNA; 23.79±1.68 vs 21.30±1.72 P<0.05 for MNCV; 17.60±1.79 vs15.92±0.65 P<0.05 for MNCV). No effect of methylcobalamin on blood glucose was shown in any given groups.
Conclusion: The study was our pilot observation that the efficacy of methylcobalamin, at least via liver IGF-1 expression upregluation contributing to serum IGF-1 level, delayed the onset of diabetic peripheral neuropathy. The effect was influenced by diabetic status, e.g., high effectiveness was achieved in a relatively good -controlled diabetic state , especially at early stage.
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1145

Angiotensin II type-1 receptor blocker olmesartan increases neuronal expression of angiotensin II receptors in dorsal root ganglia and ameliorates diabetic peripheral neuropathy in Zucker diabetic fatty rats
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Background and aims: Recent clinical studies have demonstrated that renin-angiotensin system (RAS) blockade may reduce the risk of developing type 2 diabetes and its complications. However, there has been little research on the effects of Angiotensin II type (AT)1 receptor blockers on diabetic peripheral neuropathy in type 2 diabetic animals.

Materials and methods: In the present study, 14-week-old, male, type 2 diabetic Zucker diabetic fatty (ZDF) rats were either treated orally with olmesartan (6 mg/kg/day)(n=7), an AT1 receptor blocker, or left untreated (n=7), and followed for 9 weeks. Age- and sex-matched nondiabetic lean rats served as controls (n=7).
Results: At the end of the experiment, untreated ZDF rats had higher blood glucose and HbA1c levels than control rats, and these levels were not affected by olmesartan treatment for 9 weeks. While untreated ZDF rats and control rats had comparable serum insulin and adiponectin levels, untreated rats had higher serum total cholesterol, triglyceride, free fatty acid, and leptin levels than control rats. In ZDF rats, olmesartan treatment decreased serum total cholesterol and increased serum leptin levels. Furthermore, untreated ZDF rats had increased myelinated fiber density and decreased myelin area in the distal peroneal nerve, though the densities of CD-31- and RECA-1-immunoreactive endoneurial microvessels in the sciatic nerve were comparable in untreated ZDF rats and control rats. Untreated ZDF rats developed mechanical hyperalgesia and thermal hypoalgesia, and they showed sensory and motor nerve conduction slowing in the sciatic-tibial nerves. Compared with untreated ZDF rats, olmesartan-treated ZDF rats had increased myelin area and sensory nerve conduction velocity. In dorsal root sensory neurons of untreated ZDF rats, immunoreactivities for AT1 and AT2 receptors were unchanged, but immunoreactivities for total and phosphorylated insulin receptors decreased. Olmesartan treatment increased immunoreactivities for AT1 receptors (vs. untreated ZDF rats), AT2 receptors (vs. control rats), and total insulin receptors (vs. untreated ZDF rats). The immunoreactivities for 8-OHdG, a marker of oxidative DNA damage, of dorsal root sensory neurons did not differ among the three groups.
Conclusion: These results demonstrate that olmesartan upregulates angiotensin II and insulin receptors of dorsal root sensory neurons and ameliorates sensory nerve conduction slowing and myelinated fiber pathology in ZDF rats. Further studies on the possible interaction between angiotensin II and insulin signaling systems in peripheral nerves would help elucidate the mechanisms underlying the beneficial effects of RAS blockade on diabetic peripheral neuropathy.
Supported in part by a grant from DAIICHI SANKYO COMPANY, LIMITED

1146

Loss of glyoxalase-1 promotes hyperalgesia in early diabetic neuropathy
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Background and aims: Formation of intracellular Advanced Glycation End Products (AGEs) is thought to contribute to the development and progression of diabetic neuropathy. Reactive intermediates of AGE formation such as glyoxal, methylglyoxal (MG) and other dicarbonyl compounds are detoxified by the rate-limiting enzyme glyoxalase-1 (GLO-1). Due to their high energy demand, neuronal cells metabolize large amounts of glucose through oxidative phosphorylation. This may explain why sciatic nerves from wildtype mice demonstrated particularly low levels of GLO-1 activity. However, this finding suggests that sciatic nerve may be particularly susceptible to high glucose-derived carbonyl toxicity. Therefore, we studied the contribution of GLO-1 and MG to functional changes in early diabetic neuropathy.
Materials and methods: Hyperalgesia was determined in healthy and diabetic mice using Hot Plate, Hargreaves, and Tail flick assays. GLO-1 was studied

on the level of transcription (quantitative Real time-PCR), expression (Western Blot and immunohistochemistry), and activity (enzyme-kinetic assay) in plasma, dorsal root ganglia (DRG) and sciatic nerve.
Results: Diabetes for 8 weeks reduced GLO-1 activity in sciatic nerve by 60%. A similar decrease occurred in non-diabetic heterozygous GLO-1-knock down mice (GLO-$1^{-/+}$)-mice. Decreased GLO1 activity in both models was associated with increased hyperalgesia, which was paralleled by a 2-fold increase in plasma and tissue MG. Application of GLO-1 inhibitors to sciatic nerve of wild-type mice also resulted in a significant increase in hyperalgesia, while overexpression of GLO-1 using somatic gene transfer reduced hyperalgesia in both diabetic and non-diabetic GLO1 knockdown mice. Finally, i. p. injection of increasing concentrations of MG into healthy mice caused a significant increase in hyperalgesia within 3h, which could be reduced by overexpressing GLO-1.
Conclusions: These data demonstrate that glycotoxic substrates of GLO-1 known to accumulate in diabetes play a central role in modulating neuropathic pain.
Supported by: JDRF

1147

Exercise training retarded peripheral neuropathy symptoms with heat shock protein 72 overexpression in type 1 diabetic rats
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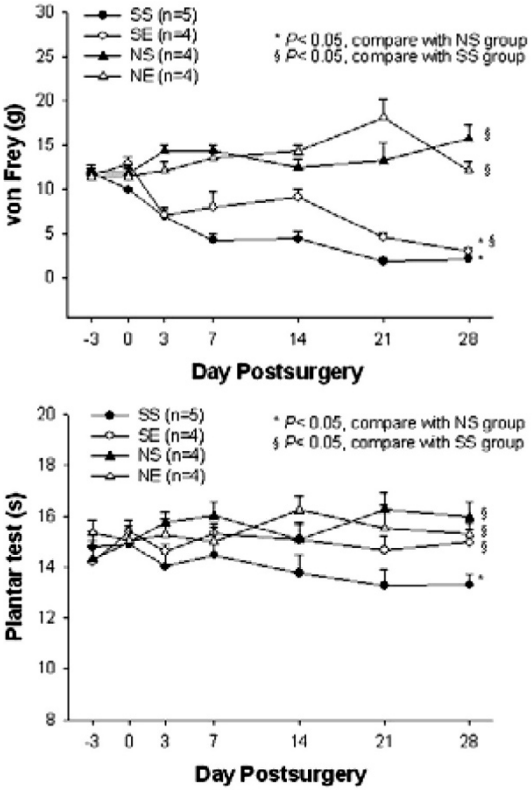
Background and aims: Diabetic patients may suffer from many complications, and one of the complications is diabetic peripheral neuropathy (DPN). Heat shock protein 72 (HSP72) has the neuroprotective function and could be induced from normal or streptozotocin (STZ)-induced diabetic rats with exercise training. Therefore, we hypothesize that exercise may induce HSP72 overexpression in peripheral nerves and reduce the symptoms of DPN in STZ-induced diabetic rats.
Materials and methods: Male Wistar rats were randomly assigned to four groups: normal sedentary group, normal exercise group, and diabetic rats with/without exercise training group. The trained rats ran on a treadmill 5 days/week, 30-60 min/day with an intensity of 20-25m/min from day 3 after STZ-administration. DPN were tested by the withdrawal responses to heat stimuli (thermal hyperalgesia) and to light touch stimuli (tactile allodynia). In addition, HSP72 expressions in sciatic and other peripheral nerves from each group were determined by western blot.
Results: Compared with normal sedentary group, normal exercise group showed no significant changes in allodynia and hyperalgesia. Exercise training modified the development of allodynia and hyperalgesia in type I diabetic rats. HSP72 expressions in sciatic and other peripheral nerves were significantly greater in diabetic rats with exercise training than those without exercise training.
Conclusion: Exercise training performed significant lags in development of DPN and increased expressions of HSP72 in peripheral nerves.



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1148

Research about inhibition of MMP9 expression in dermal fibroblast by siRNA
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Background and aims: Chronic, nonhealing skin wounds are common with significant morbidity in uncontrolled diabetes. Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases can degrade essentially all extracellular matrix components. Researches showed that MMPs activity elevated in diabetic foot, and the increased MMP9 has been confirmed to contribute to the vulnerability of diabetic skin and refractory of diabetic foot ulcer. Here to find a safe and effective way to decrease the high MMP9 expression in diabetic skin, we studied in inhibition of MMP9 expression in dermal fibroblast by siRNA in vitro.
Materials and methods: The changes of MMP9 distribution in skins of normal SD rats, STZ rats and STZ rats with skin wound were observed respectively by immunohistochemistry. Fibroblasts were obtained from the dermis of 1-day-old normal SD rats. To induce the increased MMP9 expression, the cells were cultured in the DMEM medium with high concentration of glucose and homocystein. Finally, fibroblasts were transfected with siRNA complexes or only with transfection reagents (mock) using Lipofectamine 2000 under condition with at least 75% transfection rate and 85% cell viability, which was established by transfecting FITC-labeled GAPDH siRNA, determined by inverted fluorescence microscope and MTT. 24h, 48h and 72h after transfection, RT-PCR, western blot and gelatin zymography for MMP9 were performed respectively to detect the percentages of inhibition of MMP9 mRNA, protein expression and activity.
Results: MMP9 existed extensively in epidermal layers but was not detected in dermis in the skin of normal rats. In the skin of diabetic rats, there is an increased expression of MMP9, especially around the wound. Treatment with hyperhomocysteinaemia increased the production and enzymatic activity of MMP9 in fibroblast, which was further enhanced in high glucose treatment ($p<0.05$). After a 24-h, 48-h and 72-h transfection with 40nmol/l of each MMP9-specific siRNA, significantly morphologic change of fibroblasts was not observed. RT-PCR, carried out in fibroblasts collected at different intervals, showed a remarkably reduction of MMP9 mRNA compared with

mock transfection (p<0.05), especially at 48h after that. Meanwhile, MMP9 protein abundance and activity had the same trend according to the Western blot and gelatin zymography results. Among the four MMP9 siRNAs, the sequence(5`-GGGCUUAGAUCAUUCUUCATT-3 3`-UGAAG AAUGAUCUAAGCCCAG-5) was selected out for its stable high inhibition rate.
Conclusion: The expression of MMP9 increased in the skin of diabetic rat, especially in the wound skin. MMP9 expression was inhibited remarkably after treated with MMP9 siRNA (p<0.05), especially at 48-h after transfection.

1149

Selective inhibition of disease-related thermal hyperalgesia by tapentadol in a mouse model of diabetic neuropathic pain
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Background and aims: Selective inhibition of pathological pain sensation without modification of normal sensory function is the primary aim of analgesic treatment in chronic neuropathic pain. Tapentadol [(-)-(1*R*,2*R*)-3-(3-Dimethylamino-1-ethyl-2-methyl-propyl)-phenol] is a novel analgesic with two modes of action, μ-opioid receptor (MOR) agonism (K_i = 0.1 μM for rat MOR binding) and noradrenaline (NA) reuptake inhibition (K_i = 0.5 μM for rat synaptosomal NA reuptake inhibition).
Materials and methods: Diabetic mice (streptozotocin-induced) and non-diabetic controls were tested by means of a 50°C hot plate to determine neuropathic hyperalgesia and normal nocifensive response.
Results: Tapentadol HCl (0.1-1 mg/kg iv) and morphine HCl (0.1-3.16 mg/kg iv) dose-dependently attenuated heat-induced nociception in diabetic animals with full efficacies attaining >80% at the highest doses tested. Tapentadol was more potent as compared with morphine on heat hyperalgesia (ED_{50} values [minimal effective dose], 0.32 (0.316) and 0.65 (1) mg/kg, respectively). Non-diabetic controls did not show significant anti-nociception with tapentadol up to the highest dose tested (1 mg/kg). In contrast, 3.16 mg/kg morphine, the dose that resulted in full anti-hyperalgesic efficacy under diabetic conditions, showed significant anti-nociception in non-diabetic controls.
Conclusion: Selective inhibition of disease-related hyperalgesia by tapentadol suggests an advantage in the treatment of chronic neuropathic pain when compared with classical opioids, such as morphine. It is hypothesized that this attractive efficacy profile of tapentadol is due to simultaneous activation of MOR and inhibition of NA reuptake.
Supported by: Grünenthal GmbH

PS 105 Diabetic foot

1150

Prevalence of undernutrition in diabetic foot unit
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Background and aims: Patients hospitalized for diabetic foot are at risk of undernutrition because of infection, antibiotherapy, ischemia, old age (>70 years old), renal failure or dialysis, gastroparesis, multiple hospitalizations, social problems and psychiatric disease. Undernutrition is an important factor of delayed cicatrisation, nevertheless its prevalence in diabetic foot is unknown. The aim of this study is to evaluate nutritional status in patients hospitalized for diabetic foot.
Materials and methods: We studied prospectively 252 consecutive patients hospitalized in our podology unit. Patients with hemodialysis, hepatic failure and nephrotic syndrome were excluded. According to international recommendations, diagnosis of undernutrition was established by the presence of one of the following criteria:
- clinic: loss of weight more than 10% (or 5% in the last month), BMI < 17 or 20 kg/m² (respectively for people < or > 70 years old),
- biologic: albumin < 30g/l, prealbumin < 110mg/l (for patients without inflammatory syndrome defined by CRP<15mg/l)
- clinico-biological scores
o age < 70 years old: Nutritional Risk Index NRI = 1,519 x albuminemia (g/l)+0,417 x (measured weight/usual weight)/100
o age > 70 years old: Mini Nutritional assessment MNA, well validated.
Results: Our patients' caracteristics were: 74.7% male, aged 63 ± 12 years old, 35% older than 70, HbA1c=8.2 ± 2%, BMI=26 ±5 kg/m², diabetes duration: 19 ±12 years, type 2 diabetes 85%, grade 3 wound 70%, peripheral arterial disease 60%, infection 90% (osteomyelitis 70%), lower limb amputation 6%, length of staying: 25 ±16 days. Among 252 patients, 81 (32%) were malnourished, with 43 (17%) moderately malnourished and 38 (15%) severely malnourished. Patients' loss of weight before hospitalization was 2.4 ± 6.0%. This study shows that 61.5% had inflammatory syndrome (CRP>15mg/l), 8,7% had lost more than 10% of their weight, 31.3% had albumin below 30g/l. We compared malnourished patients to normal patients: they were older (69 vs 61 years old, p<0.01), hospitalization was longer (29 vs 23 days, p<0.01), they were slimmer (weight= 72 vs 83kg, p<0.01, BMI = 25 vs 28, p<0.01), albumin was lower (30 vs 33g/l, p<0.01), foot wounds were more severe (grade D: 68 vs 45%, p<0.01). There was no difference on HbA1c, diabetes duration, prealbumin, CRP, urea, proteinuria, glomerular filtration rate, weight loss during hospitalization. Moreover patients with undernutrition died more during hospitalization: 7 (8.6%) vs 5(2.9%), p=0.046.
Conclusion: The prevalence of undernutrition in our podology unit was high despite 40% of the patients received nutritional oral supplementation. Undernutrition is associated with more severe wounds and excess of mortality. Our study proves undernutrition must be considererd as an important part of treatment of diabetic patients hospitalized for foot wound.

1151

Prevalence of risk factors in the diabetic foot ulcer
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Background and aims: Foot ulcers and lower limb amputations are the main consequences of diabetic foot complications. Around 85% of amputations start with foot ulcers, therefore having a significant increase on morbidity and mortality in this population.
Prevention programs on the diabetic foot can reduce the incidence of ulceration and lower limb amputations, as well as its socio- economic impact.The aim of this study was to determine the prevalence of risk factors for foot ulcers in diabetic patients that were observed in a 1st visit at the Portuguese Diabetic Association (APDP).
Materials and methods: In this observational study we evaluated the risk factors for foot ulcer, according to the clinical records of diabetic patients that required 1st time assistance at APDP, during the period of January through December 2007. All this patients were evaluated for the presence neuropathy, peripheral vascular disease, foot deformations and major nail alterations.

Results: We have studied 1048 patients, 495 (47,2%) female and 553 (52,8%) male, with an average age of 60,6 years old (Median 62 years old); 67 (6,4%) with type 1 diabetes, 925 (88,3%) with type 2 diabetes and 56 (5,3%) with other type of diabetes. 16,3% of patients had HbA1c equal or inferior to 6,5%, with a an average duration of disease of 11 years (Median 7 years of average duration of disease). We found foot deformations in 498 (47,5%) patients, hyperqueratosis in 522 (49,8%) and previous ulceration in 211 (20,1%) patients. It was also recorded an impairment in the vibratory sensibility, using 128 Hz tuning fork in 101 (9,6%) patients and using the Semmes-Weinstein monofilament, neuropathy was present in 168 (16,0%) patients. According to degree of risk using The International Consensus on The Diabetic Foot, we identified 67,5% of grade 0 patients, 16,0% with grade 1, 10,7% with Grade 2 and 5,8% with grade 3.
Conclusion: The high prevalence of patients with a risk degree 1, 2 and 3 found in the studied population stresses the importance and shows the need for the implementation of the right prevention strategies and foot evaluation programs, in the primary health care setting, therefore reducing the impact of this public health problem.

1152

Trends in lower extremity amputations in people with and without diabetes in England, 1996-2005
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Background and aims: To examine trends in non-traumatic lower extremity amputations over a 10-year period in people with and without diabetes in England.
Materials and methods: All individuals admitted to NHS hospitals for non-traumatic amputations between financial years 1996 and 2005 in England were identified using hospital activity data. Associated postoperative and one-year mortality were examined from financial years 2000 to 2004.
Results: There was a reduction in minor and major non-traumatic lower extremity amputations during the study interval. The number of type 1 DM- and non-DM-related minor lower extremity amputations decreased by 11.4% and 32.4%, respectively, while the number of type 2 DM-related minor amputations almost doubled. For major lower extremity amputations, there was a decline in number of type 1 DM- and non-DM-related procedures by 41% and 22.4%, respectively, whereas type 2 DM-related amputations showed a consistent upward trend increasing by 83.5 %. Median length of stay decreased significantly in people with diabetes but remained considerably longer than for patients without diabetes at the end of the study period. Overall perioperative and one-year mortality rates did not significantly change between financial years 2000 and 2004 (7.7% to 7.5%, P=0.52 and 23.8 to 23.5%, P=0.70).
Conclusion: The significant increase in lower extremity amputations in people with type 2 diabetes over the past decade has important financial and health planning implications. These findings highlight the importance of achieving further improvements in preventive care and management for lower extremity amputations in people with diabetes.
EPV is supported by an individual Marie Curie fellowship

1153

Remote areas of bone marrow oedema identified by magnetic resonance imaging in feet of subjects with diabetes presenting with neuropathic lesions are common but do not predict future Charcot neuroarthropathy
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Background and aims: We hypothesize that the spectrum of Charcot neuroarthropathy includes a subclinical phase characterised by bone marrow oedema on MRI without the classical clinical signs. We aimed to test the hypothesis by performing a retrospective cohort study to assess the prevalence of remote areas of bone marrow oedema on MRI of feet of subjects with diabetes presenting with neuropathic foot ulceration, in whom MRI had been requested to assess for underlying osteomyelitis. MRI findings were correlated with subsequent clinical outcome.
Materials and methods: All MRI scans previously performed to look for osteomyelitis associated with neuropathic foot lesions between February 2003 and January 2009 were assessed by two independent radiologists who were not aware of subsequent clinical outcomes. A third radiologist adjudicated if there was discordance. The criteria for diagnosing osteomyelitis were hyperintense signal within the bone on T2 weighted images in continuity with abnormal high signal in the surrounding soft tissues which lead to the abnormal skin and subdermal area associated with the ulcer. Remote areas of hyperintensity in the bone not associated with the soft tissue abnormality and not in direct contiguity with areas diagnosed as osteomyelitis were recorded. Subjects in whom a diagnosis of Charcot was suspected clinically at the time of imaging were excluded.
Results: Seventy MRI studies of feet with neuropathic ulcers in 66 subjects with diabetes were assessed. A second study in the same subject was of the other foot. Of the 66 subjects (mean (standard deviation) age 64 (13) years; duration of diabetes 21 (14) years; HbA1c 8.6 (2.1) %; 48 (73%) male; 8 (12%) with Type 1 diabetes; 13 (20%) on renal replacement therapy), all had clinical evidence of a peripheral neuropathy. Of the 70 neuropathic lesions (66 forefoot and 4 hindfoot), 54 (77%) healed without any form of amputation. Osteomyelitis underlying the neuropathic lesion was present in 48 (69%). Remote areas of hyperintensity in bone were present in 21 studies (30%) (5 without and 16 with concurrent osteomyelitis); in 20, the neuropathic lesion had involved the forefoot and the remote areas of hyperintensity involved the forefoot in 1 study, the midfoot in 14, the hindfoot in 3 and the midfoot and hindfoot in 2; in 1 study, the neuropathic lesion had involved the hindfoot and the remote area of hyperintensity also involved the hindfoot. Subjects with remote areas of hyperintensity were younger (56 (13) vs. 67 (12) years; unpaired t-test $p < 0.001$), were more likely to require renal replacement therapy (43% vs. 9%; Fisher's exact test $p = 0.002$) and were non-significantly more likely to have Type 1 diabetes (24% vs. 7%; $p = 0.098$). Mean duration of follow-up post-MRI was 17 (14) months. Charcot neuroarthropathy subsequently developed in only 1 of the 70 feet studied, 19 months following the index MRI which had not demonstrated remote areas of hyperintensity or osteomyelitis.
Conclusion: Remote areas of hyperintensity on MRI consistent with bone marrow oedema in feet of subjects with diabetes and neuropathic lesions are common. While the bone marrow oedema may be consistent with a subclinical Charcot process, it did not predict future clinical Charcot neuroarthropathy.

1154

Charcot neuroosteoarthropathy in patients with pancreas-kidney transplantation and pancreas transplant alone
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Background and aims: Charcot neuroosteoarthropahy (CNO) is a deforming and destructive process of the foot that can lead to increased morbidity in patients with diabetes. Pancreas-kidney transplantation (PK) and pancreas transplant alone (PTA) could have potential both decreased by improved glycaemic control, renal function and perhaps even improved vascular complications and increased risk of by corticosteroids used in early period after transplantation or for the treatment of rejection, but also by higher level of walking activity. The role of diabetic neuropathy, bone disorders and other predisposing factors developed before transplantation may also play a key role. The aims of our study were to evaluate the presence of CNO before and after PK transplantation and PTA and to assess possible difference between those two types of pancreas transplantation.
Materials and methods: All diabetic patients, in whom PK or PTA was performed in our Institute from May 1993 to December 2008, were included into our retrospective study. The study group consisted of 318 patients (279 PK transplantation and 39 PTA) with mean age at the time of transplantation 42.5±9.0 years and mean duration of diabetes 25.6±8.0 years. The presence of CNO before transplantation was assessed retrospectively from medical records, after transplantation all patients were regularly followed in foot clinic and the diagnosis of acute CNO was based on standard criteria. The mean follow-up period was 71.4±48.4 months after transplantation.
Results: There was no significant difference between manifestation of CNO in all patients before and after transplantation - 24/318 (7.5 %) vs. 23/318 (7.2%), the recurrence of CNO was seen in 6 cases. Patients with PK transplantation and PTA did not differ significantly in the frequency of CNO either before transplantation - 20/279 (7.2%) vs. 4/39 (10.6%) or during the follow-up period after transplantation - 22/279 (7.9%) vs. 1/39 (2.6%). There

were no significant differences between PK transplantation and PTA in presence of neuropathy and levels of glycosylated hemoglobin at the latest follow-up visit.
Conclusion: The frequency of CNO did not change after transplantation either PK transplantation or PTA. It is likely that other mechanisms play an important role in manifestation of acute CNO after transplantation than not only renal function and glycaemic control. PK transplantation and PTA did not increase risk of CNO in our study.
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1155

Differences in minor amputation rate in European foot centres are largely explained by differences in disease severity at presentation
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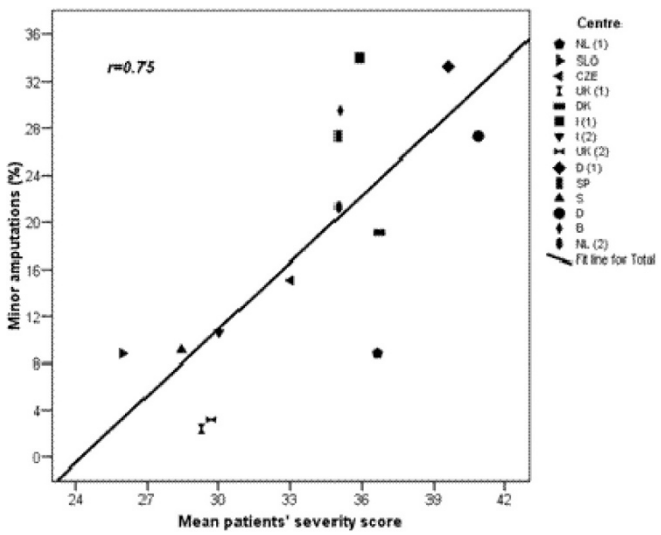
Background and aims: Minor amputation is a frequent consequence of diabetic foot ulcers. So far, determinants of minor amputation have not been studied systematically and the current literature suggests that the incidence of minor amputation may vary significantly between centres and countries. We have therefore investigated: 1) the determinants of minor amputation; 2) the differences in amputation rates between 14 centres from 11 European countries; and 3) the extent to which centre differences in amputation rates could be explained by differences in disease severity at presentation.
Materials and methods: We examined 1088 patients from the Eurodiale study, a prospective cohort study of patients with new diabetic foot ulcers who were followed, on a monthly basis, until healing, death, major amputation or up to a maximum of 1 year. Ulcers were treated according to international guidelines. Patient, foot and ulcer characteristics were obtained at baseline and data on any minor amputation were collected on a monthly basis. Multiple logistic analyses were used to ascertain the baseline characteristics independently associated with minor amputation. A severity score was then calculated, for each patient, on the basis of these characteristics. Finally, we examined the correlation between the amputation rates and the mean patient's severity scores of each centre.
Results: 194 (18%) patients underwent a minor amputation. Baseline factors independently associated with minor amputation were, depth of the ulcer (OR=6.08, CI: 4.10-9.03), peripheral arterial disease (OR=1.84, 1.30-2.60), infection (OR=1.56, 1.05-2.30) and male sex (OR=1.42, 0.99-2.04). Minor amputation rate across centres varied between 2.4% and 34% and these rates were strongly associated with the patient's severity score (figure 1: r=0.75).
Conclusion: Minor amputation is performed frequently in diabetic foot centres throughout Europe and primarily determined by depth of the ulcer, PAD, infection and male sex. Differences between the different European centres can be explained, to a great extent, by differences in patient's level of disease severity at presentation. Our findings suggest that amputation rates should not be used as an indicator of quality of care, given the large variance in patient's initial disease severity across centres, which may also be related to regional or national differences in health care organisation.



1156

Using record linkage of claims data to determine incidence of amputations in patients with and without diabetes mellitus in Austria
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Background and aims: To determine the incidence of minor and major amputations in persons with and without diabetes mellitus in the Austrian province of Styria.
Materials and methods: Styria is a province in Austria with 1.2 million inhabitants. A record linkage was performed using claims data from the year 2005 from the local insurance fund (covering approximately 70% of the Styrian population) and all publicly financed Styrian hospitals. Persons with diabetes were identified as those with claims relating to at least one prescription for anti-diabetic medicine (ATC class A10) or those with diabetes diagnoses present in hospital claims (ICD 10 codes E10-E14). Amputations were identified by means of Austrian procedure codes for inpatient services (MELs) (Major amputation: MEL 4227 femoral amputation and MEL 4408 shank amputation; minor amputation: MEL 4549 other surgery - foot, metatarsus, toes and MEL 4551 large amputation, exarticulation not covered by other codes). Amputation was classified as traumatic, if the main ICD 10 coded discharge diagnosis was included in the groups 'S','T','V', 'W','X' or 'Y' indicating a trauma as cause for hospitalisation.
Results: In 2005, ambulatory claims from 835,403 persons were available for analysis. Crude prevalence of anti-diabetic medication prescriptions was 3.56%. 138,342 persons were admitted a total of 218,517 times to hospitals. Among persons with at least one hospital admission, crude prevalence of diabetes was 7.85%. Using both data sources, a crude diabetes prevalence of 3.84% was estimated. Mean age was 66.6 ±14.4 years and 37.7 ±22.0, for persons with and without diabetes, respectively. Crude incidence of amputations (number of persons with at least one amputation per100,000 persons) is shown in table.

	Without diabetes	With diabetes	Overall
Any amputation	38.5	742.1	65.5
Any non-traumatic amputation	33.0	723.4	59.5
Non-traumatic major amputation	6.7	243.2	15.8
Non-traumatic minor amputation	26.9	536.3	46.4
Any traumatic amputation	5.7	28.1	6.6
Traumatic major amputation	0.7	9.4	1.1
Traumatic minor amputation	5.0	18.7	5.5

Table: Incidence of amputations per 100,000 persons.

Logistic regression models, with age, diabetes status and gender as dependent variables were fitted. Males and females with diabetes had, respectively, 12.8 and 5.3 fold increased risk for any amputation, compared to males or females without diabetes.
Discussion: Record linkage of health care data can be used to assess incidence rates and quality of care. Incidence of major lower limb amputations is cumbersome high for patients with diabetes mellitus in our region of Austria. Tailored programmes for tertiary prevention with the aim to reduce major amputations in diabetic subjects are urgently needed.

1157

Efficacy and safety of recombinant human platelet-derived growth factor gel for diabetic neuropathic foot ulcers: a systematic review
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Background and aims: To assess the efficacy and safety of recombinant human platelet-derived growth factor (rhPDGF) gel for diabetic neuropathic foot (DNF).
Materials and methods: We electronically searched MEDLINE, EMBASE, Evidence-Based medicine Review(Cochrane DSR, ACP Journal Club, DARE, and CCTR), China National Knowledge Infrastructure (CNKI) and VIP da-

tabase to July 2008 for randomized controlled trials (RCTs). Mata-analyses were performed with the RevMan 5.0 software from Cochrane Collaboration.For numeration data, Relative risk (RR) was used as the statistic of curative effect.Each effect number was demonstrated by 95% confidence interval (CI).Heterogeneity amongst trials was assessed by both Chi-square test and I-square test with a 10% level and a 50% level of statistical significance respectively.

Results: Six randomized controlled trials involving 1181 patients were included. Meta-analysis showed that, compared with controls, patients treated with rhPDGF at 100 ug/g had a significantly increase in complete healing rate [RR 1.36, 95%CI (1.16, 1.58)], while patients with rhPDGF at 30 ug/g didn't show an increased rate [RR 1.38, 95%CI (0.76, 2.50)]. rhPDGF gel (100 ug/g) was likely to decrease the time to complete healing and reduces the size of the ulcer more effectively. Moreover, rhPDGF gel did not cause apparent adverse effects and was relatively safe for use.

Conclusion: The currently available evidence indicated that rhPDGF gel can increase complete healing rate of DNF, shorten the time of complete healing and be helpful to shrink ulcer surface. rhPDGF gel has no apparent adverse effects.

Supported by: Department of Endocrinology and Metabolism,West China Hospital, Sichuan University

1158

The cost of treatment of neuropathic diabetic foot ulcers to healing

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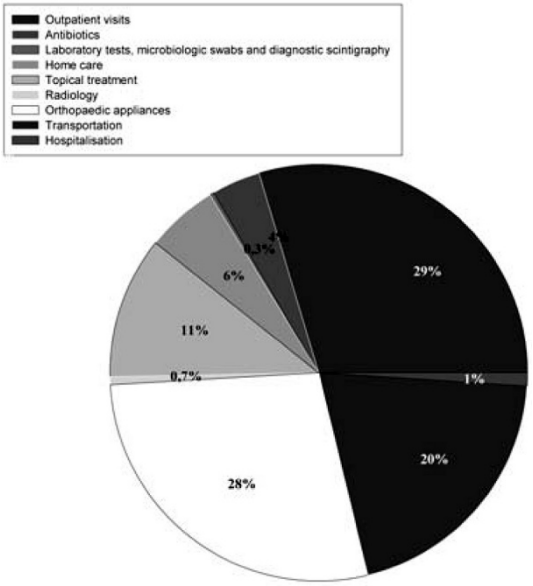
Background and aims: The burden of diabetic foot ulcers (DFU) is high, also in economic terms. We calculated the direct costs associated with the treatment of neuropathic DFUs.

Materials and methods: Costs for all resources used (outpatient visits, lab test, swabs, diagnostic procedures, transportation, hospitalisation, antibiotics, topical treatment, orthopaedic appliances, home care) in the treatment of DFU among 12 patients (mean/average age 65, 10 male, 10 type 2 diabetes) referred to a diabetes outpatient clinic in a tertiary hospital in southern Norway covering a population of 150000 inhabitants, was collected until healing. The total cost, unit costs and cost in relation to ulcer severity according to the University of Texas system for ulcer classification, was calculated.

Results: Average (min, max) healing time was 19 (4,61) weeks. The total direct cost for treatment of neuropathic DFU was Euro 5460. Outpatient visits accounted for 29 % of total costs whereas orthopaedic appliances 28%, hospitalisation 20 %, topical treatment 11 %, antibiotics 11% and home care 6%. Diagnostic procedures, transportation, lab tests and swabs only accounted for a marginal part of total costs (Figure 1). In relation to ulcer severity, costs where significantly higher for ulcers Texas grade B (n=5) than for those with grade A (n=7), Euro 10483 and 1871 (p=0.012) respectively. There was a strong correlation between total costs and time to healing (spearmans ρ=0.688, p=0.013)

Conclusion: The treatment of neuropathic DFU is associated with high costs, and time to healing and ulcer severity is cost raising factors. Seeing that even less severe DFU constitutes a significant economic burden, the importance of prevention is underpinned.



1159

Infected diabetic foot: use of actovegin to accelerate wound healing

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Background and aims: Actovegin is deproteinized hemoderivative of calf blood with low-molecular peptides and nucleic acid content. It is known as antihypoxant, activator of cell metabolism and tissues regeneration.We aimed to assess clinical efficacy of actovegin use on wound healing after surgical treatment of purulent necrotising form of diabetic foot (DF).

Materials and methods: Two matched group of type 2 diabetes (mean 12,3 and 13,1 years duration) patients with infected DF and necrotising lesion were operated. Toes or forefoot (transmetatarsal) amputation were made in 80% of patients and necrectomy - in 20% in each group. The postoperative wounds were left without sutures and healed by secondary intention. Standard care including wound, diabetes and infection treatment was given to all patients. Besides, after operations for patients of the trial group ("A-group") Actovegin was administered via IV infusion 2 g \ day in 0,9% saline during 15 days with following one month oral administration 600-1200 mg\day. A-group consisted of 41 patients (mean age 60,3 years, 14 male) included: 17 patients with neuropathic DF (NDF), 15 - with neuro-ischaemic DF (NIDF) and 9 - with ischemic DF (IDF). Control group - 27 patients (mean age 54,5 years, 9 male) included: 12 patients with NDF, 11 - with NIDF and 4 - with IDF. All patients presented comorbidity: arterial hypertension, ischaemic heart disease,prevalently. Standard examination included wound healing evaluation and TcpO2 measurement on the foot on 5-7th and 14-17th days after operation.

Results: Pre-therapy mean data of TcpO2 (mmHg) in patients of control and A-group were accordingly: NDF - 61,2 - 56,8 ; NIDF - 38,4 - 42,6; .IDF - 12,8 - 14,2. On 5-7 and 14-17 postoperative days TcpO2 significant increased in A-group patients and mean data were accordingly: in NDF - 60,2 - 62,8, in NIDF 52,9 - 59,7 ; in IDF 15,7 - 20,6 mmHg. TcpO2 in control group did not change significantly during postoperative days. The inflammation markers (local swelling, infiltration, hyperemia) disappeared 2-times earlier than in control in NDF and NIDF patients from A-group; such acceleration in IDF patients was less pronounced . Granulation appeared 3-times earlier in patients with NDF and NIDF receiving actovegin; the days of granulation appearing (mean data) in control and A-group patients were accordingly: in NDF patients 9,2 - 3,02; in NIDF 12,8 - 4,6; in IDF 19,4 - 12,8th. Acceleration of the epithelialisation in A-group was not so pronounced; days of the epitelialisation beginning in control and A-group patients were accordingly: in NDF 11,6 - 8,6; in NIDF 13,2 - 9,1; in IDF 23,2 - 18,2th.

Conclusion: Actovegin administration significantly accelerates healing of wound (2- times earlier sign of inflammation disappeared and 3-times earlier granulation beginned) after operation performed in neuropathic and neuro-ischaemic diabetic foot with purulent, necrotic lesion. Effect of actovegin on

wound in ischaemic diabetic foot was less pronounced, but significant. The revealed improvement of tissue oxygenation may be one of the possible explanation for beneficial effect of actovegin on wound healing.

1160

Use of outpatient intravenous antibiotic therapy for the treatment of diabetic foot ulcers

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Background: Foot ulcers complicating diabetes lead to hospital admission and prolonged length of stay. Parenteral out patient antibiotic therapy (OPAT) has been successfully used in clinical situations where protracted treatment is indicated. The experience of it's use in diabetic ulcers in United Kingdom is limited.
Methods: 18 patients requiring parenteral antibiotic therapy were identified at the diabetic foot clinic between March 2008 and March 2009. We used peripherally inserted central catheters (PICC) lines to enable intravenous therapy at home in these patients. Duration of prior oral treatment averaged 10 weeks. PICC line was inserted by a skilled technician and all patients underwent inpatient training and observation for 72 hours. Antibiotics were selected on basis of convenience and sensitivity data. Teicoplanin was used in 13 patients for ease of once daily administration and broad spectrum cover. 2 patients received Co-amoxiclav initially before changing to Teicoplanin and 3 had Ceftriaxone. Weekly review was arranged to prescribe antibiotics and monitor progress. Duration of therapy was 2 - 14 weeks with an average of 8.5 weeks.
Results: 15 (83.4 %) patients demonstrated healing following 6 - 8 weeks of therapy. 3 (16.6%) patients had minimal improvement after 6 weeks and two were referred to vascular surgeons. In 3 patients training was not feasible and received treatment at home administered by district nurse. One patient who left the area was lost to follow up. No line infections occurred.
Conclusion: PICC lines enabled safe and effective outpatient administration of intravenous antibiotic therapy for prolonged periods. There were no issues with compliance. 83.4 % percent demonstrated good progress or healing. This saved 675 hospital bed days with an estimated saving of £155,250.

PS 106 Somatic neuropathy - clinical

1161

Relationship between continuous variations of glucose levels and heart rate variability in patients with type 2 diabetes mellitus

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Background and aims: Heart rate variability (HRV), a marker of autonomic dysfunction, is known to be impaired in type 2 diabetic patients and tight blood glucose control has been reported to improve HRV parameters in these patients. Recent data deriving from continuous glucose measurement in animal models of diabetes mellitus have suggested that HRV may be phasically influenced by blood glucose fluctuations. Whether this potentially relevant dynamic is present in humans is unknown.
Materials and methods: Seven type 2 diabetic subjects underwent simultaneous 24-hour ECG Holter monitoring and 24-hour continuous interstitial glucose measurements by amperometric method with a needle electrode placed subcutaneously in the abdomen and acquiring data every 5 minutes (CGM, 288 per day measurements). Then, in each patient we divided the 24h-ECG and 24h-CGM in three intervals of 8 hrs and analyzed: 1) glycemic variability indices (Standard Deviation [SD], Conga 1-5h, BG rate of change and Mean) of each interval; 2) HRV parameters (time and frequency domain) obtained from the analysis of the correspondent ECG; 3) the correlations between variability indices and HRV parameters.
Results: Data from 21 correspondent intervals evidenced that 24h-mean glucose level was significantly and positively associated with several HRV parameters (SDNN-i, r-MSSD, pNN50, Band 2, Band 3, Band 4; highest correlation with pNN50, 0.63; p=0.002), as was SD (SDNN-i, r-MSSD, pNN50, Band 1, Band 2, Band 3; highest correlation with Band 2, 0.60; p=0.004). Moreover, BG rate was positively associated with SDNN, SDNN-i, r-MSSD, pNN50, Band 1, Band 2, Band 3, Band 4 (highest correlation with Band 3, 0.66; p=0.001), while, among CONGA1-5h, the highest correlation was found between CONGA5h and Band 3, 0,62; p=0.002.
Conclusion: To our knowledge, this is the first human study that demonstrates a short-term effect of glucose level on autonomic modulation of heart function. Moreover, as glycemic variability strongly correlated with HRV, this finding could explain recent data evidencing an excess of mortality in subjects treated intensively who are more likely to experience rapid glycemic excursions.

1162

The association between sudomotor dysfunction assessed by the indicator plaster Neuropad with foot ulceration in diabetes

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Background and aims: To examine the relationship between sudomotor dysfunction assessed by the Indicator Plaster Neuropad (IPN) and foot ulceration (FU) in patients with diabetes.
Methods: A total of 321 patients with either type 1 or type 2 diabetes (211 without and 110 with FU; mean age: 62±10 years) were recruited in this cross-sectional study. Assessment of PN was based on neuropathy symptom score (NSS), neuropathy disability score (NDS), vibration perception threshold (VPT) and 10g-monofilament perception. Sudomotor dysfunction was assessed using the IPN.
Results: Patients with FU had significantly longer duration of diabetes, lower values of ankle-brachial pressure index, higher values of VPT, NSS, NDS and monofilament score in comparison with patients without FU. Sudomotor dysfunction, assessed by abnormal result of the IPN, was significantly more common in the FU group (94.5%) in comparison with the group without FU (50.2%). Multivariate logistic regression analysis after adjustment for gender, body mass index, duration of diabetes, glycemic control and ankle-brachial pressure index, demonstrated that the odds (OR, 95% confidence intervals) of FU increased with measures of neuropathy such as NDS >6 (12.18, 6.96-21.29), VPT >25 Volts (23.86, 11.19-50.87), monofilament score <3 (7.64, 3.88-15.02) but was also significantly increased with abnormal IPN result (17.33, 7.28-41.27) (P<0.001).

Conclusion: Sudomotor dysfunction assessed by the IPN is associated with increased risk of FU and may be included in the screening tests for identification of the diabetic patients at risk for this complication.

1163

The diabetic neuropathy in Mongolia

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Background and aims: The aims of this study were to evaluate the diabetic neuropathy and risk factors of diabetic neuropathy among type 2 diabetic patients.
Materials and methods: This study included 285 type 2 diabetic patients (146 male, 139 female), with a mean age of 58.81±11.03 years and a mean diabetes duration of 5.06±5.68 years. The Diabetic Peripheral Neuropathy (DPN) was diagnosed by means of International Guidelines on the Outpatient Management of Diabetic Peripheral Neuropathy by Neurodiab. The DPN assessment was included brief questionnaire and clinical examination (deformities, dry skin, callus, infection and ulceration). Nerve damage examined by 128-Hz tuning fork, 10-g monofilament, pin prick, cotton wool, temperature sensation, muscle strength and achilles reflexes. The Diabetic Autonomic Neuropathy (DAN) was diagnosed by means of Position Statements from the American Diabetes Association and American Association of Neurology. The DAN diagnosed by brief questionnaire and clinical testing of Cardiovascular Autonomic Neuropathy (CAN) such as Expiration : Inspiration ratio (E:I ratio), Valsalva ratio or maneuver (VR), Heart rate response to standing (30:15 ratio) and Orthostatic Hypotension (OH).
Results: DPN and CAN were 178(68.0%) and 107(52.7%). Symptoms of genitourinary and gastrointestinal autonomic neuropathy were 72.5% and 43.7%. Sudomotor autonomic neuropathy and Impotentia in male were in 87.3% and 74.2%. diabetic patients. People with no clinical (Stages 0/1), clinical (Stage 2) and late complications of clinical (Stage 3) DPN were 84(32.1%), 138(52.7%) and 40(15.3%). Abnormal E:I ratio, VR, 30:15 ratio and OH were in 20(9.8%), 132(64.7%), 125(61.6%), and 44(21.7%) diabetic patients. Risk factors for development and progression of neuropathy such as poor glycemic control, hypertention and hyperlipidemia were 239(88.5%), 139(48.8%) and 129(52.7%). Number of cigarette smoking and alcohol consumption patients were 59(22.5%) and 61(23.3%). Mean duration of diabetes was different between groups with DPN and without DPN (5.86±6.01 vs 4.01±5.14 p<0.05). DPN related with duration of diabetes (r =0.23 p<0.01). Groups with CAN and without CAN were different for age and mean duration (63.94±9.24 vs 57.45±11.24 p<0.01), (6.36±7.31 vs 4.02±3.89 p<0.01). CAN related with age of patients and duration of diabetes (r =0.25 p<0.01 and r =0.225 p<0.01). Result of our study revealed that main differences between male and female sex were incontinence (91.4% vs. 77.4% p<0.05), sexual dysfunction (74.2% vs 26.7% p<0.01) genitourinary infections (43.1% vs. 76.5% p<0.01), dysness (20.7% vs. 47.6% p<0.01), and constipation (41.4% vs. 65.1% p<0.05).
Conclusion: The DPN and CAN in Mongolian type 2 diabetic patients were 68.0% and 52.8%. One of most important risk factors for development and progression of diabetic neuropathy poor glycemic control was in 218(87.6%) patients. DN related with diabetes duration (p<0.01).

1164

Association of serum vitamins B_{12} levels with peripheral nerve conduction abnormalities in diabetic subjects

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Background and aims: Diabetic neuropathy (DN) is a syndrome whose component disorders are still not adequately classified and the complex interaction of various metabolic, nutritional and morphological factors in producing the underlying disorders are not yet fully understood. Vitamin B_{12} is an important nutrient in maintaining proper nerve function and peripheral neuropathy related to its deficiency is a major confounder of diabetic polyneuropathy of somatic origin. Diabetic patients in Bangladesh with symptoms of DN are routinely given vitamin B Complex supplementation, but evidence for the association of vitamin B status with nerve conduction parameters in diabetic case are still rare. In the above context, the present study was undertaken to investigate the site and nature of nerve dysfunction in diabetic subjects and to explore the association of serum vitamin B_{12} with DN in general as well as with individual nerve conduction parameters
Materials and methods: A group of 33 type 2 diabetic subjects with DN and a control group of 22 type 2 diabetic subjects with non-DN, matched for glycemic status as determined by HbA_{1c} levels, were investigated for their NCV parameters and serum vitamin B_{12} levels. DN was confirmed by electrodiagnosis and type 2 DM was diagnosed by OGTT following WHO criteria. NCV was measured by EMG equipment and vitamin B_{12} was estimated by fluorescent based immunosorbent assay, HbA_{1c} was measured by a dedicated HPLC. Serum glucose (Glucose Oxidase) and serum lipids (Enzymetic method) were also measured. Anthropometric measurements were done by standard techniques.
Results: Compared to Non-DN subjects, the DN ones showed higher Ud Latency (msec, M± SD, 3.18±0.944 vs 5.28±2.32, p=<0.001), lower PCAMP(μV, 4.28±1.71 vs 2.20±1.40, p<0.001), lower PNCV (m/sec, 47154±3.40 vs 40.96±6.37, p<0.001), higher SUD latency (msec, 2.11±0.54 vs 2.40±0.24, p<0.05) and lower sensory UNCV (m/sec, 46.91±4.09 vs 42.92±5.94, p=0.005). The DN group had significantly higher age (yrs, M±SD, 38.88±11.19 vs 27.27±7.12; p=<0.001) and BMI (kg/m^2, M±SD, 25.30±3.74 vs 23.25± 2.12; p=0.012) compared to Non-DN groups. TG level was also higher in the DN as compared to Non-DN subjects. Serum vitamin B_{12} levels were significantly higher in the DN group as compare to the Non-DN one. On simple correlation, in the two separate groups, Vitamin B_{12} did not show any significant association with any of the nerve conduction parameters. However, there was a significant association of vitamin B_{12} with Mud Latency (p=0.016), PNCV (p=0.002), Sud Latency (p=0.037) and SSNAP (p=0.051) when all the subjects were pooled as a Diabetic group. On Logistic regression analysis the DN showed significant positive association (P<0.001) with serum vitamin B_{12} when the effects of age, BMI and TG were adjusted. On Multiple regression, vitamin B12 status was found to have significant association with Mud Latency (p=0.038), PNCV (p=0.005) and SSNAP (p=0.045).
Conclusion: a) Duration of DM (as reflected by higher age), body weight and TG are important determinants of neuropathy in type 2 DM subjects. b) Both ulnar (mainly motor function) and peroneal nerves (motor as well as sensory functions) are affected in middle aged type 2 DM patients, were as sural nerve are least affected and c) Serum B_{12} levels have an association with type 2 DM subjects and the vitamin seem to affect the ulnar and peroneal nerves.
Supported by: IPICS and BADAS

1165

Cerebral metabolic abnormalities in diabetes; a clue to the pathogenesis of thalamic neuronal dysfunction in diabetic neuropathy. A proton MR spectroscopic (H-MRS) study

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Background and aims: The thalamus plays an important role in somatosensory perception and H-MRS studies have demonstrated thalamic neuronal dysfunction in diabetic neuropathy (DN). Understanding the pathological changes that lead to thalamic impairment is vitally important. In this H-MRS study we explore the role of glutamine/glutamate (Glx) and astrocytes in this process. Glutamate is the primary excitatory neurotransmitter in the central nervous system, and implicated in the induction of apoptosis in neurodegenerative diseases. In neuropathic pain syndromes, long-term increase in glutaminergic signaling in nociceptive pathways has been shown to result in central sensitization. Astrocytes play a crucial role in clearance of glutamate from the synaptic cleft, thereby preventing a toxic build up. Myoinnositol (mI) an astrocyte marker can also be assessed by H-MRS.
Materials and methods: Eighteen, right-handed, subjects with type-1 diabetes (no-DN=6, painful-DN=5, painless-DN=7) and 5 healthy volunteers (HV) underwent detailed neurophysiological assessments (NIS[LL]+7 tests). H-MRS evaluation of the thalamus was performed at 1.5T. Proton spectra were obtained from a single voxel using short echo-time technique (STEAM: TE=20ms, TR=300ms. Short TE results are expressed as the areas under the Glx and mI resonances relative to that of unsuppressed water.
Results: In the full sample of subjects with diabetes, unpaired t tests revealed significantly elevated thalamic Glx in subjects with diabetes [mean(SD) 0.47(0.12)] compared with HV [0.27(0.13), p=0.001]. A one-way ANOVA revealed a main effect of group [HV, 0.27(0.14); no-DN, 0.53(0.13); DN, 0.45(0.12)] on thalamic Glx levels (p=0.002). Post hoc pairwise comparisons showed that HV had significantly lower Glx in comparison with all diabetic subgroups [No-DN (p=0.001) and DN (p=0.006)]. There was no significant difference in Glx between diabetic subgroups.

Mean mI signal of subjects with diabetes [0.38(0.16)] was lower compared with HV [0.53(0.15); p=0.67]. DN subjects had the lowest mI levels [0.36(0.16)] compared with subjects with No DN [0.42(0.17)] and HV [0.52(0.15), ANOVA p=0.14]. mI levels in subjects with DN were significantly lower compared with HV (p=0.05)

Conclusion: This cross sectional study demonstrates abnormally high levels of thalamic Glx in subjects with diabetes compared to HV. mI levels were lower in subjects with DN suggesting decreased or dysfunctional glial function. This combination may represent a susceptibility to developing thalamic neuronal dysfunction or a generalised "diabetes effect". Given the pathophysiological importance of glutamate and neuroprotective role of astrocytes, further prospective H-MRS studies are required to investigate the role of Glx and mI in DN.

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1166

Achilles tendon volume in type 2 diabetic patients with or without peripheral neuropathy: MRI study

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Background and aims: There is evidence that diabetes may increase Achilles tendon (AT) thickness and stiffness, possibly predisposing to foot ulceration. Tendon thickening may be attributable to increased density of collagen fibres as a result of chronic non-enzymatic glycation. However, the association between AT pathology and peripheral diabetic neuropathy has not been adequately investigated. Therefore, the aim of the present study was to evaluate AT in type 2 diabetic patients with vs. without peripheral neuropathy using Magnetic Resonance Imaging (MRI).

Materials and methods: This study included 19 patients (group A, mean age 63.9±7.4 years) with peripheral neuropathy and 19 patients (group B, mean age 63.6±6.1 years) without peripheral neuropathy, as well as 16 healthy age-matched controls (group C, mean age 61.6±8.4 years). Diagnosis of neuropathy was based on clinical examination using the Diabetic Neuropathy Index. Neuropathy was diagnosed in patients with DNI score higher than 2. Furthermore, moderate neuropathy was defined as DNI score 2.5-4.5 and severe neuropathy as DNI score 5-8. The maximum AT thickness and AT volume were measured in sagittal T_1 weighted MRI images. AT volume was calculated by the sum of the tendon surface area of all contiguous sections multiplied by the slice thickness.

Results: Overall, diabetic patients had significantly (p<0.001) greater AT volume than controls (9742.0±2034.9 mm^3 vs. 7323.8±1918.2 mm^3). On the contrary, AT thickness did not differ between diabetic patients and controls (7.2±1.0 mm vs. 6.7±1.5 mm, p= 0.194). The difference in AT volume was observed both in men (p=0.030) and in women (p<0.001). AT volume was significantly greater in group A vs. C (9503.9±1764.8 mm^3 vs. 7323.8±1918.2 mm^3, p=0.003) and in group B vs. C (9980.2±2297.3 mm^3 vs. 7323.8±1918.2 mm^3, p<0.001), but there was no difference between groups A and B (p=0.469). Finally, in group A increased AT volume was significantly (p=0.041) associated with clinical severity of neuropathy.

Conclusion: Type 2 diabetic patients have increased AT volume as compared to controls. There is no difference in AT volume between patients with and without neuropathy. However, in neuropathic patients increased AT volume is associated with severity of neuropathy.

1167

New markers of diabetic neuropathy?

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Background and aims: The plasma levels of the N-terminal fragment of brain natriuretic peptide (NT-proBNP) recently gained extreme importance as marker of myocardial dysfunction in type 2 diabetes mellitus (T2DM). In a previous work, we observed that NT-proBNP levels also display a significant association with the Diabetic Neuropathy (DPN), even after correction for age and cardiovascular disease (CVD). Scarce data is available regarding the association between diabetic polyneuropathy (DPN) and other peptides as Transforming Growth Factor Beta 1 (TGFβ1) and Tumor Necrosis factor (TNF). TGFβ1 manifested several important roles in diabetic nephropathy and TNF in insulin resistantce but its relationship to DPN is not quite understood.

Materials and methods: Hence, we set forth a cross-sectional pilot study, to assess the hypothetical relationships between NT-proBNP, TNF and TGFβ1 levels, CVD and DPN in a group of T2DM patients (N=190; W:46 %) randomly selected from the North Catalonia Diabetes Study (NCDS). CVD and DPN (clinical and neurophysiology evaluation) prevalence were assessed. NT-proBNP was measured by immunoassay, total TGFβ1 by ELISA and TNF by inmunoassay.

Results: Patients' age: 65.6±7.2, T2DM duration: 11.2±4.5 years, HbA1c: 7.3±1.7; DPN: 48.6%. Log-transformed NT-proBNP levels (logNT-proBNP) were significanlly higher in T2DM patients with CVD (*P*<0.004) and DPN (*P*<0.000) and also log-transformed TGFβ1 (Log TGFβ1) was higher in CVD (p< 0.001) and DPN (p< 0.04) patients, but no differences was observed in log-transformed TNF. Log NT-proBNP manifested an important correlation with log TGFβ1 (p<0.0001) and correlated with: age (*P*<0.001), microalbuminuria (p<0.03), CVD (*P*<0.000) and DPN (*P*<0.000) -even after correction for age and CVD (p<0.001)-. Log TGFβ1 correlated with creatinine (p<0.001), microalbuminuria (p<0.001), C-reative protein plasma levels (CRP) (p<0.02), CVD (*P*<0.006) and DPN (*P*<0.000) -even after correction for microalbuminuria, creatinine and CVD (p<0.014), but log TNFa only correlated to: age (0<01), BMI (0<0.1) and CRP (p<0.05) but not to CVD and DPN.

Conclusion: Noteworthy,TGFβ 1 and NT-proBNP plasma levels appear as important molecules for diabetic micro-angiopathic complications' development and screening although TNF could not play and important roler in diabetic neuropathy. Further prospective trials are warranted.

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1168

5% lidocaine medicated plaster vs pregabalin in patients with painful diabetic polyneuropathy (DPN): efficacy and tolerability results from a randomized, controlled trial

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Background and aims: Comparison of efficacy, tolerability and safety of topical 5% lidocaine medicated plaster with systemic pregabalin for the treatment of neuropathic pain in patients with painful DPN.

Materials and methods: In a pan-European study, 213 patients with painful DPN were randomized 1:1 to 5% lidocaine medicated plaster or pregabalin (titrated to effect: 300 or 600mg/day). The safety set (SAF) comprised 210 patients, the per protocol set (PP) 193 patients. Responder rates were measured as a ≥2 point reduction from baseline in NRS-3 or an overall score of ≤4 after 4 weeks' treatment. Additionally change in NRS-3 values and AEs were reported.

Results: Responder rates were comparable between lidocaine plaster and pregabalin groups in patients with painful DPN (66/99, 66.7% vs. 65/94, 69.1%; PP). The NRS-3 decreased significantly by 2.5 points in both treatment groups (lidocaine plaster: 2.5+-2.0, pregabalin: 2.5+-1.8; baselines were lidocaine plaster: 6.9+-1.3, pregabalin: 6.6+-1.2). Significantly fewer lidocaine plaster patients than pregabalin patients experienced drug-related adverse events: 3.8% (4/105) versus 36.2% (38/105) (p<0.0001). Overall, only 1.9% (2/105) patients administering 5% lidocaine medicated plaster experienced drug-related adverse events leading to discontinuation, compared with 21.9% (23/105) of patients treated with pregabalin.

Conclusion: 5% lidocaine medicated plaster delivers continuous analgesic therapy of neuropathic pain symptoms associated with DPN with low frequency of drug-related adverse events and discontinuations. 5% lidocaine medicated plaster shows comparable analgesic efficacy to pregabalin with improved tolerability in patients with painful DPN.

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1169

Risk of microvascular events following initiation of insulin glargine or NPH insulin in type 2 diabetes in the US

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Background: It is unknown if glycemic control with different insulins is associated with differential risk for subsequent microvascular events (ME).
Methods: A database of 47 US managed care plans was analyzed to determine the incidence of ME following initiation of glargine (GLAR, n=10,667) or NPH (n=2,009) in patients treated with oral antidiabetic drugs between 2001 and early 2008. MEs were defined based on ICD9-CM codes for diabetes specific eye, kidney or nerve complications. Patients with MEs or no HbA1c value available during the 1 year period (baseline) prior to insulin initiation were excluded.
Results: Patients initiating GLAR vs NPH were older (53 vs 48 years), predominantly male (57 vs 36%), had a higher mean HbA1c (9.3 vs 8.3%), fewer specialist visits (18 vs 29%), and lower Charlson comorbidity score (0.86 vs 1.0). Initial crude, unadjusted ME incidence estimates were 225 vs 201 events/1,000 patient-years (Rate Ratio=1.12, p=0.02) with mean follow up of 15 vs 16 months from initiation of GLAR vs NPH, respectively. Unadjusted 1-year HbA1c change was similar between the groups (-1.0 vs -0.96%). Analysis adjusted for multiple confounders showed an increasing risk for ME with baseline HbA1c (Hazard ratio (HR)=1.07, p<0.001) and the risk trended slightly lower in patients achieved HbA1c < 7% vs those who did not during first year follow-up (HR=0.95, p=0.32). After accounting for substantial baseline group differences, 1:1 propensity matched comparisons (Table) showed a 15% lower risk of ME in patients who initiated GLAR (n=1,589) instead of NPH (n=1,589).
Conclusion: These provocative data suggest a lower incidence of ME following initiation of GLAR vs NPH after adjusting for important baseline confounders. This effect of GLAR appears incompletely explained by the differences in HbA1c lowering. Further well controlled studies are necessary to evaluate this hypothesis.

Comparisons on incidence of microvascular events in propensity matched (1:1) GLAR vs NPH initiator groups

Agent	N	Baseline A1C %	% Patients with ME	OR (95% CI)	McNemar S (P)
Match criterion: difference in estimated propensities between glargine and NPH < 0.1					
Glargine	1589	8.64 (p=0.49)	27.6	0.85 (0.73, 0.99)	4.12 (0.04)
NPH	1589	8.70	30.8		
Match criterion: difference in estimated propensities between glargine and NPH < 0.05					
Glargine	1523	8.66 (p=0.16)	27.8	0.83 (0.71, 0.97)	5.26 (0.02)
NPH	1523	8.77	31.6		
Match criterion: difference in estimated propensities between glargine and NPH < 0.01					
Glargine	1449	8.70 (p=0.08)	28.2	0.82 (0.70, 0.96)	6.05 (0.01)
NPH	1449	8.84	32.4		

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1170

Decreased adiponectin levels in diabetic patients with neuropathy

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Background and aims: The mechanism underlying Diabetic peripheral neuropathy still remains unclear. Adiponectin, is an adipocytokine, having several beneficial and protective effects like anti-inflammatory, vasculoprotective and anti-diabetic effects. Little is known about the relationship between adiponectin and diabetic peripheral neuropathy. Therefore, the aim of this study is to investigate the role of adiponectin on diabetic peripheral neuropathy in type 2 diabetic subjects.
Materials and methods: Thirty-eight Type 2 diabetic patients and twenty-three non-diabetic healthy controls were included in the study. We studied EMG and neuropathy symptom score in all study subjects. All subjects underwent a plasma adiponectin level measurement by ELISA method.
Results: The level of adiponectin was found statistically different among all groups (healthy control, diabetic patients with or without neuropathy). Adiponectin levels were found lower at the diabetic patients when compared with healthy controls and the lowest levels have been measured at the diabetic patients with neuropathy. Serum adiponectin levels were found negatively correlated with neuropathy symptom score and also with the diabetes duration, serum fasting glucose, body mass index, triglyceride, hs-CRP and sedimentation rate at type 2 diabetic patients.
Pathogenetic mechanisms underlying the progressive nerve fiber loss seem to be multifactorial, including polyol pathway, reactive oxygen particles, some adipocytokines, and altered protein C kinase activity. Our data imply that adiponectin can be a protective factor against neuropaty at the diabetic patients and further studies should be made about increasing adiponectin levels for the protection of diabetic patients without neuropathy from newly developing neuropathy.
Conclusion: Pathogenetic mechanisms underlying the progressive nerve fiber loss seem to be multifactorial, including polyol pathway, reactive oxygen particles, some adipocytokines, and altered protein C kinase activity. Our data imply that adiponectin can be a protective factor against neuropaty at the diabetic patients and further studies should be made about increasing adiponectin levels for the protection of diabetic patients without neuropathy from newly developing neuropathy.

	Diabetic patients without neuropathy	Diabetic patients with neuropathy	p value
Number	15	23	
Age	54.33±6.23	59.78±8.24	0.027
Body mass index	28.41±2.67	28.7±3.91	0.8
Diabetes duration (year)	4.87±3.18	12.48±4.06	0.0001
Adiponectin (ng/ml)	109.58±39.83	85.15±26.27	0.048
Serum fasting glucose (mg/dl)	123.4±27.5	170.40±53.21	0.001
HbA1c (%)	6.68±0.99	8.31±1.30	0.0001
Total Cholesterol (mg/dl)	212.5±63.8	215.95±42	0.86
Triglyceride (mg/dl)	175±135	211.4±121.3	0.42
HOMA-IR	5.20±3.44	5.33±6.08	0.93
Neuropathy Symptom Score	1.57±0.5	1.57±0.5	0.002

PS 107 Gestational diabetes - pathophysiology

1171

Distinct regulation of fetal adiponectin and adipo-cytokines in pregnancy complicated by maternal obesity

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Background and aims: Obesity in pregnancy is associated with metabolic imbalance in the fetus, leading to enhanced in utero growth and adiposity. The aim of this study was to investigate whether modifications of adipo-cytokines production by fetal tissues are associated with the fetal anabolic state.
Methods: 121 women with singleton pregnancy (53 lean ; BMI : 22.0 ± 1.9) and 68 obese ; BMI : 38.4 ± 6.3) were recruited at the time of term elective C-section delivery (38.8 ± 0.6weeks). They had no sign of infection and had normal glucose tolerance but features of chronic low grade inflammation. Umbilical blood was sampled within 10 min of delivery and neonatal anthropometrics were recorded within 24 hrs of birth. Plasma cytokines concentrations were measured by ELISA.
Results: Neonates of obese mothers had similar body weight 3217±452 vs 3320± 460 g (p=0.22) but increased fat mass 384 ±150 vs 448±175 g (p=0.04) at birth. They were insulin resistant (HOMA-IR : 1.9±0.3 vs 1.2 ±0.2). Total adiponectin (30.8 ± 10.0 vs 30.6 ± 9.7 µg/ml (p=0.94) and adiponectin index defined as the ratio of HMW/total adiponectin (0.35±0.06) were not different between groups. Plasma leptin was increased in neonates of obese women 14.7 ± 13.6 vs 8.2 ± 4.7 ng/ml (p=0.001) and so were IL-6 concentrations 3.5 ± 2.3 vs 2.4 ± 1.4 ng/ml (p=0.02). Circulating umbilical TNF-alpha and C-reactive protein concentrations were similar in both groups. When assessing the relationships between circulating adipo-cytokines and body composition only leptin displayed a positive correlation with fetal adiposity (y = 1.04 + 0.02x; r = 0.30, p=0.001).
Conclusion: These results delineate specific regulations of adipo-cytokines in fetuses of obese women. Despite the development of insulin resistance in the fetus, adiponectin concentrations remained unchanged and there were only minor signs of inflammation. Since maternal cytokines do not cross the placenta these data suggest that obese fetuses are effectively protected against maternal inflammation. However, the combination of high insulin and high adiponectin levels may have functional consequences in creating a favorable environment for enhanced fetal adiposity.
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1172

YKL-40, a biomarker of inflammation, is not increased in patients with gestational diabetes mellitus

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Background and aims: Gestational diabetes mellitus (GDM), defined as disturbance in glucometabolism with onset during pregnancy, complicates about seven percent of all pregnancies and its prevalence is increasing. Up to now the importance of inflammatory processes during pregnancy is not fully clarified. YKL-40, a novel marker which is produced by macrophages is elevated in various inflammatory processes as well as in atherosclerotic processes. Recent studies demonstrated increased YKL-40 levels in patients with type 2 diabetes mellitus (T2DM) compared to healthy controls independent of the BMI. Thus, we were interested if inflammatory alterations being involved in T2DM might be as well associated with GDM.
Materials and methods: We performed a cross-sectional and a longitudinal study, in which 28 patients with GDM (BMI 33.1± 5.8 kg/m², mean age 33±6 years) as well as 30 pregnant women without GDM (NGDM, BMI 28.1± 5.4 kg/m², mean age 32±6 years) were included. Apart from weight and standard risk factors for GDM (Family History, BMI pre-pregnancy, GDM in previous pregnancy, high birth weight) a standard oral glucose tolerance test (75g), lipid and insulin parameters for the calculation of HOMA were assessed at 28±4 weeks of gestation as well as 8 weeks postpartum in both groups. Blood samples for YKL-40 were obtained at the same time points and were determined by a commercial ELISA.
Results: YKL-40 levels in GDM during pregnancy did not differ significantly from NGDM (74.1±62. vs 60.3±30.10 ng/ml). A non-significant decrease of YKL-40 levels postpartum in GDM (63.4±30.5 ng/ml) whereas a non-significant increase of YKL-40 levels in NGDM was observed (66.9±32.7 ng/ml). During pregnancy there were the following differences between the two groups (GDM vs NGDM): fasting glucose (74.6±9.0 vs 89.4±13.4 mg/dl; p<0.001), glucose two hours postprandial (110.4±27.3 vs 161.1±35.9 mg/dl; p<0.001), HbA1c (5.0±0.4 vs 5.5±0.6%; p<0.001), HOMA (1.5 vs 3.2; p<0.001), BMI (28.1±5.4 vs 33.1±5.8kg/m2; p<0.001). In a correlation analysis delta YKL-40 (the change of YKL-40 between pregnancy and postpartum) was associated with BMI (r= 0.423, p=0.04) and insulin one hour postprandial (r=0.511, p=0.008).
Conclusion: YKL-40 levels did not differ significantly between the groups, suggesting that the inflammatory state - as evaluated by macrophage marker YKL40 - is not different between women with or without gestational diabetes. An alternative explanation might be that the abnormal glucometabolic state in pregnancy might be too short to lead to changes in YKL-40.

1173

Normal secretion of the glucagon-like peptide-1 (GLP-1) during gestational diabetes mellitus

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Background and aims: Gestational Diabetes (GDM) predisposes women to future development of type 2 diabetes and the two conditions share a similar spectrum of metabolic alterations. Recent observations suggest that a defective glucose stimulated insulin secretion by glucagon-like peptide-1 (GLP-1), plays a role in the pathogenesis of type 2 DM. Whether such a defect in incretin secretion is impaired in women with GDM remains to be ascertained. Therefore, we have determined GLP-1 secretion in response to oral glucose load in pregnant women with normal glucose tolerance (NGT) and with GDM.
Materials and methods: 100g-3h OGTT (evaluated according to Carpenter and Coustan criteria) was performed in 12 GDM and 16 NGT women at 27.3±4.1 weeks of gestation, for determination of plasma GLP-1, glucose, insulin and C-peptide at 0',60',120',180' min. Insulin sensitivity (ISI) and insulin secretion (first and second phase); as well as insulin sensitivity-secretion index (ISSI) were also derived from the OGTT.
Results: NGT and GDM women were comparable for age (35.2±23.2 vs 34.5± 3.2 years), pre-pregnancy BMI (23.36± 2.86 vs 25.45± 4.26 kg/m²) and weight gain (7.88±3 vs 6.45± 3.8 kg, respectively). As expected GDM women had higher value of the area under the glucose curve (AUC glyc: 27575.5±3448 vs 20685.88±2715 mg/dl min, p<0.01), but lower first phase insulin secretion (993.12±367 vs 1376.61±423 , p<0.05) and ISSI compared to Controls (3873.23±1185 vs 6232.13±1734, p<0.001). In contrast nor AUC GLP-1 did not significantly differ between the two groups (NGT 6542.01 ±2104 vs GDM 5863.5 ±1313 pmol/l min) nor mean GLP-1 values during OGTT were different in the two groups. Only plasma GLP-1 at 180' tended to be lower in GDM (NGT:37.08 ±23.4 vs GDM: 23.19 ±8.28 pmol/l; p=0.07). Univariate regression analysis showed no correlation between AUC GLP-1 and AUC glic, first phase of insulin secretion, ISSI, age and prepregnancy BMI.
Conclusion: Our data show that GLP-1 secretion in response to OGTT in GDM and NGT is not different in spite of a difference in glucose tolerance and b-cell function. Therefore our data do not support a primary role of defect in incretin-mediated insulin secretion in gestational diabetes.

1174

The expression of peroxisome-activated receptor-gamma in fat and placental tissue is not altered in patients with gestational diabetes

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Background and aims: Recent studies have implicated adipokines and ligand-activated transcription factors, such as peroxisome proliferation-activated receptors (PPARs), as the key regulators of lipid and glucose metabolism

that allow adaptation of the mother to the nutritional requirements of the fetus. In the present study we aimed to investigate whether there are significant differences in the expression of PPAR-gamma, transcription factor 7-like 2 (TCF-7L2) and various adipokines in subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT) and placental tissue obtained from pregnant women with gestational diabetes mellitus (GDM) and normal glucose tolerance (NGT).
Materials and methods: The expression of adiponectin, leptin, visfatin, resistin, PPAR-gamma and TCF-7L2 mRNA was measured using quantitative real-time PCR Serum adipokines concentrations were estimated by RIA or ELISA.
Results: Serum adiponectin and visfatin concentrations were significantly lower in the GDM than in the NGT subjects (27.3 [23.6-40.3] vs 35.0 [28.0-45.0] μg/ml, p=0.04 and 2.75 [0.7-4.6] vs 5.2 [3.9-5.9] ng/ml, p=0.02, respectively). The patients with GDM had also lower adiponectin (p=0.008) and TCF-7L2 mRNA expression (p=0.04), as well as higher resistin mRNA expression in VAT (p=0.02) in comparison with the healthy women, whereas PPAR-gamma mRNA expression did not differ significantly between the groups studied. One-way ANOVA revealed that PPAR-gamma and TCF-7L2 mRNA expression in SAT and VAT was significantly higher than in placental tissue (p=0.0003, p=0.0009, p=0.04 and p<0.0001, respectively). Leptin mRNA expression was markedly higher in SAT than in VAT and placental tissue (p<0.0001), whilst adiponectin mRNA expression did not differ between SAT and VAT. No detectable amount of adiponectin mRNA in any of the placental tissue samples was found. There was a significant correlation between the expression of PPAR-gamma and TCF7-L2 mRNA in SAT (R=0.60, p<0.0001), VAT (R=0.64, p=0.001) and placental tissue (R=0.56, p=0.0005). There were also correlations between PPAR-gamma mRNA and adiponectin mRNA expression in SAT (R=0.77, p<0.00001), PPAR-gamma mRNA and visfatin (R=0.43, p=0.009) and leptin (R=0.64, p=0.001) mRNA expression in VAT, as well as between PPAR-gamma mRNA and visfatin (R=0.79, p<0.0001) and leptin (R=0.44, p=0.01) mRNA expression (R=0.56, p=0.0005) in placental tissue.
Conclusion: Low circulating adiponectin and visfatin, as well as decreased adiponectin and TCF-7L2 mRNA expression in VAT seem to play a pathogenic role in GDM, whereas the expression of PPAR-gamma in fat and placental tissue is not altered in GDM patients.
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1175

Plasma MR-proADM concentrations in women with gestational diabetes
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Background and aims: Gestational diabetes (GDM) is associated with distinct differences in placental perfusion and maternal hemodynamics. Adrenomedullin (ADM), a potent vasoactive peptide, is implicated in the regulation of vascular function and related to glucose intolerance and obesity. Previously, ADM was found to play a role in the development of gestational hypertension and pre-eclampsia. Midregional pro-ADM (MR-proADM) is secreted equimolarily with ADM but features a longer half-life. The objective of the present study was to evaluate whether GDM *per se*, i.e. uncomplicated by hypertension or pre-eclampsia, is associated with changes in circulating MR-proADM levels as compared to pregnant women with normal glucose tolerance (NGT).
Materials and methods: In a cross-selectional study, plasma MR-proADM concentrations were measured by ELISA in 101 women with GDM and 87 pregnant women with NGT at gestational week (GW) 10-23, 24-30 and 31-38 (timepoints 1-3). In a longitudinal study, changes in MR-proADM levels between timepoints 1 and 2 were evaluated in 12 NGT and 5 GDM as well as in 23 NGT and 28 GDM between timepoints 2 and 3.
Results: In the cross-selectional study, HOMA-IR was significantly increased in GDM as compared to NGT at all timepoints (p<0.05, respectively). Plasma MR-proADM concentrations were similar in GDM and NGT at GW 10-23 (0.53±0.22 vs. 0.43±0.20 nmol/l, p=0.012), however lower in GDM at GW 24-30 (0.72±0.33 vs. 0.84±0.40 nmol/l, p=0.088) and GW 31-38 (0.82±0.35 vs. 0.99±0.42 nmol/l, p=0.032). At GW 24-30 circulating MR-proADM levels correlated with midregional pro-atrial ANP levels (MR-proANP; r=0.197, p=0.043) and at GW 31-38 with HOMA-IR (r=-0.305, p=0.009) and MR-proANP (r=0.499, p<0.0001). In the longitudinal study, the increase in ΔMR-proADM between timepoints 1 and 2 as well as timepoints 2 and 3 was more pronounced in NGT than GDM (p=0.05; p=0.076) and correlated positively with Δweight (r=0.32, p=0.023) and ΔMR-proANP (r=0.46, p<0.0001) between timepoints 2 and 3.
Conclusion: Although previous studies reported on increased plasma ADM concentrations in type 2 diabetic or obese subjects, our data indicate that GDM women feature lower MR-proADM concentrations in late pregnancy as compared to pregnant subjects with NGT. Such decrease in ADM was related to an increase in insulin resistance. Thus, it might be suggested that during late pregnancy glucose intolerance might curb the physiologic increase of circulating ADM levels.

1176

The activity of lysosomal n-acetyl-beta-hexosaminidase during pregnancy in type 1 diabetes mellitus and gestational diabetes mellitus
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Background and aims: Hyperglycaemia causes chronic tissue damage and might influence on the activity of lysosomal N-acety-β-hexosaminidase (HEX), which catalyzes the removal of N-acetylglucosamine or N-acetylgalactosamine residues from the non-reducing end of glycoconjugates oligosaccharide chains. HEX is a sensitive marker of diabetic vascular complications. The aim of our investigation was to determine the changes in the activity of lysosomal HEX in type 1 diabetes during pregnancy and gestational diabetes mellitus.
Materials and methods: Serum and homogenates of placenta tissue of 20 women with gestational diabetes mellitus (GDM), 10 with type 1 diabetes (DM1) and 15 women in physiological pregnancy and serum of 15 non pregnant women as a control groups. The diabetic women was well controlled. HEX activity (pKat/ml of sample or μKat/kg of protein) was measured by Chatterjee *et al* method in modification by Zwierz *et al*.
Results: We observed a statistically significant increase of HEX activity in the serum in physiological pregnancy (1221,32±282,3) compared to the non-pregnant women (428,92±86,2; p=0,000000), statistically significant increase of HEX activity in the serum of GDM women (1843,64±495,86) compared to physiological pregnancy (p=0,000124). In placenta tissue we found statistically significant increase of Hex activity in GDM (321,5±135,95) as compared to non-complicated pregnancy (138,23±30,3; p=0,000021). There was no statistically differences of HEX activity in serum (p=0,477) and placenta (p=0,7) between type 1 diabetes and gestational diabetes mellitus. The activity of lysosomal HEX in serum 3 months postpartum was compared to control group non-pregnant women.
Conclusion: Our study might suggest that diabetes might have an influence on metabolism of gycoconjugates that leads to increase in their degradation. The mechanism of hyperactivity of HEX is probably the same in DM 1 and GDM. It is suggested that damage of vascular endothelium is the major source of the enzyme in diabetes.
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1177

Role of maternal iron status in the pathogenesis of gestational diabetes mellitus
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Background and aims: Gestational diabetes mellitus (GDM) is one of the commonest complications of pregnancy; but its pathophysiology is still not fully understand. Recently attention has been focused on the relation between iron metabolism and glucose intolerance in the genesis of GDM. The present study was conducted to investigate the association of body iron store with various covariates of metabolic syndrome.
Materials and methods: A total 100 subjects were included in this study: 43 were healthy nondiabetic and nonanemic pregnant women (Control group) and 57 were pregnant women having Gestational Diabetes Mellitus (GDM group). The subjects were investigated for glycemic (fasting & 2h ABF glucose and HbA_1c), insulinemic (fasting C-peptide) and body iron (serum iron,

ferritin & transferrin) status. Glucose level was measured by using glucose-oxidase method, fasting serum C-peptide by chemiluminescent enzyme immunoassay, HbA_{1c} by using a modified HPLC method and insulin sensitivity (HOMA%S) and insulin secretory capacity (HOMA%B) were calculated by Homeostasis Model Assessment. Serum transferrin receptor (STfR) was measured by Enzyme-Linked Immunosorbent Assay (ELISA), and serum ferritin level was assessed by Microparticle Enzyme Immunoassay (MEIA) technology. Serum iron concentration was measured by IRN method.
Results: The age of the study groups were found to be matched (p=0.522). Gestational weeks and parity of the study groups were significantly higher in GDM than Controls (p=0.004 and p=0.015 respectively). HbA_{1c} level (%, M±SD) was significantly higher in GDM group (6.09±1.1) as compared to Control (5.8±0.7, P=0.001). Serum insulin level [ng/ml, Median (range)] as assessed by C-peptide, was significantly higher in GDM group [2.5(1-6.6)] compared to Control [1.5(1-3.3), P<0.001], but the insulin secretory capacity (HOMA% B) was not different between the two groups [median (range) 144(102-270) in GDM vs 165(100-281) in Control]. HOMA%S [Median (*range*)] was significantly lower in GDM group [38(20-90)] as compared to Control [60(32-99), P<0.001]. The lipidemic status of the GDM group did not show any difference from Control . Among the marker of body iron status hemoglobin level showed no difference between GDM (11±1.25) and Control groups (10.6±0.8), but serum iron concentration [median (range)] was significantly lower in GDM group [6(2-19)] as compared to Control [12(2-36)]. There was no significant difference between the two groups in their levels of serum ferritin and STfR. On Pearson's correlation coefficient analysis Ferritin level had significantly negative correlation with HbA_1c (P=0.046). No other correlation was found among other variables.
Conclusion: From the above data it may be concluded that GDM in Bangladeshi subjects does not seem to be associated with iron less deficiency or elevated body iron store. GDM subjects may show lower serum iron, but this is probably related to chronic inflammatory state of diabetes rather than iron deficiency itself. The data also suggest that interpretation of body iron status in this condition, using the available markers (like serum iron, serum ferritin and STfR), needs to be made with caution due to the presence of concurrent factors like pregnancy and chronic inflammatory status.
Supported by: IPICS and BADAS

1178

Homeostatic insulin sensitivity indices in the detection of gestational diabetes mellitus

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Background and aims: Early identification of GDM is strongly warranted for prevention of both maternal and fetal complications, but well known disadvantages of the present methods based on oral glucose challenge reduces the compliance and applicability of these methods in the screening of the disorder. The present study aimed to assess FBG-based insulin sensitivity indices (ISIs) regarding their suitability as alternatives of 2 hour 75-g OGTT. Other related glycemic and insulinemic indices as suitable alternatives were also explored. McNamara test was used to calculate sensitivity, specificity, PPV and NPV of various tests against the gold standard of OGTT.
Materials and methods: A group of pregnant mothers, at 24 to 32 weeks of gestation, were recruited from BIRDEM (the tertiary hospital of Diabetic Association of Bangladesh) and Dhaka Medical College Hospital were screened for GDM by adapting WHO criteria of 75-g OGTT. Out of 300 subjects, 112 had GDM. Finally 84 GDM and 82 normal mothers were analyzed through a nested case control design. Serum glucose was measured by glucose oxidase method and insulin was assayed with a chemluminescence based ELISA. Insulin sensitivity indices as well as glycemic and insulinemic indices were calculated and tested for their ability to detect GDM. Homeostatic formulas were used to quantify insulin sensitivity and B-cell function.
Results: The GDM group had higher age as compared to control (years, M±SD, 28.9±3.8, vs. 26.7±4.6, p<0.001). HOMA%B was significantly (p<0.001) lower in GDM (113.3±51.4) than their non-GDM counterparts (207.9±91.3). QUICKI of GDM was 0.52 ±.03 and that of control was 0.55±.05; the difference was statistically significant (p<0.001). HOMA%S showed no significant difference (p=0.158) between GDM and non-GDM groups. In Pearson's correlation analysis HOMA%B had a significant correlation with age, FBG, 75-g OGTT and fasting insulin level. HOMA%S showed statistically significant correlation with FBG, 75-g OGTT, fasting insulin, HOMA%B and QUICKI. Logistic regression analysis provided significant association of HOMA%B with GDM (p=0.002) after adjusting the effect of the confounders. The value of different screening markers for predicting GDM was explored. HOMA%S at optimum cut-off value of 50 showed sensitivity of 50% and specificity of 56%, with PPV and NPV 56% and 55% respectively. QUICKI had sensitivity and specificity of 28% and 31% respectively at an optimum cut-off value of 0.54. Fasting insulin showed sensitivity and specificity of 54% and 49% respectively at cut-off value of 12.9μU/ml with PPV 50% and NPV 50%. At an optimum cut- off value of 5mmol/l, the sensitivity, specificity, PPV and NPV of FBG was 82%, 78%, 79% and 81% respectively The corresponding value for combined fasting glucose and fasting insulin were 84%, 79%, 82% and 82%.
Conclusion: The data suggest that insulin sensitivity indices do not seem to be reliable alternatives for oral glucose based tests for the detection of GDM. Simple fasting blood glucose with a cut-off value of 5.0mmol/l, for Bangladeshi population, seems to be an acceptable test in the screening of GDM.
Supported by: IPICS and BADAS

PS 108 Gestational diabetes - maternal and foetal outcome

1179

Increasing incidence of abnormal glucose tolerance in women with gestational diabetes (GDM) or mild gestational diabetes (MGH) in France: DIAGEST 2 study

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Background and aims: Gestational diabetes (GDM) is characterized by impaired glucose metabolism during pregnancy. After pregnancy, these women do not completely normalise their metabolism and maintain an increased risk of later maternal diabetes (DM) and vascular disease. Mild blood glucose abnormalities during pregnancy may be linked to later glucose tolerance abnormalities or diabetes mellitus. Our aims were 1) to evaluate the prevalence of diabetes mellitus (DM), impaired glucose tolerance (IGT), and impaired fasting glucose (IFG) 11 years later after delivery in women with various blood glucose status during pregnancy 2) to determine the risk factors of diabetes in this population.
Materials and methods: We compared long-term outcomes among control women (n= 221), women with mild gestational hyperglycaemia (n=322) and women with gestational diabetes (GDM) (n=466). Women were recruited from 15 public maternity units in north of France wich belong to the Diagest group. Between delivery and the follow-up visit (6 years and 11 years), women with prior GDM or MGH had not received specific advice, particularly about nutrition or physical activity. Women with prior GDM or MGH and control subjects were offered a 75-g OGTT. Women who refused the OGTT contributed only a fasting plasma glucose. Rates of DM, IGT and IFG were compared between the groups (American Diabetes Association criteria).
Results: Eleven years after delivery, clinical and biological parameters were be determined in 225 GDM, 122 MGH and 76 controls. Rates of DM, IGT and IFG were respectively 3.9%, 4.5% and 1.3% in the control group. In GDM women, the rates of DM, IGT and IFG are respectively 35.6%, 8.9% and 5.3%. In MGH women, the rates of DM, IGT and IFG are respectively 12.3%, 15.7% and 10.7%. Among 178 GDM who were seen at 6 years and at 11 years, the prevalence of DM was 30.3% at 6 years IC 95% [23.6-37.1] and 42.7% at 11 years IC 95% [35.4-50.0]. At 11 years, predictors for DM are: family history of diabetes, previous GDM, BMI > 27 kg/m^2, fasting glucose during pregnancy > or = 5.5 mmol/l, the severity of hyperglycaemia during pregnancy defined by the number of abnormal blood glucose values fasting, 1, 2 and 3 h during the glucose tolerance test at diagnosis of GDM, and insulin therapy during pregnancy.
Conclusion: This study has identified a high prevalence of glucose tolerance abnormalities after GDM and MGH. Compared to GDM women, women with MGH have also a high risk of later diabetes. After 6 years, the prevalence carries on to increase. Thus, women with previous glucose tolerance abnormalities represent a high-risk group and a target for intervention to postpone or prevent the development of overt diabetes in France.
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1180

Metabolic syndrome in early pregnancy and risk of gestational diabetes mellitus

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Background and aims: Gestational diabetes mellitus (GDM) is a prevalent disease associated with adverse outcomes of pregnancy. The association between metabolic aberrations in early pregnancy and the development of GDM remains little explored. The purpose of this study was to determine the extent to which, maternal metabolic syndrome in early pregnancy is a risk factor for GDM.
Materials and methods: The mother-child "Rhea"study in Crete is a prospective cohort examining pregnant women (Greek and immigrants) residents at the prefecture of Heraklion that became pregnant during one year starting in February 2007 and initiated prenatal care before 15 weeks of gestation. Five hundred and eight women with sigleton pregnancies without a diagnosis of diabetes mellitus or GDM in a prior pregnancy were included in the analysis. Maternal fasting serum samples were collected and blood pressure was measured at the time of the first major ultrasound (Mean: 12 weeks, SD: 1.5). Metabolic syndrome in early pregnancy was defined according to the NHLBI/AHA criteria. Pregnant women were screened for GDM between 24 and 28 weeks of gestation, and GDM was defined by the criteria proposed by Carpenter and Coustan. Multivariable log-binomial regression models were used to estimate the effect of metabolic syndrome in early pregnancy on the risk of GDM after adjusting for confounders.
Results: Women with metabolic syndrome were in high risk for GDM (RR=3.17, 95 percent CI: 1.06, 9.50), whereas among the components of metabolic syndrome, the most significant risk factors were impaired fasting glucose (RR=4.92, 95 percent CI: 1.41, 17.23), and pre-pregnancy obesity (RR=2.65, 95 percent CI: 1.23, 5.70). An elevation of 10mmHg in systolic or diastolic blood pressure increased the relative risk for GDM by 49% (RR=1.49, 95 percent CI: 1.10, 2.02) and 34% (RR=1.34, 95 percent CI: 1.04, 1.73) respectively, while a per unit increase in the BMI pre-pregnancy increased the relative risk for GDM by 6% (RR=1.06, 95 percent CI: 1.01, 1.12).
Conclusion: These findings suggest that women with metabolic syndrome in early pregnancy had higher risk for GDM.
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1181

Undiagnosed pre-gestational diabetes among women with gestational diabetes mellitus

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Background and aims: Among women diagnosed with gestational diabetes mellitus (GDM), a subpopulation is presumed to have undiagnosed pre-gestational diabetes. Well aware of the severe adverse pregnancy outcome in women with pre-gestational diabetes, such as malformations, preterm delivery and perinatal mortality, women with undiagnosed pre-gestational diabetes are assumed to be equally vulnerable to complications in pregnancy. This implies that GDM women with undiagnosed pre-gestational diabetes deserve intensified pregnancy surveillance, and therefore need to be identified as early as possible in pregnancy.
Materials and methods: A cohort of 215 women diagnosed with GDM in 2007 was investigated. Two women were classified as having had undiagnosed pre-gestational diabetes due to continuous need for insulin treatment after delivery. In addition, 10 women were diagnosed according to the WHO criteria of oral glucose tolerance tests performed in 141 cases (66%) two months post partum.
Results: At diagnosis of GDM, all women were instructed in diabetes diet, resulting in a restricted mean weight gain of 1.6 kg from GDM diagnosis to delivery, seven weeks (0-35) later. Sixty-one of these women (43%) were insulin treated due to insufficient glucose control on diet treatment alone.
Patients with undiagnosed diabetes in pregnancy were characterised by higher 2 hour plasma glucose value (13.8 mmol/l (SD 3.9) vs. 10.7 (SD 1.8), p<0.001), higher HbA1c value at GDM diagnosis (6.7 % (SD 1.5) vs. 5.5 (SD 0.7), p<0.001), and being diagnosed earlier in pregnancy (median 19 weeks (11-37) vs. 31 (5-39), p<0.001), while age and BMI were comparable (34.5 years vs. 33.6 and 32.6 kg/m^2 vs. 31.7). In eight cases with baseline HbA1c>6.5% and GDM diagnosis before 35 weeks, four (50%) had diabetes and four (50%) had pre-diabetes (IGT or IFG) post partum. Among women treated with insulin, 10 (16%) had diabetes, and 24 (39%) had pre-diabetes two months post partum, in comparison to two (2%) and 17 (21%) among women treated with diet alone.
Conclusion: The presence of women with undiagnosed pre-gestational diabetes among women with GDM is a significant clinical problem. These women can be distinguished among GDM patients by higher 2 hour plasma

glucose and HbA1c values, early onset of GDM, and the necessity of insulin therapy during pregnancy. Special attention should be paid to GDM patients with these characteristics in order to prevent severe pregnancy outcome due to undiagnosed pre-gestational diabetes.
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1182

Obstetrical outcomes in pregnancies with gestational diabetes: what benefits? Which patients?
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Background and aims: Up today, no international consensus was found for gestational diabetes mellitus (GDM) diagnosis. We have previously reported that O'Sullivan test has a low relevance for GDM screening in our Mediterranean population. Thus we adopted a universal one-step screening strategy with a 75 grams OGTT. We report here our first results of obstetrical outcomes since we apply this new screening strategy at our University Hospital.
Materials and methods: We conducted a prospective and descriptive study between 01/01/2008 and 12/31/2008. Over nine months (January to September 2008), all pregnant women (n=2014) were screened with a 75 gr OGTT between 24th and 28th week of amenorrhea (wa). When GDM was diagnosed (fasting plasma glucose (FPG) > or = 95 mg/l and/or 2h post-load (PLG) > or = 140 mg/l), women had an intensive management including dietary advice, glucose self-monitoring and insulin therapy as necessary.
Results: GDM was screened at mean term of 27 wa. Prevalence of GDM was increased from 2,98 % to 7,78 % (n = 157). Mean age was 32 years (20 - 45). Mean preconceptional BMI was 25,8 kg/m² (16,8 - 44,6) but above 27 kg/m² in 38,2 % of patients. Insulin was initiated in 21 % (n = 33). Delivery occurred at 39 wa (32-42), spontaneous in 58 %. Caesarean section rate was 26,8 % (vs 19,2% in global population). Prematurity rate (under 37 wa) was 10,8 %. Mean birth weight was 3218 gr (1640-5120). Macrosomia was present in 5,7 % (birth weight (BW) > or = 4000 gr) and in 8,3 % (BW > or = 90th percentile for a standard French population). No major neonatal event was reported.
Insulin therapy was more frequent in patients with BMI > or = 27 kg/m² and/or FPG > or = 95 mg/l (p < 0,001) and was associated with higher rates of macrosomia (RR = 5,2; p = 0,006) and caesarean section (RR = 3,5; p = 0,003). Labor induction was most frequent in insulin treated GDM and induced caesarean section in 20 %.

	GDM with insulin therapy	**GDM without insulin therapy**	p
Percentage	21 %	79 %	< 0,01
Mean BMI (kg/m²)	**30,3 ± 6,9**	**24,5 ± 4,7**	< 0,001
BMI ≥ 27 kg/m²	**66,7 %**	**30,6 %**	< 0,001
FPG (mg/l)	100 ± 21	85 ± 12	< 0,001
2h PLG (mg/l)	176 ± 45	153 ± 21	< 0,001
BW > 90th perc.	**21,2 %**	**4,8 %**	< 0,001
BW < 10th perc.	12,1 %	14,5 %	NS
Global caesarean section rate	**48,5 %**	**20,9 %**	< 0,001
Primary caesarean section rate	24,2 %	12,1 %	< 0,001
Labor induction rate	**91 %**	**29 %**	< 0,001

Preconceptional BMI above 27 kg/m² was only associated with higher rate of insulin therapy (OR = 4,48; p or = 95 mg/l was associated with higher rates of insulin therapy (OR = 4,26; p < 0,001) and caesarean section (OR = 2,43; p = 0,02); no correlation was found between FPG or preconceptional BMI and macrosomia.
34 % of patients had no risk factors for GDM and were characterized by lower glycemia levels (mean FPG 83 mg/l; mean 2h PLG 151 mg/l), lower BMI (21,2 kg/m²) and lower rates of insulin therapy (7,1 %), caesarean section (17,8 %) and macrosomia (3,6 %).
Conclusion: Intensive management of GDM is efficient but leads to a high rate of caesarean section. Preconceptional weight excess and FPG appear as prognostic factors of obstetrical outcomes. It is not obvious that intensive management of GDM impacts obstetrical prognosis of women without risk factors, who are characterized by lower glycemia levels. This should trigger reflection on diagnostic thresholds and therapeutic intervention strategy.

1183

Maternal and foetal outcomes of gestational diabetes in a tertiary hospital Sri Lanka
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Background: Maternal and foetal outcomes of gestational diabetes mellitus (GDM) in peripheral hospitals in Sri Lanka are not well documented.
Materials and methods: This case control study was conducted in an antenatal clinic from 1st of February 2008 to 31st January 2009. Objectives of the study are to describe the underlying socio-demographic factors of GDM and to determine the maternal and foetal outcomes of GDM at Provincial General Hospital (PGH), Badulla, Sri Lanka. Cases were women with singleton pregnancy and GDM according to the WHO criteria. Controls were age, BMI and parity matched women with singleton pregnancy with no GDM. GDM was diagnosed using a 75g OGTT when plasma glucose was >140mg/dl in 50g glucose challenge test (GCT) at one hour.
Results: 87 women out of 7934 (1.1%) had GDM. Mean age of those with GDM was 31.6 years. 65.6% of cases had a Body Mass Index over 23. There was no difference in the income between cases and controls (p=0.745).Polyhydramnios was seen in 16.9% of cases vs. 3.6% of controls (p=0.05). Caesarean sections were carried out in 67.5% of cases and in 62.5% of controls (p=0.515). Temperature more than 98.4^{0}F was seen in 3.6% of cases and 9.6% of controls (p=0.119). Vaginal candidiasis was seen in 4.8% of cases and 6% of controls (p=0.732). Urinary tract infection and pre term labour were equally present in cases and controls, 3.6% and 6% respectively. No birth traumas were reported.Neonatal hypoglycaemia was reported in 4.8% of cases but not in controls (p=0.043).Two still births were reported in the cases while one was reported in the controls (p=0.56). Birth weight more than 3.5Kg was reported in 12% of cases and 3.6% of controls (p=0.043). Congenital anomalies were reported as 1.2% both in cases and controls. Neither neonatal deaths nor shoulder dystocia were reported.
Conclusion: In this hospital based population from peripheral Sri Lanka, the prevalence of GDM (1.1%) was lower than that shown in the studies from urban or semi-urban areas (5.5 - 8.4%). Polyhydramnios, birth weight >3.5kg and neonatal hypoglycaemia were found significant in this study.Caesarean sections, Temperature >98.4F, Vaginal candidiasis, Urinary tract infection, pre term labour were not significantly associated with GDM as maternal outcomes. Congenital anomalies, shoulder dystocia, still births, neonatal deaths, were also not significantly associated with GDM pregnancies compared to the others as Foetal outcomes. These findings are useful in establishing national policies on screening for GDM.

1184

Metabolic syndrome and adiponectine in women with prior gestational diabetes
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Background and aims: Women with prior gestational diabetes mellitus (pGDM) are at increased risk of developing type 2 diabetes and associated vasculopathy. The relationship between insulin sensitivity, parameters of subclinical inflammation, and plasma concentrations of adipocytokines was investigated in pGDM both at 3 months and 12 months after delivery
Materials and methods: We prospectively studied 198 women with pGDM .According to OGTT, we divided into two groups: pGDM with normal glucose tolerance post-partum (NG) (66,8%) and pGDM with abnormal glucose tolerance post-partum (AG) (33,2%). Insulin sensitivity was derived using HOMA. Metabolic syndrome (MS) was defined by ATP-III We collected obstetric history, personal history of pGDM and personal and family history of cardiovascular risk factors Plasma concentrations of ultrasensitive C-reactive protein (CRP), adiponectin, and leptin were measured
Results: The mean age was 33,45 ± 4,5 yrs.; 26,25% smoking, 15,6% history of dyslipemia, 30% had history of previous abortions. 62,34% of women had first-degree relatives with type 2 DM and 59,25% had family history of hypertension The prevalence of MS was higher (32,3%) in AG group than in NG (14,7 %); p<0.005. In addition, AG group had higher values of BMI and WC vs. NG group (28,86 ± 4,5 vs 22,70 ± 3,1 Kg/m²; p<0.005 and 93,76 ± 6,1 vs 80,34 ± 4,6; p<0.05, respectively) . Fasting glucose and 2h-glucose levels

were higher in AG group than NG (98,85 ± 7,7 vs 80,2 ±5,6 mg/dl, p<0.005 and 166,57± 23 vs 119,4 ± 20 mg/dl). Fasting insulin and HOMA values were significantly higher in AG than NG; p<0.005. AG showed lower (P < 0.0001) plasma adiponectin (5.8 +/- 0.2 microg/ml) than NT (8.9 +/- 0.6 microg/ml) and a decreased (P < 0.003) but increased plasma leptin (P < 0.003) and CRP (P < 0.03). adiponectin correlated positively with HOMA (P < 0.003) and HDL cholesterol (P < 0.0001) but negatively with plasma glucose (2-h oral glucose tolerance test [OGTT]) (P < 0.0001), leptin (P < 0.01) and CRP (P < 0.007). In addition, AG group presented a higher prevalence of family history of type 2 DM (68,7% vs 48%, p<0.01)

Conclusion: Women with pGDM have a high prevalence of abnormal glucose tolerance and MS and lower values of adiponectin, particularly those with AG.. Thus women with pGDM and family history of type 2 DM may be especially suitable interventions aimed at preventing or reducing type 2 DM and probably cardiovascular events.

1185

Glycaemic variability and foetal growth in diabetic pregnancy

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Background and aims: Fetal overgrowth is the most important complication of diabetic pregnancies (gestational (GDM) and pregestational (preGDM)). Aim of the study was to evaluate the relationship between glycemic profile, detected with continuous glucose monitoring (CGM), (Glucoday, Menarini Diagnostic, Firenze, Italy) and parameters of fetal growth

Materials and methods: 80 pregnant women (32 with preGDM, 31 with G DM, 17 with normal glucose tolerance (NGT)) were evaluated. PreGDM women measured glucose profile by CGM at the first, second and third trimester of pregnancy, GDM and NGT women at the second and third trimester.As for mothers, age, prepregnancy BMI, HbA1c and glucose monitoring were recorded. As for newborn, birth weight, ponderal index (gr/cm^3), large± for gestational age (LGA) parameters were recorded.

Results: Mean age was 33± 6 yrs for preGDM, 35± 4yrs for GDM, 33±5 yrs for NGT women, prepregnancy BMI was respectively 29± 3, 26± 4, 21± 4; HbA1c were 6.8± 1.2 %, 7.2± 1.1%, 6.0± 0.8 % for preGDM at each trimester, 5.2 ± 0.5%, 5.5± 0.4%, for GDM at second and third trimester. As for fetal growth, mean birtweigth was 3096 ±583 in preGDM, 3240± 582 for GDM, 3142± 386 for NGT; ponderal index was 2.88 ± 0.9 in preGDM, 2.61± 0.6 in GDM and 2.46± 0.4 in NGT . As for glycemic variability, we took into consideration MAGE (mean amplitude of glycemic excursion) and M-value (a quantitative index od the deviation of blood glucose determination in 24 hrs period from an arbitrariluy selected standard). M-value, at the third trimester, was related to birth weigth in NGT(p=0.05), furthermore M-value was related to birth weigth and ponderal index at the second trimester in preGDM patients (p=0.04, p=0.018 respectively),MAGE at the second trimester was related to birth weigth in GDM (p= 0.05).

Conclusion: Our data show that glycemic variability evaluated by M-value and MAGE is important in determining fetal overgrowth, suggesting that the second trimester of pregnancy in diabetic women is critical for fetal growth and so a good metabolic control needs to be obtained in this period in order to normalize fetal weigth.

1186

Impact of incidental use of oral antidiabetics on pregnancy outcome

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Background and Objectives: Except for patients with Polycystic Ovary Syndrome who are continued on metformin even after getting pregnant, other oral antidiabetic agents are not advised during pregnancy. Because of obvious ethical considerations a prospective controlled study of oral antidiabetics during pregnancy can not be conducted in humans. In this pilot study we followed 93 pregnant diabetic women who continued to use their oral medications for variable periods of time before realizing they were pregnant. Once pregnancy was confirmed, they were shifted to standard therapy with diet and/or insulin.

Subjects and methods: The study included 93 diabetic ladies with type 2 diabetes mellitus.Their ages ranged from 26 to 41 years with a mean ± SD of 37±7.9 years. The mean duration of oral antidiabetic use before shift to other treatment modality ranged from 2 weeks to 2 months. The oral antidiabetic medications used were metformin in 89 patients (95.7%), one of the sulphonyl ureas (glibeneclamide, glimepride or gliclazide) in 67 patients (72%), acarbose in 4 patients (4.3%) and thiazolidinediones (TZD's) in 6 (6.5%) of the study group (4 on pioglitazone and 2 on rosiglitazone). Concomitant use of other medications was recorded in 7 patients who received antihypertensive drugs and 5 patients who received antacids for heartburn. SMBG, frequent oral and post-prandial plasma glucose measurements or glycated hemoglobin level determination were used to asses the degree of diabetes control during pregnancy.

Results: 81patients (87%) were able to reach full term, while 12 patients (13%) had abortions or miscarriages. Normal vaginal delivery was possible in 21 patients of those who completed their pregnancy (25.9%), while 60 patients (74.1%) were delivered by elective CS. Maternal complications in the form of toxemia of pregnancy was recorded in eight patients (8.6%). Regarding fetal complications, macrosomia or large for gestational age weight at birth was recorded in 23 newborn infants (28.4%) and gross fetal anomalies diagnosed immediately after birth were found in 7 of the newborn babies (8.6%).In this latter group two of them were born to mothers who used TZD's in their 1st trimester (one was using rosiglitazone and another was using pioglitazone). The majority of the newborn babies required glucose infusion post-natally to correct for hypoglycemia. Regarding the degree of control of the diabetic state, the mean glycated hemoglobin level was 8.7% on booking to the clinic, 6.3 % at the end of the 1st trimester, 7.2% at the end of the 2nd trimester and 7.1% at the end of the 3rd trimester.

Conclusion: Except for probably thiazolidinediones, the use of oral hypoglycemic agents, specially metformin and SU's may be safe during pregnancy. However cumulative experience from different centers should be collected and carefully analyzed before reaching a solid conclusion.

1187

Short acting insulin analogues versus regular human insulin in women with gestational diabetes - patient-relevant outcomes: systematic review

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Background and aims: Gestational diabetes mellitus (GDM) is associated with risks of complications during pregnancy and birth. A therapy with insulin is recommended as a therapy option for possibly lowering these risks. Short acting insulin analogue use for diabetic patients is still controversial. Therefore, possible beneficial effects of short acting insulin analogues (SA-I) compared to human insulin (HI) on maternal and perinatal patient-relevant outcomes were evaluated.

Materials and methods: To assess the effects of SA-I in gestational diabetes, a systematic literature search was conducted in 18 databases up to 10/2008 for controlled clinical trials (CCTs) and randomized controlled trials (RCTs). Trial selection and evaluation of study quality were carried out independently by two reviewers. A systematic review was performed.

Results: In 3 RCTs fulfilling our inclusion criteria 134 subjects were included. For 2 studies the intervention was a therapy with Insulin Lispro (IL) and for 1 trial with Insulin Aspart (IA), both in comparison to HI. In terms of the methodological quality all 3 studies were classified as having a high risk of bias. The differences in HbA1c-values between treatment groups were not statistically significant at any time. The incidence of a caesarean section delivery was similar in SA-I and HI. Findings on maternal hypoglycaemia, reported in 2 studies for the intervention with SA-I, were conflicting: While in one study for the HI-group they were higher, they were lower in the other trial. Maternal hyperglycaemic episodes were reported only in one study and indicated a statistically significant difference (IL: 4.0 vs. HI: 5.5, p=0.019). 1 perinatal death occurred in the IA-group and 2 children with malformations were identified in the HI-group of the same trial. In 2 studies no children were classified as being large-for-gestational-age (LGA), in the third the incidence of this outcome was not statistically significant. Data for perinatal hypoglycaemic events was found only in Jovanovic 1999 (IL: 0% vs. HI: 0%).

Conclusion: Based on this data, there is no evidence that SA-I have benefits with regard to maternal and perinatal outcomes compared to HI when it comes to the therapy of GDM. To answer this question and to evaluate safety more evidence on patient-relevant outcomes is urgently needed.

Results of SA-I vs. HI

Maternal outcomes		**Jovanovic** 1999[a] (n=42)	**Mecacci** 2003[a] (n=65)	**Pettitt** 2007[b] (n=27)
HbA1c (1st trimester)	%	5.1 vs. 5.2	-	4.2 vs. 4.8
HbA1c (3rd trimester)		-	5.2 vs. 5.1	5.2 vs. 5.2
Caesarean section delivery	events	7 vs. 6	7 vs. 6	-
Hypoglycaemia		0.9 vs. 2.2 (% of measurements)	-	11 vs. 5 (persons with events)
Severe hypoglycaemia	events	-	-	0 vs. 0
Hyperglycaemia	events	4.0 vs. 5.5* (% of measurements)	-	-
Perinatal outcomes				
Neonatal death	events	-	-	1 vs. 0
Malformations	events	0 vs. 0	-	0 vs. 2
Hypoglycaemia	events	0 vs. 0	-	-
LGA / Macrosomia	%	0 vs. 0[c]	8 vs. 12.5[c]	0 vs. 0[d]

[a] IL vs. HI, [b] IA vs. HI, [c] LGA, [d] Macrosomia (not specified),
- = not available; * = statistically significant difference

Supported by: IQWIG

PS 109 Pregnancy - complications

1188

Pregnancy and type 1 diabetes: St Vincent declaration 20 years later, an alarming observation

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Background and aims: In 1989, the St Vincent's declaration stated that the outcome of diabetic pregnancy should approximate the one of non diabetic pregnancy within 5 years. What is the situation 20 years later ?
Materials and methods: We conducted a retrospective study in a French regional university maternity on the outcomes and management of 117 pregnancies between 2000 and 2007. These 117 pregnancies occurred among 85 diabetic women (maternal age = 29.6 ± 5.4 years, diabetes duration = 14.4 ± 7.0 years, pregestational BMI = 24.1 ± 4.0 kg/m^2, preconceptional HbA1c = 8.1 ± 1.7 %). The data were compared to the results of the 2003 French perinatal survey.
Results: 100 births were reported. There were 19 spontaneous abortions. A preconception care program was implemented in only 42% of the pregnancies. Gestational hypertension or pre-eclampsia occurred in 22.2% of the women (6.1% in the general population). Perinatal mortality (4.3%), severe congenital malformations (3.9%) and prematurity (37%) were above corresponding rates in the general population (respectively 0.7%, 2.2%, 7.2%). Caesarean section rate was increased 2.5-fold in diabetic women (54 vs 20%). A preconception HbA1c level < 7% (26.2% of the pregnancies), was associated with a significant reduction in preterm delivery (p=0.05). This level of blood glucose control depended mostly on the implementation of a preconception care program.
Conclusion: Twenty years after the St Vincent declaration, pregnancies in patients with Type 1 diabetes remains at high risk (perinatal mortality x 6, prematurity x 5, vascular complications x 3.6, caesarean section x 2.5, severe congenital malformations x 2).
The implementation of a preconception care program remains insufficient despite the knowledge of the risks and the availability of means to reduce the obstetrical and neonatal complications. The reasons for this insufficient preconception care remain partially unknown. Counselling women with Type 1 diabetes before conception is essential. They should be provided with a management plan that will help them to live a nice pregnancy.

1189

Prognostic importance of the intensive diabetes mellitus control prior to conception and pregnancy for mother and child

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Background and aims: Therapeutic "near normalization" of metabolism and of clinical status positively influence in a specific way many life functions of the person with diabetes mellitus. It could be assumed, that it involves also the complex factors of the reproduction in women with diabetes mellitus type 1: 1) early in the preconception and conception and 2) later during pregnancy periods. The application of the intensive therapy in the early (1) period has the preventive influence and is connected with planning the pregnancy. Intensive therapy applied during already existing pregnancy comes too late, is less effective in shaping the prognosis for mother and child. In order to examine this thesis the study involving both types of intervention time was executed.
Materials and methods: In Diabetological-Obserical Centre based on close cooperation between both sides - diabetological and obstetrical - of care the cohort at 118 women with diabetes mellitus type 1 was formed. They did not present any of organ complications of diabetes mellitus beside simple retinopathy (class B, C and D). This cohort underwent the routine clinical examination and entered the study program. The cohort was subdivided into 2 subgroups:1) 31 women planning conception and pregnancy - the preventing subgroup and 2) 88 women already pregnant in a non-planned group - the late intervention subgroup. They were similar in typical clinical parameters - age, BMI, absence of additional diseases and therapies, smoking and behavio-

ral parameters. In both subgroups the intense metabolic and clinical therapy program was applied. All women in the planning group become pregnant after - in average - 1 year of the program and continued it during pregnancy according to the study protocol.
Results: Diabetological data - mean values of HbA1c in consecutive trimesters were statistically lower in the group planning the pregnancy than in the non planning group (6,4%, 5,84%, 5,67% vs 7,0%, 6,33%, 6,28%; p<0,05, p<0,05 i p<0,001). There were also significant differences in the general clinical parameters like number of hyperglycemia episodes, blood lipids concentration and in compliance behavior. 8 put of 9 cases of the worsening of retinopathy occurred in the non-planning - late intervention subgroup. Obstetrical data.1) Late intervention (non-planning) subgroup: in 88 women occurred 2 spontaneous abortions, in 86 delivered children 8 had major congenital abnormalities. The early (1 month) death rate of children in this group was 3,4%. This was accompanied by the Hb1c average level of 8.23%. Planning, preventive subgroup: no spontaneous abortions or congenital malformations occurred. The average HbA1c level was 6,92% (p<0,01). Early death at children was not observed.
Conclusion: Planning the fertilization and pregnancy enabling the introduction of intensive metabolic and clinical therapy in average 1 year before conception significantly improves the metabolic and clinical parameters of diabetes control. It is correlated with the decrease of the risk of worsening of retinopathy, spontaneous abortion and congenital malformations. The preconceptional intensive therapy program applied in average 1 year before pregnancy is effective in improving the prognosis for mother and the child.
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1190

Pregnancy-induced increase in IGF-I is associated with progression of diabetic retinopathy
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Background and aims: To evaluate the influence of Insulin-like Growth factor-I (IGF-I) and placental growth hormone on progression of diabetic retinopathy during pregnancy in women with type 1 diabetes.
Materials and methods: Prospective study of 94 consecutively included pregnant women with type 1 diabetes for median 16 years (range 1-36) and median HbA_{1c} 6.7% (range 5.2-10.5) in early pregnancy. At 8, 14, 21, 27 and 33 gestational weeks blood samples were drawn for IGF-I, placental growth hormone and HbA_{1c} analyses and blood pressure was recorded. Fundus photography was performed at 8 and 27 weeks. Diabetic retinopathy was classified in five stages. Progression was defined as at least one stage of deterioration of retinopathy and/or development of macular oedema on at least one eye and/or loss of visual acuity ≥0.2 on Snellen's chart.
Results: Placental growth hormone and IGF-I increased throughout pregnancy and progression of diabetic retinopathy occurred in 24 (26%) women. In a multivariate logistic regression analysis, progression of diabetic retinopathy was associated with a steeper increase in IGF-I levels from 8 to 33 gestational weeks (p=0.02) and higher systolic blood pressure at 8 gestational weeks (p=0.01) independent of placental growth hormone and HbA_{1c} levels. At 8 gestational weeks IGF-I levels were comparable in women with and without progression (p=0.40). However, the steeper increase resulted in 11% higher IGF-I levels (p=0.049) throughout pregnancy in women with progression compared with women without progression while similar levels of placental GH (p=0.70) and HbA_{1c} (p=0.74) were observed.
Conclusion: Pregnancy-induced increase in IGF-I levels is associated with progression of diabetic retinopathy independent of changes in HbA_{1c}.

1191

Prevalence and progression of diabetic retinopathy during pregnancy in women with type 2 diabetes
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Background and aims: To study the prevalence and risk of progression of diabetic retinopathy during pregnancy in women with type 2 diabetes.
Materials and methods: In 80 out of 108 (74%) consecutively referred pregnant women with type 2 diabetes retinopathy status was available. Fundus photography was performed at median 10 (range 6-21) and 28 (27-37) gestational weeks. Diabetic retinopathy was classified in five stages. Progression was defined as at least one stage of deterioration of diabetic retinopathy and/or development of macular oedema on at least one eye between the two examinations. Significant loss of visual acuity was defined as a deterioration of ≥0.2 on Snellen's chart and macular oedema was defined as retinal thickening and/or hard exudates within the macula area of 1500 um in diameter. Clinical parameters were obtained from the medical records.
Results: Diabetic retinopathy was present in 11 (14%) women in early pregnancy, mainly mild retinopathy. Duration of diabetes was 3.7 years (3.3) (mean (SD)), HbA1c 6.6% (1.1), BP 121 (12) / 72 (9) mmHg in early pregnancy and 22 (28%) women were on insulin treatment prior to pregnancy. During pregnancy 74 (93%) were treated with insulin and 11 (14%) with antihypertensive treatment. At 34 weeks mean HbA1c was 5.7% (0.6%) and BP 124 (16) / 75 (9) mmHg. During pregnancy progression of diabetic retinopathy (including new-onset) was seen in 11 (14%) women. Mainly mild progression occurred (table 1), but one woman progressed in both eyes from mild diabetic retinopathy to sight-threatening proliferative retinopathy, clinical significant macular oedema and impaired vision. She had a diabetes duration of five years and became unplanned pregnant with a HbA1c of 13.2%, uncontrolled hypertension and proteinuria. Another woman developed preproliferative changes. Two cases with macular oedema in early pregnancy regressed during pregnancy. Women with progression of diabetic retinopathy during pregnancy were characterized by a longer duration of diabetes (p = 0.03), more prevalent insulin therapy prior to pregnancy (p = 0.004). At 10 weeks a higher HbA1c and a higher diastolic blood pressure in women with progression did not reach statistical significance (p = 0.13 and p = 0.12).
Conclusion: Despite a low risk of progression of retinopathy in pregnant women with type 2 diabetes, development of macular oedema with impaired vision and proliferative retinopathy did occur. Screening for diabetic retinopathy in pregnancy in women with type 2 diabetes should follow the recommendations for type 1 diabetes.

Stage of retinopathy for **every single eye** (n=160) in early and late pregnancy in 80 women with type 2 diabetes.

Eye examination at 28 weeks \ Eye examination early in pregnancy	No diabetic retinopathy	Mild NPDR	Moderate NPDR	Preproliferative NPDR	Proliferative diabetic retinopathy
No diabetic retinopathy	132	4	1	0	0
Mild NPDR	6	4	0	0	0
Moderate NPDR	7	0	3	0	0
Preproliferative NPDR	0	1	0	0	0
Proliferative diabetic retinopathy	0	2	0	0	0

NPDR = nonproliferative diabetic retinopathy.

1192

Preeclampsia; prevalence and risk factors in women with diabetes mellitus
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Background and aims: The rate of preeclampsia(PE) is 4-7% in non-diabetic subjects. The aims of this study are to determine the rate of preeclampsia in diabetic women and the association between preeclampsia and maternal age, parity, duration of diabetes and glycemic control, during the first trimester, in type 1 and type 2 Diabetes Mellitus (DM).
Materials and methods: Analysis of retrospectively collected data of 726 patients was performed. Patients were stratified according to the type of Diabetes they have. Those who are known to have hypertension or nephropathy were excluded from the analysis.
Results: A total number 619 women had type 1 DM while 107 had type 2 DM. Preeclampsia was diagnosed in 11.6%(72/619) and 13.1%(14/107)of women with type 1 and type 2 DM respectively.

PE found to be higher in younger women (P= 02), nulliparous women (P=0.0002) with type 1 DM. Women with type 2 DM were found to be older (P=0.04) and to be multiparous(P= 0.001) compared to those who did not have preeclampsia. Women with type 1 Diabetes Mellitus had higher fructoseamine (P=0.04) and higher insulin requirement (P=0.003) in the first trimester compared to those who did not have preecalmpsia. Women with type 2 DM had higher HbA1c% (P=0.03) than those who did not have preeclampsia. Duration of DM was not found to be associated with preeclampsia.
Conclusion: We concluded that the rate of preeclampsia in women with DM is double that of non diabetic women. Maternal age, parity and glycemic control, in the first trimester, are significant risk factors for the development of preeclampsia in women with DM

1193

Efficacy and safety of continuous subcutaneous insulin infusion therapy in pregnancy complicated by type 1 diabetes

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Background and aims: Excellent glycemic control during pregnancy in women with type 1 diabetes (T1DM) is required to improve maternal and neonatal outcomes. Two regimes of insulin therapy are used to achieve this goal: multiple daily injections (MDI) and continuous subcutaneous insulin infusion (CSII). We report the results of a large, observational study that compared efficacy and safety of CSII and MDI in pregnant women with T1DM.
Materials and methods: From 2002 to 2008, we examined 255 women with T1DM. There were 147 subjects on MDI and 108 patients on CSII. The groups did not differ by age (mean 29.0±4.0 vs. 28.4 years±6.0; p=0.3, respectively) and duration of T1DM (mean 12.4±7.3 vs. 11.4±7.4 years; p=0.3, respectively). In 59.3% of CSII patients, pump therapy was initiated before the conception, while the rest underwent training and received the insulin pumps through the first trimester. We compared HbA1c level achieved by each treatment regimen, insulin dose, weight gain, birth weight, frequency of adverse pregnancy outcomes and number of C-sections. The estimated differences between treatment regimens were adjusted for baseline characteristics, such as age, diabetes duration, BMI and baseline HbA1c.
Results: The CSII did not differ from MDI by HbA1c in the 1st trimester (6.8% vs 6.9%, p=0.7). It similarly improved in both groups during 2nd and 3rd trimester to 5.8% (p=0.9). However, women on CSII gained more weight during pregnancy than those on MDI (14.5 vs. 12.5, p=0.002). There was no difference in duration of gestation and birth weights (38.2 weeks vs. 38.2 weeks, p=0.4; 3.46 kg for CSII vs. 3.47 kg, p=0.8, for CSII and MDI, respectively). Similarly, no difference occurred between CSII and MDI in Small for Gestational Age and Large for Gestational Age frequencies (6.7% vs. 9.0%, p=0.8 and 19.0% vs. 18.8%, p=0.3, respectively). The frequency of stillbirths and fetal malformations was similar in both groups (5.6% vs. 9.3%, p=0.7). The frequencies of C-sections did not differ significantly between the study groups (p=0.3).
Conclusion: In summary, both CSII and MDI can provide excellent glycemic control in pregnant women with T1DM, thus, limiting the risk of adverse pregnancy outcomes. This model, however, seems to predispose to a larger weight gain in mothers.

1194

Relationship between mean blood glucose values and HbA_{1c} in diabetic pregnant women

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Background and aims: Previous studies have confirmed a close relationship between mean blood glucose (MBG) derived from self blood glucose measurements and HbA1c in patients with type 1 and 2 diabetes. Recently it was proposed (ADAG study) that HbA1c can be expressed as estimated average glucose values. The strength of this relationship during pregnancy is unclear due to various physiological changes. The aim of the study was to establish the strength of the association between MBG profiles in diabetic pregnancies and HbA1c at each trimester of pregnancy.
Materials and methods: In 42 pregnant women with type 1 DM a 7-point glycaemic profile (before and 1h after meals, and at bedtime) was performed daily throughout pregnancy with a portable glucose meter. Target BG values were: 70-90mg/dl before meals, 100-130 mg/dl after meals. To achieve this, intensified insulin therapy with frequent adjustments was applied. MBG of each trimester was correlated with HbA1c measured at the end of each trimester (HPLC Menarini-Arkray HA8160). The accuracy of self monitoring of glucose measurements was tested at hospital visits where each patient had simultaneous measurements of blood glucose by her portable meter and by the laboratory method (hexokinase). Their correlation was highly significant (r=0.9444, p<0.001).
Results: Mean HbA1c, MBG and number of self measurements at each trimester are shown in Table 1. The range of HbA1c values was 4.1-7.8%. In the 1st trimester a positive correlation was observed between MBG (y) and HbA1c (x), where: y= 12.57 x + 54.68 (r=0.522, p<0.001). In the 2nd trimester the correlation was y =14.75 x+41.41 (r=0.5834, p<0.001) and in the 3rd it was y=11.85 x+51.51 (r=0.531, p<0.001). These equations were different to the ADAG study. No correlation was found between HbA1c and MBG of pre- or post-meal measurements.
Conclusion: The relationship between MBG and HbA1c in pregnant women with pre-existing diabetes differs significantly from that of non-pregnant diabetic subjects, as observed in the ADAG study. Possible explanations were that pregnancy is not a glucose stable state, also the range of HbA1c observed in pregnancy was narrower than in non-pregnancy, and finally the physiological changes per se that occur in pregnancy.More data are needed to confirm these findings.

Table1. Mean HbA1c, MBG and Self-Measurement

	MBG (mg/dl)	BG Measurements	HbA1c (%)
	x±SD	n	x±SD
1st Trimester	129 (19.3)	343	5.9 (0.8)
2nd Trimester	120 (16.0)	686	5.3 (0.6)
3rd Trimester	114 (14.2)	441	5.3 (0.6)

1195

A real-time continuous glucose monitoring for diabetic women during the delivery

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Background and aims: Optimal maternal glucose control before and during delivery is recommended to avoid neonatal hypoglycaemia in newborns of diabetic mother. This can be prevented by frequent measurements of maternal glycaemia and coordinated glucose/insulin infusion. Continuous Glucose Monitoring (CGM) allows time-to-time glucose assessment and, therefore, it may provide a better opportunity for maternal glycaemic control at delivery. The aim of this study was, then, to evaluate CGM efficacy during the delivery on maternal glucose control and on the prevention of neonatal hypoglicaemia.
Materials and methods: We studied 13 diabetic women treated with intensive insulin therapy (DM1: n 8, DM2: n 2 and GDM: n 3), undergoing delivery with elective C-section (CS). In all women CGM (GUARDIAN®) was applied 24 hours before delivery. On the morning of the CS a dual channel infusion pump started for intravenous infusion of 5% dextrose and insulin (0.5 UI/ml). To achieve the glycaemic target (90-120 mg/dl), infusions were adjusted by predefined algorithms (ADA recommendations 2008) based on the glucose values registered by CGM.
Results: Average delivery time was 36±1 weeks of gestation, six women having preterm delivery. Mean glucose values at 24, 8 and 1 hours before delivery were 119±14, 115±22 and 108±11 mg/dl, respectively. At birth time maternal glycaemia was 103±19 mg/dl and 1 and 2 hours after the delivery increased to 110±29 and 143±35 mg/dl, respectively. All babies were alive with birth-weight of 3203±406 g.(3 newborns were LGA) with Apgar at 5' of 8.8. Neonatal hypoglycaemia (<30 mg/dl 1h after birth time) occurred in 2 cases (both in preterm newborns). The neonatal plasma glucose value at 60' after the birth (37±6 mg/dl) was inversely related to maternal glucose levels at 24, 8 and 1 hours before the delivery and at birth time (all the p<0.01). After multivariate analysis, only the maternal glicaemia at birth time remains independently correlated with the neonatal glycaemia (F test = 20.4%; p<0.01)
Conclusion: This observation, although limited, support the possibility that common use of CGM during the delivery may help to control maternal glycemia and prevent neonatal hypoglicaemia.

PS 110 Intrauterine development and observations in children

1196

Maternal undernutrition during the first half of gestation accelerates the age-related insulin resistance, and promotes hepatic lipid deposition in the adult offspring in a sex-specific manner

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Background and aims: Epidemiological studies have linked low birth weight with the risk of a number of chronic diseases in adulthood. In this context, it has been proposed that intrauterine malnutrition enhances the risk to develop diabetes in later life. Since the accumulation of fat depots in maternal tissues takes place during early pregnancy, we hypothesized that it may play a key role in the availability of nutrients to the foetus with long-term consequences. Thus, the aim of this work was to determine whether an impairment of the mother to build up fat depots during early pregnancy would affect lipid metabolism, and alter the glucose/insulin axis in adult offspring.
Materials and methods: From mating, one group of pregnant rats was fed ad libitum (controls, C) whereas another group was allowed to eat 60% the amount fed by C until day 12 of pregnancy (underfed, U). From this time on, all the animals were fed ad libitum. During lactation, litters were adjusted to 9 pups per dam and they were weaned at 21 days of age. From this time to 19 months age (19 m), body weight and biochemical parameters were determined. At different ages, insulin sensitivity index (ISI) was quantified by means of glucose and insulin values in OGTT. Insulin-stimulated phosphorylation of the insulin receptor was analyzed in adipose tissue of 4 m old pups as index of tissue insulin sensitivity. Hepatic lipid content and the expression and activity of enzymes involved in lipid synthesis were analyzed in 19 m old pups.
Results: At 12 d of pregnancy, lumbar adipose tissue of U mothers weighed less than C. When compared to the C group, newborns of U dams had lower body weight, this effect disappeared at weaning. The ISI values appeared lower in 4 m old males than in females and whereas it was even lower in U males than in C pups, with no difference between U and C in females. At this age, the insulin-stimulated Tyr-phosphorylation of the insulin receptor in adipose tissue was also lower in male U than in C, whereas no difference was found in the females. Values of ISI declined faster with age in males than in females and at 8 m of age, in both males and females, were lower in U than in C pups. The lowest ISI was found in pups from 15 m of age, with no difference between U and C groups of either gender. At 19 m lipid accumulation in liver was higher in the females offspring of the U mother, due to a higher accumulation of TG and NEFA. By the contrary, no change, or even a decreased in Cholesterol and NEFA, was observed in the hepatic lipids in the U males. This gender-specific lipid deposition in the liver seems to be caused by the delay in the age-dependent decrease of ISI in U females vs. males, causing a differential modulation of mRNA/protein expression and activity of AMPK, ACC and other enzymes involved in hepatic lipid metabolism in males and females.
Conclusion: Maternal undernutrition during the first half of pregnancy impairs the accumulation of maternal fat depots and compromises normal foetal growth, which permanently alters insulin/glucose relationships in the offspring, accelerating the age-related deterioration of insulin sensitivity, appearing the effect earlier in males than in females. Furthermore, this nutritional alteration in the mother favours liver steatosis in female but not in male offsprings later in life.
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1197

Intrauterine milieu influences the mRNA expression and the wiring of anorexigenic POMC cells in the arcuate nucleus of offspring from diabetic and/or obese dams in mice

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Background and aims: We tested how altered perinatal metabolic milieu due to maternal hyperglycemia/obesity influences 1) the phenotype of offspring and 2) the wiring and mRNA expression of POMC (pro-opio-melanocortin) cells in the arcuate nucleus.
Materials and methods: We placed 8-week old females on high fat (HF) or low fat normal diet (ND). When body weights (bw) and glucose levels were different ($p < 0.005$) females were set up as breeders. Offspring's bw, POMC-mRNA, glucose and insulin levels were monitored. At day 21, mice were processed for synaptic analysis of POMC. mRNA were collected at birth and 21 days. At weaning progeny were placed on ND and followed for 18 weeks.
Results: At birth the body weights of all groups were similar (no gender differences). After postnatal day 10, both males and females of HF mothers weighed more than those from ND during lactation. After weaning bw difference diminished and remained indistinguishable. After birth POMC-mRNA levels of HF mothers' offspring were lower than ND counterparts. Blood glucose of newborn pups of HF mothers, was significantly lower than that from ND dams, that difference diminished at day 21 however both males and females of HF mothers had significantly higher insulin levels than the ND offspring. The POMC expression did not differ significantly between male groups, but there was a significantly lower POMC mRNA expression in female newborn pups of HF mothers. Synaptic input organization of POMC neurons of 21-day-old mice from HF mothers were different from that of ND-mice: inhibitory synapses shifted towards excitatory contacts corresponding with electrophysiology. At 21 days, male mice from HF diet mothers had a higher POMC mRNA concentration, but the difference is not significant. In female mice, there seems to be no difference between the two groups, however the percentage growth of POMC mRNA from newborn age is bigger in the HF group. At day 144, POMC cells number and volume was smaller in HF mothers' offspring than in ND counterparts.
Conclusion: Synaptic input organization of the POMC cells is altered in offspring from obese, diabetic mothers, suggesting that increased excitatory input on POMC cells might be due to their altered number and function. This might contribute to diet induced obesity later in life. The body-weight changes, however, showed that overfeeding of mothers does not result in obesity of offspring while they are on ND. POMC mRNA expression is decreased in newborn female pups from HF/diabeitc mothers which may indicate a gender specific sensitivity to the intrauterine milieu and/or the different effect of female and male hormones on the development of the melanocortin system under altered intrauterine conditions. The POMC cell number and volume differences found at older age might indicate the long term effect of these early intrauterine conditions.
Supported by: NIH

1198

FTO is expressed in human placenta and is related to birth weight and placental visfatin

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Background and aims: The fat mass and obesity associated gene (FTO) has been found to contribute to obesity risk in adult subjects. The loss of the FTO gene in mice leads to postnatal growth retardation and to a significant reduction in fat and lean mass. Visfatin, a newly described adipokine, is strongly expressed in fetal membranes. In adipose tissue, the expression of visfatin correlates with that of FTO gene. Our aim was to assess whether the FTO

gene is expressed in human placenta and whether it correlates with fetal growth and placental visfatin.
Materials and methods: Human placentas from 83 Caucasian women with uncomplicated pregnancies were obtained at delivery after informed consent. Clinical variables of the newborns were assessed. FTO, visfatin and housekeeping genes (TATA box binding protein and Succinate dehydrogenase complex, subunit A) were quantified in placental tissue by real-time PCR using TaqMan probes. A relative quantification was used using the delta-delta Ct formula.
Results: FTO was highly expressed in human placenta and was independently and directly related to newborn's weight and length (both $p<0.001$) and to the fetal-to-placental weight ratio ($p<0.05$). FTO gene expression was also directly related to visfatin gene expression ($p<0.0001$).
Conclusion: For the first time to our knowledge, we describe that the FTO gene is expressed in the human placenta and is directly associated with measures of fetal growth and to placental visfatin expression. The role of the FTO gene in the regulation of body weight is herein suggested to include prenatal development.
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1199

Maternal fatty acid status during pregnancy and risk of type 1 diabetes in the offspring
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Background and aims: A recent prospective study found that n-3 fatty acids in the diet and in membranes of red blood cells of children predicted a lower risk of islet autoimmunity, and we have previously found an association between use of cod liver oil by the mother during pregnancy or by the child in the first year of life and lower risk of type 1 diabetes (T1D). Cod liver oil is a rich source of the long chain n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the Norwegian population. We aimed to test whether maternal EPA and DHA in the phospholipid fraction of serum samples taken in pregnancy (as marker of EPA and DHA dietary intake) predicted lower risk of T1D in the offspring.
Materials and methods: In a prospective cohort of nearly 30 000 pregnant women who gave birth in Norway during 1992-94, we analyzed serum samples from 106 pregnant mothers whose child developed T1D and 125 mothers whose child did not develop T1D before 15 years of age. The sera were stored at -20°C until analysis in 2008/9. Cases were identified by record linkage to The Norwegian Childhood Diabetes Registry. Controls were randomly chosen. Fatty acid status was expressed as the proportion of EPA and DHA of the total phospholipids in serum, analyzed using solid phase extraction and gas chromatography (GC) with flame ionization detection.
Results: There was no evidence for any association between EPA or DHA in maternal serum and risk of T1D in the offspring. Odds ratio (OR) for upper vs. lower quartile for EPA: OR=0.9 (95% CI 0.4-2.0), test for trend: p=0.6, and for DHA: OR= 0.7 (95% CI 0.3-1.5), test for trend p=0.5. The median level of both EPA (0.2% in cases and 0.2% in controls) and DHA (1.1% in cases and 1.1% in controls) was similar in cases and controls.
Conclusion: There was no association between maternal EPA or DHA status during pregnancy and risk of T1D in the offspring.
Supported by: Oslo Diabetic Research Centre

1200

Insulin resistance and secretion indexes in healthy Italian children and adolescents: a multicentre study
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Background and aims: Type 2 diabetes mellitus (T2DM) is a metabolic disorder of heterogeneous etiology with social, behavioural and environmental risk factors. Studies in populations at risk of T2DM reported that insulin resistance is an early abnormality detectable in the normoglycemic, pre-diabetic state, and that the worsening of insulin resistance leads to fasting hyperglycemia, impaired glucose tolerance and clinical diabetes mellitus. We aimed to establish normal values of insulin resistance, secretion and sensitivity indexes i.e. HOMA-IR, HOMA-β% and QUICKI in healthy Italian children and adolescents, based on fasting samples.

	N	2.5th	5th	10th	25th	Median	75th	90th	95th	97.5th
HOMA-IR										
TS 1	73	0.28	0.42	0.55	0.74	1.28	1.67	2.11	2.20	2.69
TS 2-3	45	0.37	0.38	0.57	0.81	1.20	1.87	3.08	3.61	4.02
TS 4-5	24	0.42	0.73	0.79	1.08	1.71	2.59	3.63	3.64	4.36
HOMA-β%										
TS 1	73	32.8	43.3	48.9	67.8	100.2	131.3	157.0	192.1	683.9
TS 2-3	45	24.6	34.4	41.2	55.0	100.4	163.7	259.4	523.4	548.8
TS 4-5	24	32.8	51.2	55.5	113.7	183.9	265.0	403.4	421.8	487.4
QUICKI										
TS 1	73	0.33	0.34	0.34	0.35	0.37	0.40	0.43	0.45	0.49
TS 2-3	45	0.31	0.32	0.32	0.35	0.37	0.40	0.42	0.46	0.46
TS 4-5	24	0.31	0.32	0.32	0.33	0.35	0.38	0.40	0.40	0.45

Methods: We determined HOMA-IR, HOMA-β% and QUICKI at baseline in 142 healthy subjects from Pediatric Centres, aged 2.7 to 19 years (10.6±3.8, Mean±SD), with different Tanner pubertal stages (TS). None had hypo/hyperglycemia (fasting plasma glucose ranging from 3.6 to 5.6 mmol/l), obesity (BMI 17.9±2.4 kg/m^2 M±SD), or family history for diabetes mellitus. The following formula were used: for HOMA-IR: fasting plasma insulin in mU/l × FPG in mmol/l/22.5; for HOMA-β%: 20 × (fasting insulin in mU/l)/(fasting glucose in mmol/l - 3.5), for QUICKI: 1/($\log_{10}$ fasting plasma insulin in mU/l + $\log_{10}$ glucose in mg/dl).
Results: The HOMA-IR index slightly increases with Tanner stage. As regards HOMA-β% and QUICKI, a weak variation throughout puberty was observed. No significant correlation was observed between HOMA-IR, HOMA-β%, QUICKI and BMI-SDS or chronologic age.
Conclusion: Normal values of HOMA-IR, HOMA-β% and QUICKI are useful tools in the clinical and epidemiological practice for baseline screening and follow-up of subjects at risk for type 2 diabetes mellitus.

1201

Coeliac disease precedes the development of type 1 diabetes in children with both diseases
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Background and aims: The prevalence of celiac disease (CD) is higher in patients with type 1 diabetes (T1D). It is assumed that T1D develops first accompanied by an increased risk of developing CD and other autoimmune diseases. However, we hypothesised that untreated CD may lead to the subsequent development of T1D in some children. The HLA-DQ genotype distribution in T1D has changed over time in Sweden, the risk HLA shared by both diseases has increased with 7% during a ten years period. One of the reasons for the increased incidence of T1D might be the increased consumption of gluten.
Materials and methods: Anti-tTG were measured in serum samples from 169 T1D children collected at T1D onset, 88 siblings of the T1D children, and 96 age and sex matched control children. CD was confirmed with a small intestinal biopsy showing villous atrophy. Antibodies against the islet autoantigens, GAD64 and IA-2, were also measured.
Results: In total, 17/169 T1D children, 6/88 siblings and 3/96 control children had elevated serum levels of anti-tTG. Five children had confirmed CD before T1D onset, and 12 children were diagnosed after T1D onset. Notably, 11/12 children had elevated anti-tTG levels at T1D onset, and the remaining child was positive in a follow up sample six months later. The prevalence of biopsy confirmed CD at T1D onset was 10.1%. None of the siblings had symptoms indicating CD, despite anti-tTG positivity, and so far CD has been confirmed in 4/88 (4.5%) of the siblings. Antibodies against IA-2 were detected in 70% of the T1D children and in 5% of the siblings. Against GAD65 the corresponding figures were 60% and 13.5%. There were no correlation of CD and T1D related antibodies.

Conclusion: The presence of anti-tTG at T1D onset indicates that CD precedes T1D in children with both diseases. The leaking gut syndrome with sub clinical inflammation is associated with T1D onset (Vaarala O 2008) and infections with enteric viruses such as enterovirus has for long been associated with T1D and in children with CD the spread of enteroviruses to other organs, such as the pancreas, would be facilitated with the viral receptor, a protein of the tight junction, made accessible to the virus. Moreover, the lack of correlation between the CD related and the T1D related autoantibodies implies that this is not a general autoimmunity behind these diseases. If this hypothesis is correct and inflammation of the gut precedes T1D, it would be possible to prevent the onset of the disease in children with CD by a population-based screening program.
Supported by: Gillbergska Foundation, Swedish Diabetes Foundation, Swedish Medical Research Council, EU 7th Framework Program

1202

WITHDRAWN

1203

Genetic dependence of urinary enzyme, neutral alpha-glucosidase, in diabetic nephropathy in Uzbek children and adolescents with type 1 diabetes mellitus
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Aim: To assess clinical-diagnostic value of urinary α-glucosidase activity in predicting chronic renal insufficiency in type 1 diabetes mellitus in Uzbek children and adolescents.
Materials and methods: We examined 152 children and adolescents of the Uzbek population, 122 children and adolescents with type 1 diabetes mellitus (51 male and 71 female). By proteinuria the patients were divided into 3 groups: with normoalbuminuria (NAU) (n=52), with microalbuminuria (MAU) (n=50) and marked proteinuria ((MPU) (n=20). 30 children and adolescents of matched age and sex were included into the control group. The urinary neutral α-glucosidase activity was measured by rate of glucose formation from maltose.
Results and discussion: In Uzbek children and adolescents with typ1 diabetes mellitus high incidence of D-allele was observed as diabetic nephropathy progressed. II Genotype was registered in 41.6% of cases on the MAU stage, but no cases were observed on MOU stage to be the evidence for high protection of the ACE gene genotype in chronic renal insufficiency progression and consistent with data of studies conducted in other ethnic populations. For the first time the study on interconnection between activity of urinary neutral α-glucosidase activity and ACE gene genotype was performed. It was established that urinary neutral α-glucosidase activity depended not only on diabetic nephropathy (DN) severity, but on all DN stages in persons with ID and DD genotype it was confidently higher than in those with II genotype. That is the activity of the enzyme was turned out genetically determined by ACE gene.
Conclusion: The findings broaden diagnostic opportunities in assessment of urinary neutral α-glucosidase activity not only in early diagnosing of preclinical DN in children and adolescents with type 1 diabetes mellitus, but also in predicting progression of terminal DN stages in patients with high susceptibility to chronic renal insufficiency. Registration of increase in the urinary neutral α-glucosidase in children and adolescents with type 1 diabetes mellitus on the pre-MAU stage as a marker of early pre-clinical DN stage will allow solving problem of identification of a group with confidently high DN predicting and progression risk as well as preventive therapy of the complication in question.

PS 111 Treatment in nephropathy, hypertension and macrovascular disease

1204

Treatment with lisinopril reduces E-selectin and increases adiponectin in type 1 diabetic patients with diabetic nephropathy
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Background and aims: New potential treatment targets are evolving from knowledge on biomarkers and their involvement in inflammation and endothelial dysfunction. Patients with type 1 diabetes and diabetic nephropathy have a high overall risk, and potentially they could benefit from new renoprotective treatment modalities. Knowledge on how biomarkers of inflammation and endothelial dysfunction are influenced with treatment are scarce, thus we sought to examine if treatment with high doses of the ACE-inhibitor lisinopril influenced biomarkers in these high risk patients.
Materials and methods: A total of 56 type 1 diabetic patients with diabetic nephropathy were included in this double-masked randomized crossover trial consisting of three treatment periods. At inclusion, ongoing antihypertensive treatment was discontinued and replaced with slow-release furosemide in individual but fixed doses. After 2 months wash-out period, patients were treated in random order with lisinopril 20, 40, and 60 mg once daily, each dose for 2 months. The biomarkers C-reactive protein (hsCRP), soluble vascular cell adhesion molecule (s-VCAM-1), soluble intercellular adhesion molecule (s-ICAM-1), von Willebrand Factor (vWF), soluble E-selectin, and HMW adiponectin was measured after the wash-out period (baseline), and after each treatment period.
Results: Forty-nine patients completed the study. Seven patients were excluded due to mild reversible adverse events with no specific dose-relationship. All doses of lisinopril effectively reduced albuminuria and blood pressure. E-selectin was reduced 11.4% with treatment (p for change with treatment <0.001), and adiponectin increased 33.9% with treatment (p=0.008 and p<0.001). There was no correlation between change in urinary albumin excretion and E-selectin or adiponectin during treatment. There were no significant differences in levels of any biomarkers between different doses of lisinopril. Levels of sVCAM-1, sICAM-1, vWF, and hsCRP were not significantly changed with RAS-blockade.
Conclusion: E-selectin, a cell adhesion molecule that binds leukocytes to the endothelial cell during inflammation was reduced with ACE-inhibitor treatment. Finally, HMW adiponectin was significantly increased with RAS-blockade. Adiponectin is thought to have beneficial effects in regards to cardiovascular disease and the elevation may reflect a compensatory mechanism.
Adiponectin was kindly measured by Georg Hess and Dietmar Zdunek, Roche Diagnostics

1205

Renoprotective mechanism of ARB in diabetic nephropathy compared with TZD
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Background and aims: Although two types of agents, angiotensin II receptor blockers (ARB) and thiazolidinediones (TZD), are known to reduce proteinuria in diabetic nephropathy, the precise mechanisms by which these renoprotective effects are exerted remain unclear. To clarify the differences between these two agents, we compared their biochemical and pathological effects on diabetic nephropathy.
Materials and methods: Two animal models of diabetic nephropathy were employed: 16-week old STZ mice, type 1 DM models, were administered STZ at 4-weeks of age, and 16-week old db/db genetically obese mice served as type 2 DM models. At 16 weeks of age, these mice were given pioglitazone (PGZ) or losartan (LOS)-containing chow for 2 weeks (n=4, each).
Results: Vehicle-treated diabetic mice showed early stage diabetic nephropathy: 3, 92 and 362mg albumin/gCr, in non-diabetic, STZ and db/db mice, respectively. In treated mice, albuminuria improved significantly, showing

83% (PGZ) and 62% (LOS) reductions in STZ mice, and 38% (PGZ) and 45% (LOS) reductions in db/db mice. Thus, there were no significant differences in albuminuria reduction between the two agents. On light microscopic analysis, glomeruli were significantly enlarged only in db/db mice, a finding which normalized with PGZ treatment, but not with LOS. On the other hand, scanning electron microscopy (SEM) revealed podocyte projections to be so shortened and thinned that areas in the gaps between the projections were enlarged by 22% in STZ and by 26% in db/db mice, presumably resulting in albumin leakage. Interestingly, these conformational changes of podocytes showed marked improvement in LOS-treated mice, i.e., the gap areas between projections were diminished to the size of those in control mice, while no significant changes were observed in mice given PGZ. The expressions of podocin and nephrin were not significantly changed at either the transcription or the protein level in these murine diabetic models, suggesting that podocyte destruction is not associated with albuminuria at least in the early stage of diabetic nephropathy. The extracellular matrix markers, type IV collagen and TGF-β, were both increased in diabetic mice, and both normalized with PGZ or LOS treatment. On the other hand, inflammation markers, ICAM-1 and MCP-1, were both increased in diabetic mice, and normalized with PGZ, but not LOS, treatment.
Conclusion: Taken together, these observations of renoprotective mechanisms indicate that ARB reverses podocyte conformational damage presumably through hemodynamic change, while TZD reduces mesangial expansion through an anti-inflammatory action.

1206

Optimal antiproteinuric dose of the direct renin inhibitor in type 2 diabetes: a randomized crossover trial

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Background and aims: The optimal renoprotective dose of the direct renin inhibitor aliskiren is not known. The aim of this study was to determine the maximum antiproteinuric dose of aliskiren in type 2 diabetic patients with albuminuria.
Materials and methods: After one month washout, 26 patients with type 2 diabetes, hypertension and albuminuria (>30 mg/day) were randomised to four consecutive, 2-month treatment periods, with aliskiren once daily:150 mg, 300 mg, 600 mg and placebo, in random order. Patients also received furosemide to control sodium retention and blood pressure (BP). The primary endpoint was change in urinary albumin excretion rate (UAER) calculated as the geometric mean of three consecutive 24h collections at the end of each treatment period. Secondary measures included change in 24h BP and glomerular filtration rate (GFR, ^{51}Cr-EDTA plasma clearance). Placebo (baseline) mean UAER was 350 mg/day, mean 24h BP was 137/81 mmHg, GFR was 87 ml/min/1.73 m^2.
Results: Treatment with aliskiren 150, 300 and 600 mg daily reduced UAER significantly by 36%, 48% and 54%, respectively (p<0.001) compared to placebo. The 600 mg dose produced a significantly larger reduction in UAER compared to 150 mg (p=0.025), but not compared to 300 mg, 8% lower during 600 mg (95% CI 29,-19) (p=0.517). 24h systolic BP was reduced by 4.5, 8.0 and 9.2 mm Hg compared to placebo, significant reductions for the 300 mg (p=0.001) and 600 mg (p<0.001) groups. 24h diastolic BP was reduced 3.0, 4.1 and 4.4 mm Hg respectively, all doses significantly different from placebo (p=0.019, p=0.001 and p<0.001). There was no difference between the 300mg and 600 mg doses of aliskiren in either systolic BP (p=0.598) or diastolic BP (p= 0.814) GFR was reduced by 3.0, 5.1 and 6.5 ml/min1.73 m^2, significant reductions compared to placebo for 300 and 600 mg (p=0.007 and p<0.001). All doses were well tolerated. No instances of hyperkalemia were observed. The most frequent side effects were dizziness without hypotension (four patients, one in each treatment group) and fatigue (two patients, one in the 300 mg and one in the 600 mg group).
Conclusion: In this small study in patients with type 2 diabetes, hypertension and albuminuria, the maximum antiproteinuric effect was obtained with aliskiren 300 mg, also the maximal antihypertensive dose approved for clinical use. Increasing the dose to 600 mg gave marginal non-significant additional gain in antiproteinuric effects and BP lowering.
Supported by: Novartis

1207

Protection Against Nephropathy in Diabetes with Atorvastatin (PANDA): trial results

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Background and aims: A sub-group analysis of the Treating to New Targets (TNT) study (N=9656) suggested that in patients with coronary disease high-dose atorvastatin reduces the progression of nephropathy when compared to low dose therapy. There have been no randomised controlled trials (RCT) of high- vs low-dose statin therapy to prevent renal dysfunction in diabetes.
Materials and methods: We performed a double-blind RCT of atorvastatin 80-mg/day vs 10-mg/day in 119 patients with Type 2 diabetes (T2DM) with microalbuminuria or proteinuria (albumin creatinine ratio (ACR) >5 mg/mmol). We excluded those with 24-hour protein excretion >2g or serum creatinine >200 μmol/l. We assessed renal function 3-6 monthly by several methods and compared the global progression of nephropathy between groups at these time points over 2 years using a linear mixed model for longitudinal data adjusting for differences in baseline covariates including renal function. Blood pressure was actively managed (target <130/75 mm Hg) prior to randomisation and during the trial including therapy with angiotensin II receptor blockers and/or angiotensin converting enzyme inhibitors.
Results: At baseline, mean (SD) age was 64 (10) years, 83% were male; HbA_{1c}: 7.7 (1.3) %; BP: 131/73 mm Hg. Baseline estimated glomerular filtration rate (eGFR) by the Modification of Diet in Renal Disease (MDRD) equation was 68 (24) ml/min/1.73 m^2; median (IQR) urinary albumin excretion (AER) was 77 (39-174) μg/min. Over 2-years there were 10 (8%) drop-outs and 7 deaths leaving complete follow-up data in 102 (86%) patients. During follow-up the MDRD eGFR declined by 0.84 vs 0.62 ml/min/1.73 m^2/year in the 10-mg vs the 80-mg group; the mean (95% CI) between-group difference in MDRD eGFR was not significant (2.2 (-1.1 to 5.4) ml/min/1.73 m^2, p=0.20; positive difference suggests benefit from 80-mg). Similarly, we observed no differences in Cockcroft and Gault-estimated creatinine clearance (2.5 (-2.4 to 7.3) ml/min, p=0.32); creatinine clearance from serum creatinine/24 hour urine collections (4.0 (-4.8 to 12.7) ml/min, p=0.38); cystatin C (p=0.69); log urine AER (p=0.93); log ACR (p=0.79) or 24-hour urine protein excretion (p=0.92). After adjusting for baseline levels, patients taking 80-mg atorvastatin had lower total cholesterol (mean difference 0.7 mmol/l, p=0.01); higher HbA_{1c} values (0.3%, p=0.03), and similar BP (p>0.7) compared to those taking 10-mg. We recorded no significant between-group differences in deaths or adverse events.
Conclusion: In T2DM patients with well-controlled BP and early renal disease we found no difference in the rate of renal dysfunction taking 80-mg vs 10-mg atorvastatin. We cannot rule out an effect of <1.6 ml/min/1.73m^2/year on MDRD eGFR, or an effect if BP was less well controlled. The benefits of 80-mg atorvastatin on lipoproteins, which we are currently investigating, are likely to outweigh any deleterious effect on HbA_{1c}.
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1208

Aliskiren/valsartan combination lowers blood pressure effectively irrespective of diabetic status compared to the component monotherapies: a *post-hoc* analysis

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Background and aims: Hypertension frequently coexists with diabetes mellitus, accounting for 75% of the added cardiovascular disease risk in such patients. Patients with diabetes often have difficulty achieving the recommended blood pressure (BP) goal (<130/80 mmHg). The aim of this post-hoc analysis was to assess the antihypertensive efficacy of aliskiren (a direct renin inhibitor)/valsartan (an angiotensin receptor blocker) combination compared to the component monotherapies in patients with and without diabetes.
Materials and methods: In this double-blind study, 1797 patients (193 with diabetes and 1604 without diabetes) with mean sitting diastolic BP [msDBP] 95 to <110 mmHg were randomized to receive once-daily aliskiren/valsartan 150/160 mg, aliskiren 150 mg, valsartan 160 mg, or placebo. After 4 weeks patients were forced-titrated to double the initial doses. At Week 8 endpoint, change from baseline in msDBP, mean sitting systolic BP (msSBP), and the proportion of patients achieving the control rates <140/90 mmHg (<130/80 mmHg if with diabetes) were assessed in the intent-to-treat population.
Results: Baseline characteristics were comparable between the subgroups except patients with diabetes had high rate of BMI ≥30 kg/m^2 (65.3% vs. 46.0%) compared to patients without diabetes. Baseline SPB was slightly higher in patients with diabetes compared to patients without diabetes in all treatment groups, as expected. At the week 8 endpoint the mean reductions from baseline in sitting BP for the aliskiren/valsartan combination were largely similar for each subgroup (Table). Blood pressure reductions and control rates in the aliskiren/valsartan group were greater compared to the component monotherapies or placebo in patients with and without diabetes. Overall rates of adverse events (AEs) were comparable across the treatment groups and were not notably affected by the presence of diabetes (with, 31.6%; without, 36.1%). The rate of AEs in diabetic patients were less for the combination group than placebo (22.7% vs. 42.5% respectively). However rate of AEs with combination were similar to placebo groups in patients without diabetes (36.3% vs. 36.1%, respectively). Headache was the most frequently observed AE. Aliskiren/valsartan combination was associated with less headache versus placebo in patients with (6.8% vs. 10.0%) and without diabetes (4.0% vs. 8.9%).
Conclusion: Combination therapy with aliskiren/valsartan reduced BP regardless of diabetic status, provided additional BP-lowering effects compared to component monotherapies and was well-tolerated.

Table

	Aliskiren/ valsartan	**Aliskiren**	**Valsartan**	**Placebo**
Patients with Diabetes (N)	44	59	49	39
Mean baseline BP	157.8/100.9	157.3/100.4	156.9/99.6	158.1/100.0
Change in msSBP, mmHg [a]	-18.4±13.93*	-13.2±14.10	-12.9±13.06	-2.2± 14.81
Change in msDBP, mmHg[a]	-12.90±9.92*	- 9.8±9.28	-9.9±8.36	-4.00± 9.88
Control rate, % (<130/80 mmHg)[b]	20.5¶	13.6	6.1	2.6
Patients without Diabetes (N)	394	371	404	416
Mean baseline BP	152.1/100.0	153.5/100.2	153.9/100.4	153.9/100.5
Change in msSBP, mmHg[a]	-16.8±14.93*†‡	-13.00± 15.16	-13.1±13.22	-5.00±13.66
Change in msDBP, mmHg[a]	-12.2 ±9.21*†‡	-9.0±8.53	- 9.9±7.96	-4.2±8.33
Control rates, % (<140/90 mmHg)[b]	49.7*‡§	38.3	34.4	16.8

*p<0.0001 vs. placebo; †p<0.0001 vs. aliskiren; ‡p<0.0001 vs. valsartan; §p<0.05 vs. aliskiren; ¶p<0.05 vs. placebo and vs. valsartan
[a] Mean±SD change from baseline to week-8 endpoint; P-values obtained from ANCOVA adjusting for region and baseline. [b]Control rates were analyzed by logistic regression.

Supported by: Novartis Pharmaceuticals Corporation, East Hanover, USA

1209

Renin-angiotensin system blockade improves the inflammation and insulin resistance in patients with hypertension and metabolic syndrome

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Background and aims: Insulin resistance (IR) is highly prevalent in arterial hypertension, and plays a central role in the pathogenesis of the metabolic syndrome. Epidemiological evidence suggests that renin-angiotensin system (RAS) blockade may improve insulin resistance and influence on the release of proinflammatory cytokines. The aims of the present work were to evaluate the effect of olmesartan, an angiotensin receptor blocker (ARB), on insulin sensitivity and inflammatory markers in patients with hypertension and metabolic syndrome (MS)
Materials and methods: We studied 36 hypertensive patients with MS, according to NCEP-ATPIII criteria. After 4 weeks of run-in period, all patients were allocated randomly to one of the three treatment: once-daily olmesartan (40 mg), once-daily olmesartan 80 mg or placebo, and followed for a period of 20 week. If the target blood pressure was not achieved (SBP>140 mmHg or DBP >90 mmHg), diuretics agents were prescribed. We measured the BMI, waist circumference, systolic and diastolic blood pressure, lipid profile, CRP, adiponectin, and microalbuminuria. IR was assessed by Homeostasis Model Assessment (HOMA) index
Results: Mean age was 61±9,8 yrs. BMI 30,9±4,98 Kg/m^2, waist circumference (WC) 105,6± 12,3 cm and 98 ±11 cm in male and female respectively. Baseline mean SBP/DBP: 154,34±8,7/93,6±6,43 mmHg. After 24 weeks of treatment, both OLME-40 and OLME-80 significantly reduced SBP and DBP compared with their baseline values (14±8/9±3 mmHg and 18±9/16±5.3 mmHg for OLME-40 and OLME-80, respectively (p<0.01). Olmesartan treatment resulted in a significant decrease in fasting insulin (17.1±3.73 vs 9,8 ±3,9; P < 0.05), HOMA index (5,8±2,5 vs 3,9± 2,8; P < 0.05). A significant reduction of high-sensitivity C-reactive protein levels, 3.27 ±0.07 *vs* 1.8 ±0.06; p<0.05) and an increase in adiponectin (3.97 ± 0.46 vs 5,14 ± 0.51 mU/ml; p<0.05) . We observed positive correlations between abdominal obesity and insulin levels (0.47; p<0.01) and HOMA index (r=0.56;p<0.01) ; by contrast, inversely correlations were found with PCR and adiponectina (r=-0.48; p<0.01). Both OLME-40 and OLME 80 were well tolerated, without any serious adverse event reported.
Conclusion: Hypertensive patients with MS have a high prevalence of IR, and chronic inflammation. The ARB olmesartan improves IR and inflammation markers in these patients.

1210

The beyond effects of antihypertensive drug on diabetes, using calcium channel blocker and angiotensin II receptor blocker

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Background and aims: It is controversial whether calcium channel blocker (CaB) or Angiotensin II receptor blocker (ARB) is superior as anti-hypertensive drug in a lot of clinical and epidemiological studies. For example, "the VALUE Study" shows ARB will decrease the appearance of disease rate in diabetes, in spite of inferior prevention in myocardial infarctions and strokes. On the contrary, "the JIKEI Heart Study" shows that ARB will decrease strokes and chronic heart failure. Our propose of this study is which drug is suitable as a view of extra-cardiovascular factor such as diabetes, atherosclerosis and endocrine factors.
Materials and methods: We randomized 22 patients to CaB (amlodipine) and ARB (valsartan) treatments, who took first therapy of hypertension. After 6 month, they were switched drug treatments each other. We performed echocardiograph, pulse wave velocity (PWV) test, biochemical examinations containing adiponectin, BNP (brain natriuretic peptide), dehydroepiandrosterone-sulfate (DHEA-S), high -sensitive C reactive protein (H-CRP) before and after treatment for 6 months and 12 months.
Results: There were no differences in blood pressure and heart rate on every 6-month period between CaB and ARB groups, also any differences in Echocardiographic measurements (Ejection Fraction, LV size, LV mass in-

dex, etc.,) and PWV. As for blood samples measurements, adiponectin, BNP, DHEA-S and serum insulin were not different between two groups. But H-CRP was significantly decreased in ARB treatments (594±108 μg/dL to 467±106 μg/dL, p<0.05) and the increment of HbA1c for 6 months in ARB treatments was lower than in CaB treatments (CaB vs ARB : 0.19±0.04 vs 0.10±0.09(mg/dL), p<0.05).
Conclusion: The treatments of ARB (valsartan) for hypertension may have prevented the development of diabetes and atherosclerosis, and it is suggested that ARB has the beyond effects on not only hypertension but also diabetes and atherosclerosis.

1211

Arterial stiffness on ramipril therapy in patients with type 2 diabetes mellitus and arterial hypertension
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Background and aims: Cardiovascular disease is the main cause of death in type 2 diabetes mellitus (T2DM). Increased arterial stiffness may be one important pathway linking diabetes to the increased cardiovascular risk. The aim of the study was to evaluate the arterial stiffness on ramipril therapy in high risk patients with T2DM and arterial hypertension (AH).
Materials and methods: T2DM patients with inadequately controlled blood pressure (BP) on any antihypertensive treatment were included. Inclusion criteria: systolic BP (SBP) >130 and/or diastolic BP (DBP)>80 mmHg. After 2-week wash-out period (if patients used inhibitors of angiotensin-converting enzyme (IACE) or angiotensin II receptor antagonists (ARA)) ramipril 5-10 mg per day was added. Ambulatory blood pressure monitoring (ABPM), urine albumin excretion rate (UAER) and arterial stiffness were assessed before and after 12-week treatment period. Pulse wave analysis was performed by using reactive hyperemia peripheral arterial tonometry technique. Augmentation index (Aix) - the indirect parameter of arterial stiffness was estimated from the difference between the second (P2) and first (P1) systolic peaks and expressed as a % of pulse pressure (PP) ([P2-P1]/PP)x100(%).The study was approved by local Ethical Committee and informed consent was obtained from all the patients.
Results: 30 patients with T2DM and AH were examined: median age 65.2±6.9yr, M/F 5/25, AH duration 10.1±6.9yr, mean BP 148/85 mmHg, mean HbA1c 8.1±1.8% (HbA1c>7%, 6.5-7%, <6.5%: 72%, 21% and 7% patients) with one or more additional cardiovascular risk factors: smoking 10%, microalbuminuria 20%, ischemic heart disease (IHD) 43%, familial history of cardiovascular disease 76.7%. Endothelial dysfunction by reactive hyperemia was found in 93% of patients. We observed significant decrease by ABPM in mean SBP during active period: 147/78 vs 135/74 mmHg, p<0.001, as well as maximal SBP: 183/105 vs.166/99 mmHg, p<0.001 and mean SBP and DBP during passive period: 135/74 vs. 129/65 mmHg, p<0.05. The target BP level <130/80 mmHg was attained in 44% of patients. We have also found statistically reliable differences in parameters of arterial stiffness: Aix (39.9 vs 35.3%, p<0.05) and time of ejection period (T [ED/mc]: 295.8 vs. 268.9, p<0.001).
Conclusion: We conclude that treatment with IACE ramipril in patients with T2DM and hypertension with high cardiovascular risk not only has the antihypertensive effect but can reduce arterial stiffness - the significant factor of endothelial dysfunction and cardiovascular morbidity.

PS 112 Other complications

1212

Long-term complications in newly diagnosed patients with type 2 diabetes by regions of Serbia
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Background and aims: Diabetes and its associated complications are a major health and economic burden, particularly in developing world. Type 2 diabetes is usually recognized years after hyperglycemia develops. This delay in diagnosing the disease results in a high prevalence of chronic complications at the time of actual diagnosis. The aim of this study was to determine the prevalence of complications in newly diagnosed type 2 diabetes patients by regions of Serbia.
Materials and methods: A population-based, cross-sectional study of all newly diagnosed type 2 diabetes patients in year 2007, in 8 districts of Vojvodina and Central Serbia (n=5482) identified through a Serbian Diabetes Registry (SDR) database. Age-standardized prevalence was calculated by using a direct-method to the 2002 Serbian census data. To examine differences in variables between the two groups, Student's t-test was performed for continuous variables, the X^2 test for dichotomous variables.
Results: Over a half of the patients in both study regions (63.3% in Vojvodina and 73.5% in Central Serbia) had at least one of the studied complications by the time of diagnosis type 2 diabetes mellitus. The mean age of the patients was 61.8±11.0 years in Central Serbia and 60.7±10.6 in Vojvodina (p<0.01). Long-term complications were more common among females than males in both study groups (p<0.05). Higher age-standardized prevalence of microvascular complications was observed in Central Serbia (4.8% vs. 2.4%, p<0.01), including retinopathy (6.2% vs. 2.3%, p<0.001), and neuropathy (6.9% vs. 3.1%, p<0.001). Rates of nephropathy were similar. Although there were regional differences in the prevalence of macrovascular complications, the difference was not statistically significant.
Conclusion: The findings in this study indicate that significant proportion of newly diagnosed type 2 diabetes patients in both study regions had multiple complications at diagnosis, and were diagnosed at an older age. High prevalence of complications may be related to prevalence of undiagnosed diabetes in two regions. This underlines the importance to increase public awareness and secure earlier detection of diabetes and chronic complications by appropriate screening methods especially in high-risk groups. Also, prevention programs should focus on disproportionately affected regions (central Serbia) and disproportionately affected genders (females). This will help to prevent or delay the vascular complications and thus reduce the clinical, social and economic burden of the disease.

1213

Oral health status in type 1 and type 2 diabetes patients - results of a national multicentre survey: Teeth in Diabetes (TID) study
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Background and aims: Diabetes is a known risk factor for periodontal and dental diseases. However, little data on oral health in type 1 and type 2 diabetes patients are available. We conducted a nationwide cross-sectional study aiming at assessing oral health status using teeth number as a general index of thereof.
Materials and methods: During first two weeks of March 2009 diabetes patients attending 14 diabetes care centres were invited to fill in a questionnaire answering questions regarding their gender, age, diabetes duration, diabetes type, insulin treatment, smoking status, education level, number of their own teeth, and most recent HbA1c value.

Results: The study group comprised 2514 (1342 [53%] women, mean [±SD]age 56±17 years) diabetes patients: 680 (27%) with type 1 diabetes (T1D) (mean age 38±14 years, diabetes duration 14±11 years, HbA1c 7.95±1.85%, 167 [25%] smokers) and 1834 (73%) with type 2 diabetes (T2D) (mean age 63±11 years, diabetes duration 11±8 years, HbA1c 7.70±1.58%, 1057 [58%] treated with insulin, 291 [16%; p<0.0001 vs T1D patients] smokers). Mean number of teeth in T1D and T2D patients were 23±10 and 12±10, respectively (p<0.0001). T1D patients who were smokers had significantly fewer teeth (20±10 vs 24±10; p<0.0001) and higher HbA1c (8.72±1.85 vs 7.7±1.79%; p<0.0001) than non-smoking individuals. T2D patients who smoked had slightly greater HbA1c (7.91±1.6 vs 7.65±1.56%; p<0.05) than non-smokers, though no difference in teeth number was found. In T1D patients there was a statistically significant correlation between teeth number and HbA1c (r= -0.22; p<0.05), diabetes duration (r= -0.47; p<0.05) and age (r= -0.62; p<0.05) as well as education level i.e. patients who completed primary, secondary or higher education level presented with 14±11, 22±10 and 26±8 teeth, respectively (p<0.0001; after adjustment for the diabetes duration, age and HbA1c). In T2D there was also a significant correlation between teeth number and glucose control (r= -0.11; p<0.05), diabetes duration (r= -0.27; p<0.05) and age (r= -0.42; p<0.05). Patients with T2D who completed primary, secondary or higher education level had 8±9, 13±10 and 17±11 teeth, respectively (p<0.0001, adjusted). Multifactorial analysis showed that the age, diabetes duration, HbA1c, education level, and smoking status, were determinants of oral health as assessed through teeth number.
Conclusion: People with diabetes, even the young ones with T1D, present with significant teeth loss. Poorly controlled subjects with long-standing T1D, low level of education and smoking are at particularly high risk of oral cavity diseases. This group of subjects should be placed under intensive dental care so as to prevent oral health deterioration.

1214

Impaired lung function in type 2 diabetes correlates with insulin resistance and metabolic control. A case-control study

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Background and aims: In a recent case-control study we have demonstrated that type 2 diabetes (T2D) adversely affects breathing during sleep, becoming an independent risk factor for severe nocturnal hypoxemia in obese patients. However, there is little information linking the presence of insulin resistance and the blood glucose control with the severity of pulmonary function. The aim of the study was to evaluate the role of insulin resistance and the degree of glycemic control on the pulmonary function in type 2 diabetic patients.
Materials and methods: In this case-control study 30 type 2 diabetic women (fasting glucose 162.4 ± 62.9 mg/dl; HbA1c 7,7 ± 1,1 %) were compared with 60 non-diabetic women closely matched by age (43.23 ± 8.05 vs. 42.10 ± 8.0, p = 0.529), body mass index (49.18 ± 6.35 vs. 49.15 ± 6.46, p = 0.989), waist circumference (130.83 ± 12.27 vs. 129.83 ± 13.43, p = 0.746), and basal oxygen saturation. The exclusion criteria for this study were type 1 diabetes, chronic diseases others than those accompanying metabolic syndrome, and active treatment with continuous positive airway pressure. Forced spirometry and static pulmonary volume measurements were performed using a MasterLab apparatus, and values were expressed as a percentage of the predicted value. All tests were performed following the European Respiratory Society guidelines. Static pulmonary volumes were measured using the plethysmography method. Insulin resistance was evaluated as HOMA-IR. Statistical analyses: Student *t* test, Pearson linear correlation test, and stepwise multiple regression analysis.
Results: No differences in PaO_2 (82.3 ± 11,4 vs. 83.6 ± 8.1 mmHg, p = 0.574), $PaCO_2$ (39.7 ± 5.2 vs. 38.9 ± 3.9, p = 0.441), forced vital capacity (FVC, 87.3 ± 1.1 vs. 89.9 ± 13.7 %, p = 0.447), forced expiratory volumen in 1st second (FEV1, 91.2 ± 20.2 vs. 97.3 ± 14.3 %, p = 0.114), total lung capacity (TLC, 97.2 ± 14.0 vs. 94.3 ± 11.6 %, p = 0.369) were observed between diabetic and non-diabetic patients. However, in diabetic patients an almost significantly decrease in the FEV1/FVC ratio (81.4 ± 9.15 vs. 85.0 ± 5.0, p = 0.058) and a significantly increase in the percentage of RV (100.1 ± 22.8 vs. 79.7 ± 18.1, p < 0.001) were observed in comparison with non diabetic subjects. When all patients were grouped, in univariate analysis RV was positively correlated with fasting glucose (r = 0.471, p < 0.001), HbA1c (r = 0.585, p = 0.001), and HOMA-IR (r = 0.429, p = 0.001). Stepwise multiple regression analyses showed that type 2 diabetes was independently associated with RV.
Conclusion: The present study is the first to demonstrate a relationship between pulmonary function and the degree of insulin resistance or blood glucose levels. Interventional studies lowering blood glucose levels and increasing insulin sensitivity are needed to demonstrate the eventual reversibility of pulmonary dysfunction.
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1215

Study of lung function in adults with and without impaired glucose tolerance (IGT). Lung could be a target organ for diabetic complication

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Background and aims: Cross-sectional and/or longitudinal studies in the western countries have significantly demonstrated lower lung function(i.e. forced vital capacity (FVC), forced expiratory volume in 1s(FEV1)) in diabetic patients than in the non-diabetic counterparts.
In Japan, however, only a few studies on lung function in diabetic adults have been investigated . We conducted an analysis of the medical checkup data to determine whether adults with IGT have impaired lung function.
Material and methods: This cross-sectional study was carried in central Japan. 570 individuals aged ≥45 yrs (237 males and 333 females) took part in this study. None of these subjects had sustained asthma and chronic obstructive pulmonary disease (COPD). Spirometry was performed in a standard fashion using an electronic spirometer. We then investigated the relationship between the results of pulmonary function tests and glucose tolerance defined by HbA1c and fasting plasma glucose (FPG) levels. Subjects were divided into the normal glucose tolerance (NGT) group (HbA1c<5.5% and FPG<110mg/dl) and the IGT group (HbA1c≥5.5% or FPG≤110mg/dl) . FEV1% and %FVC were calculated by the measured values (i.e. FVC and FEV1). Statisitical analysis was carried out using Students t-tests and linear correlation analysis. Differences in these pulmonary functions (i.e.FVC,FEV1,FEV%) between NGT and IGT subjects were compared utilizing t tests for continuous variables. Furthermore, multivariable analyses were used to assess the comparison of NGT groups with IGT groups in the models adjusting for age, sex, BMI, height, waist circumference and so on. The subgroups of subjects divided into males and females were evaluated in the same way.
Results: We found 116 NGT men, 77 IGT men, 219 NGT women and 61 IGT women in this survey. The subjects with IGT had significantly lower %FVC (103%v.s.109%, P=0.0497) and FEV1.0% (97%v.s.103%, P=0.0081) than those without IGT. We also found that the subjects with IGT had significantly lower %FVC (P=0.0075), FVC (P=0.0439), FEV1.0% (P=0.0004) and FEV1.0 (P=0.0261) than those without IGT in the multivariable models adjusting for age, sex, BMI, height, waist circumference some parameters. While significant negative correlations were found between lung functions (%FVC and FEV1.0%) and two parameters (BMI and visceral fat) in men, a significant correlation was found between %FVC and muscular volume in women (correlate coefficient r=0.306593 , P<0.0001) .
Conclusion: Japanese people with IGT had significantly lower lung function than those with NGT. Consequently, lung could be a target organ for diabetic complication regardless of ethnicity. Additional investigation is required to reveal the processes how lung function is deteriorated as they become glucose intolerant.

1216

Direct effect of change in insulin sensitivity on bone formation

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Background and aims: Correlation between insulin sensitivity (IS) and bone mineral density (BMD), ceasing with progression of glucose intolerance have recently been identified. Decrease in insulin esistance results in increased bone formation, however insulin sensitizer rosiglitazone increases bone fracture risk. To determine if change in IS, or a direct bone effect of rosiglitazone is responsible for increased fracture risk, the effect of a non-glitazone insulin sensitizer BGP15 on bone formation was investigated among insulin resistant volunteers. The hydroxylamine derivative BGP-15 have been shown to elevate the level of heat shock proteins and nitric oxide synthase and significantly improved insulin sensitivity in phase II clinical studies.

Materials and methods: 27 volunteers with elevated HOMA (3,8 ± 2,3) were treated with 400 mg (n=16) or placebo (n=11) for 28 days. Total body glucose utilization (M)- assessed by hyperinsulinemic -euglycemic clamps-,body composition, lumbar and femoral bone mineral density (BMD), serum N-MID osteocalcin, ß-crosslaps, total P1NP, a katepsin-K, osteoprotegerin, sRANKL, leptin, resistin, adiponectin were measured.
Results: M values increased in active group (6.6 ± 2.2 vs 7,9± 2,4, p=0.003; placebo:7,6 ± 3.1 vs. 6,8 3.1, ns), while katepsin K (4.51 ±1.4 vs 3.17 ± 1.1 ng/ml, p=0.0012) and ß-crosslaps (0.41 ± 0.18 vs 0,36 ± 0.1ng/ml,p=0.031) decreased, and P1NP increased (37± 18 vs 40,9 ± 15 ng/ml, p= 0.040).
BMU index (formation markers/resorption markers to characterize the activity of the bone metabolic units: = (OGPxP1NP) x (β-crosslaps x catepsin-K x sRANKL) $^{-1}$) was also increased in active groups (270 ± 195 vs 484 ± 392, p=0.004). The relationship between osteocalcin and M values was highly significant (r = 0.5939, p<0.001). Neither change in adipocytokines levels nor their correlation with the change in M values were observed.
Conclusion: Our data strengthened the role of osteocalcin in the energy homeostasis-bone formation axis, and suggest that improved insulin sensitivity is beneficial for bone formation. The increase in bone fracture risk observed during rosiglitazon treatment should be the direct bone effect of the drug and not related to insulin sensitivity, indicating that development of insulin sensitizer different from glitazone family would be important to save the bones of 2DM patients.

1217

Glucose tolerance, insulin sensitivity, subclinical inflammation and endothelial dysfunction in subjects with chronic pancreatitis

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Background and aims: Chronic pancreatitis (CP) is often associated with glucose intolerance or diabetes. So-called 'pancreatic diabetes' is often difficult to treat due to impaired gastrointestinal absorption and erratic food pattern caused by recurrent abdominal pains. Moreover, cardiovascular risk of subjects with pancreatic diabetes is frequently underestimated, partly because of relative young age and low body weight of these patients. We studied glucose tolerance in patients with established chronic pancreatitis aiming at identifying typical features of glucose (in)tolerance associated with CP. In addition, the aim of the study was to assess endothelial dysfunction and subclinical inflammation as early markers of cardiovascular risk.
Materials and methods: The study group was 27 non-diabetes individuals with recently diagnosed CP (mean age 46.8±10.9 years, BMI 21.5±3.2 kg/m2), 18 age- and body weight-matched healthy subjects served as controls. All subjects underwent oral glucose tolerance test (OGTT) according to WHO protocol with plasma glucose and insulin measurements. HOMA index and fasting plasma adiponectin, TNF-alfa, interleukin-6 (IL-6), interleukin-1beta (IL-1b), E-selectin, thrombomodulin, adhesion molecules ICAM and VCAM, and high-sensitive CRP were assessed.
Results: CP and control subjects plasma glucose values in OGTT were at 0 min 101±21 and 84±12 (p<0.05), 60 min - 166±44 and 93±24 (p<0.001), 120 min - 126±43 and 88±16 (p<0.001) mg/dl, and plasma insulin at 0 min - 3.3±1.3 and 8.1±5.3 (p<0.01), 60 min - 17.5±6.9 and 29.9±18.5 (p<0.01), 120 min - 16.1±9.2 and 25.3±14.4 mIU/l. CP patients presented also with significantly greater insulin sensitivity as measured with HOMA than controls (0.79±0.36 vs 1.98±1.25; p<0.01). Moreover, CP subjects as compared with controls had significantly greater plasma adiponectin (13580±6449 vs 6327±4211 ng/ml; p<0.001), TNF-alfa (45.9±48.1 vs 16.5±13.2 pg/ml; p<0.05), IL-6 (8.2±3.8 vs 3.2±1.0 pg/ml; p<0.01), thrombomodulin (1.8±0.5 vs 1.0±0.4 ng/ml; p<0.01), ICAM (586±371 vs 336±71 ng/ml; p<0.001) and VCAM (1219±505 vs 793±306 ng/ml; p<0.001) concentrations.
Conclusion: In conclusion, chronic pancreatitis is associated with elevated fasting and early post-challenge plasma glucose as well as significant insulin sensitivity. Moreover, despite absence of insulin resistance, CP subjects present with clinical markers of subclinical inflammation and endothelial dysfunction. Therefore, regular assessment of patients with CP towards glucose tolerance and level of cardiovascular risk should be recommended.

1218

Plasma levels of chemerin and adipose triglyceridelipase in newly diagnosed type 2 diabetic patients and type 2 diabetic patients with hypertension

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Background and aims: Chemerin is a chemoattractant protein that serves as a ligand for the G protein-coupled receptor CMKLR1 and showed a strong and independent association with key markers of the metabolic syndrome including obesity, plasma triglycerides and blood pressure. Adipose triglyceridelipase has an important role in TG lipolysis and energy metabolism in cultured adipocytes and weight maintenance in mice and other lower-order organisms.But Its pathophysiologic role in humans remains unknown. In this study, we have investigated whether or not plasma chemerin and adipose triglyceridelipase level were different in patients with type 2 diabetes mellitus, type 2 diabetes mellitus patients with hypertension and control subjects.
Materials and methods: 81 subjects with newly diagnosed T2DM (42 men, 39 women, age 53±12 years, T2DM group), 33 newly diagnosed T2DM patients with hypertension (18 men, 15 women, age 61±8 years, T2DMH group) and 60 normal control subjects(22 men, 38 women, age 51±12 years, NGT group) participated the study. All patients were newly diagnosed and were not treated with oral hypoglycemic agents or diet control. The body composition and metabolic parameters were assesses.Blood samples were drawn after an overnight fast and Plasma insulin, FFA, HbA1c, TG, TC, LDL-C, HDL-C were measured. Plasma chemerin and ATGL levels were determined were evaluated using a commercially available ELISA. The homeostasis model assessment of insulin resistance (HOMAIR) and the homeostasis model assessment of β-cell insulin secretion (HOMAIS) were calculated.
Results: Plasma chemerin levels were found to be markedly increased in type 2 diabetes mellitus patients with hypertension compared with type 2 diabetes mellitus and NGT (P<0.01). Multiple regression analysis showed that waist circumference, Diastolic blood pressure, 2 h plasma insulin after glucose overload and HbA_1c were independent related factors influencing plasma chemerin levels. Plasma adipose triglyceridelipase levels were significantly increased in type 2 diabetes mellitus patients with hypertension compared with type 2 diabetes mellitus (P<0.01), whereas only Diastolic blood pressure, cholesterol and homeostasis model assessment of insulin resistance were independent related factors with plasma adipose triglyceridelipase levels.
Conclusion: The present work indicates the potential link of chemerin and adipose triglyceridelipase with the pathogenesis of insulin resistance and type 2 diabetes mellitus.
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PS 113 Macrovascular complications

1219

Prevalence of asymptomatic coronary artery disease in type 2 diabetic patients with micro/macroalbuminuria

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Background and aims: Type 2 diabetic patients with albuminuria have a poor prognosis, primarily due to cardiovascular disease. Early detection of asymptomatic cardiovascular disease is essential to improve treatment and prognosis, however a valid screening strategy has not been established. The present study examines the prevalence of asymptomatic significant coronary artery disease (CAD) in type 2 diabetic patients with albuminuria and investigates possible screening strategies, including the use of NT-proBNP levels for risk stratification.
Materials and methods: In a cross-sectional study, we identified a consecutive cohort of 200 type 2 diabetic patients with elevated urinary albumin excretion rate (micro- or macroalbuminuria), but normal P-creatinine and without known cardiovascular disease. Patients with P-NT-proBNP levels above the median and/or coronary calcium scores (CCS) > 400 were stratified as high risk patients (n=133), all others as a low risk group (n=67). High risk patients were examined by myocardial perfusion imaging (SPECT) (n=108) and/or CT-angiography (CTA) (n=20) and/or coronary angiography (CAG) (n=81).
Results: Patients were 59 (SD 9) years of age, diabetes duration was 13 (7) years, BMI 33 (6) kg/m2, and 74% were males. Albuminuria was 109 mg/24h (geom. mean; range 3-8318), P-creatinine 76 (18) µmol/l and 62% had retinopathy. Medical treatment included statins (94% of patients), aspirin (90%) and RAAS blockade (98%). HbA_{1c} was 7.9 (1.3) %, total cholesterol 3.9 (0.9) mmol/l, systolic blood pressure 130 (17) mmHg, and diastolic blood pressure 75 (11) mmHg. The median P-NT-proBNP level was 43.2 (range 5.1-575.4) ng/l and the median CCS was 183 (0-4529). In 62/108 (57%) of high risk patients, SPECT showed myocardial perfusion defects and in 25/101 (25%) examined with CTA or CAG had significant CAD. In 76 out of all 200 patients (38%) or in 76/133 (57%) of high risk patients, SPECT, CTA and/or CAG demonstrated myocardial perfusion defects or significant CAD. Revascularization procedures were performed in 9 patients. Among high risk patients, those with P-NT-proBNP >100 ng/l and CCS > 800 (n=14) had more significant CAD (88%) than patients with P-NT-proBNP and CCS below these cut-off values (53%) p=0.013**.**
Conclusion: Despite optimal medical treatment, type 2 diabetic patients with albuminuria have a very high prevalence of asymptomatic significant CAD. P-NT-proBNP and CCS may provide important information for the identification of patients with significant CAD.
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1220

Relation between coronary and peripheral artery disease in type 2 diabetic patients with micro/macroalbuminuria

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Background and aims: Type 2 diabetic patients with micro/macroalbuminuria have a poor prognosis, primarily due to cardiovascular and systemic macrovascular disease. Both coronary (CAD) and peripheral artery disease (PAD) results in end organ damage and therefore have very important clinical implications. It is unclear, whether atherosclerosis in diabetes progress as a universal process throughout the vasculature or show regional differences in its manifestations. This question is important for the determination of future screening and treatment strategies, particularly because both CAD and PAD also occur in asymptomatic patients. The present study examines the relation between coronary calcium score and peripheral artery disease in type 2 diabetic patients with micro/macroalbuminuria.
Materials and methods: In a cross-sectional study, we identified a consecutive cohort of 200 type 2 diabetic patients with micro/macroalbuminuria but normal p-creatinine and without known cardiovascular disease at Steno Diabetes Center. We performed clinical and biochemical evaluation, coronary calcium Agatston score (CCS) and distal systolic blood pressure measurements at toe level (PBP)(worst big toe).
Results: Patients were 59 (SD 9) years of age, diabetes duration was 13 (7) years, BMI 33 (6) kg/m2, and 74% were males. Albuminuria was 109 mg/24h (geom. mean; range 3-8318), P-creatinine 76 (18) µmol/l and 62% had retinopathy. Medical treatment included statins (94% of patients), aspirin (90%) and RAAS blockade (98%). HbA_{1c} was 7.9 (1.3) %, total cholesterol 3.9 (0.9) mmol/l, systolic blood pressure 130 (17) mmHg, and diastolic blood pressure 75 (11) mmHg. Median CCS was 183 (0-4529). Mean PBP was 121 (2) mmHg and toe:arm ratio was 88 (1.7)%. Patients with toe:arm ratio<67% (n=35) had a median CCS of 485 (0-2406), whereas patients with ratio>67% had 112 (0-4529), p=0.007. Patients with CCS >100 (n=109) had a mean toe:arm ratio of 81 (2)% compared to 98 (2)% in patients with CCS < 100, p<0.001. We found a linear correlation between CCS and toe:arm ratio (CCS = -9 x toe:arm ratio + 1276), R=0.29, p<0.001. In a multiple linear regression model, systemic systolic blood pressure and toe:arm ratio where associated with CCS (R=0.41, p<0.001), whereas albuminuria, HbA_{1c} and cholesterol level did not influence level of CCS. In a similar model CCS and cholesterol level were associated with toe:arm ratio (dependent variable).
Conclusion: Coronary calcium score and distal blood pressure are related in type 2 diabetic patients with micro/macroalbuminuria. This may help identify high risk patients with significant CAD and PAD.
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1221

Contribution of body weight, glycaemic control and insulin resistance to platelet clopidogrel responsiveness in patients with non-ST segment elevation acute coronary syndrome

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Background and aims: Poor response to clopidogrel is a risk factor for the recurrence of ischemic complications in patients with non-ST segment elevation acute coronary syndrome (NSTE-ACS) undergoing coronary stenting. However, it is still unknown whether body weight, glycemic control, inflammation or insulin resistance are implicated in this suboptimal response or not. The aim of this study was to determine the clinical and biological factors implicated in clopidogrel platelet response in patients with NSTE-ACS.
Materials and methods: 441 consecutive NSTE-ACS patients (174 diabetics and 267 non-diabetics) undergoing coronary stenting were included in this study. All patients received loading doses of 250 mg aspirin and 600 mg clopidogrel at least 12 h before percutaneous coronary intervention. Post-treatment maximal intensity of ADP-induced platelet aggregation (ADP-Ag), platelet reactivity index of vasodilator-stimulated phosphoprotein (PRI-VASP), fasting plasma glucose and insulin, HbA1c, lipid parameters, adiponectin, and high sensitive CRP were analyzed. For non-diabetic patients, a simplified homeostatic model assessment (HOMA) of insulin resistance was calculated. Clopidogrel non-responses were defined by an ADP-Ag >70% and PRI-VASP >53%.
Results: ADP-Ag and PRI-VASP were strongly associated with body mass index (BMI) (r=0.28, p<0.0001 and r=0.26, p<0.0001, respectively). Clopidogrel non-responders (ADP-Ag> 70% or PRI-VASP>53%), had a significantly higher BMI than responders: 28.2±0.5 vs. 26.5±0.2 kg/m^2, p=0.005 27.8±0.3 vs. 26±0.3 kg/m^2, p=0.0003 respectively. Obese patients also had a significantly lower response to clopidogrel than non-obese patients. High levels of CRP were significantly associated with non-response to clopidogrel (p=0.049 and p=0.003 for ADP-Ag and PRI-VASP, respectively). Clopidogrel non-responders had significantly increased levels of CRP compared to clopidogrel responders: 18.3±2.2 vs. 3.6±0.4 mg/L, p<0.0001 (PRI-VASP). PRI-VASP but not ADP-Ag was correlated with lipid parameters known as cardiovascular risk factors: HDL cholesterol (r=-0.14, p=0.01), triglycerides (r=0.14, p=0.009), and with markers of insulin resistance: fasting plasma in-

sulin (r=0.18, p=0.001) and HOMA (r=0.17, p=0.001). Clopidogrel non-responders (PRI-VASP >53%) had increased HOMA compared to responders (2.9±0.3 vs. 4.4±0.6, p=0.0003). No correlation was found between platelet tests and fasting plasma glucose or HbA1c. Diabetes mellitus was not associated with non-response to clopidogrel although there was a trend towards an association with ADP-Ag (p=0.07). After multivariate analysis, only BMI and CRP remained statistically associated with platelet tests.
Conclusion: BMI and CRP are strong predictive factors for clopidogrel non-response in NSTE-ACS. These findings indicate that clopidogrel loading dose should be weight-adjusted and reevaluated during infections to prevent ischemic events.

1222

High glucose acutely reduces the aspirin effects on platelets by impairing the aspirin-induced activation of the NO/cGMP pathway

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Background and aims: Atherothrombotic vascular disease in type 2 diabetes recognizes as a major cause platelet hyperactivity, justifying the recommendation of antiplatelet therapy with aspirin. In intervention studies, however, the number of diabetic patients who benefit from aspirin is lower than in the general population, suggesting a resistance to aspirin in diabetes. Hyperglycaemia is known to influence platelet function: therefore, a potential role of high glucose on platelet responses to aspirin should be hypothesized. Aim of the study is to evaluate if platelet exposure to high glucose *in vitro* modifies the anti-aggregating effects of aspirin by influencing the arachidonate metabolism and the activation of the anti-aggregating nitric oxide (NO)/cyclic GMP pathway, i.e. the two mechanisms of aspirin action.
Materials and methods: We studied 45 healthy volunteers (25M and 20F; age:22.8±0.4 yrs; body mass index: 22.6±0.4 kg/m^2), non smokers, with normal glucose tolerance and insulin sensitivity (HOMA IR:1.7±0.1). In platelet-rich plasma (PRP) and washed platelets (WP) preincubated with 5 and 25 mmol/l D-glucose, we evaluated the influence of a 30-min exposure to lysine acetylsalicylate (LAS) (1-300 μmol/l) on: i)aggregation induced by sodium arachidonate (NaAA;1 mmol/l) and ADP (20 μmol/l) (Born's method); ii)thromboxane B_2 (TxB_2) synthesis (RIA); iii)NO synthase (NOS) activity (conversion of ^{3}H-arginine to ^{3}H-citrulline) and cGMP levels (RIA). Aggregations were repeated in presence of the NOS inhibitor L-NMMA (100 μmol/l). As osmotic control, mannitol was used. Data are expressed as mean±SEM.
Results: Exposure to 25 mmol/l D-glucose decreased LAS inhibition of platelet responses: in particular, LAS IC-50 (μmol/l) for NaAA-induced aggregation was 31.4±5.2 with 5 mmol/l glucose and 47.8±8.3 with 25 mmol/l glucose (p<0.01) and inhibition exerted by 300 μmol/l LAS on the ADP-induced aggregation was 43.7±3.1% with 5 mmol/l glucose and 25.5±2.9% with 25 mmol/l glucose (p<0.0001). With 300 μmol/l LAS: i)TxB_2 production (ng/ml) in WP was similar in the presence of 5 and 25 mmol/l glucose: 28.0±2.8 vs 30.0±2.5 after NaAA stimulation (n.s). and 17.6±1.4 vs 17.0±1.7 after ADP stimulation (n.s.); ii)the NOS activity increase from 0.13±0.01 to 0.24±0.02 pmol ^{3}H-citrulline/min/mg protein (p<0.0001) in the presence of 5 mmol/l glucose was blunted by 25 mmol/l glucose (0.11±0.009 vs 0.10±0.01 pmol ^{3}H-citrulline/min/mg protein, ns), iii)the intraplatelet cGMP increase from 10.2±1.8 to 25.6±4.0 pmol/10^{9}plts (p<0.003) observed in the presence of 5 mmol/l glucose was blunted by 25 mmol/l glucose (11.8±2.3 vs 13.1±1.9 pmol/10^{9}plts, ns). Pre-incubation with iso-osmolar mannitol did not modify platelet responses to LAS. The role of NO in the anti-aggregating effect of LAS was confirmed by the fact that, in the presence of 5 mmol/l glucose, L-NMMA decreased the percentage of LAS-induced inhibition on the aggregating responses to both NaAA (57.1±5.2 vs 69.4±5.2%; p<0.006) and ADP (66.4±1.7 vs 90.0±4.7; p<0.0001).
Conclusion: This study shows that a short-time platelet exposure to high glucose reduces the aspirin anti-aggregating effect, mainly inhibiting the drug action on the NO/cGMP pathway, suggesting that hyperglycaemia plays a role in aspirin resistance.
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1223

Left ventricular systolic and diastolic function in type 2 diabetic patients with cardiac autonomic neuropathy

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Background and aims: Patients with type 2 DM appear to have an increased incidence of early subclinical left ventricular (LV) dysfunction. To assess the relationship between cardiac autonomic neuropathy and systolic and diastolic function in patients with type 2 diabetic.
Materials and methods: 139 type 2 diabetic patients (48M/91F, age 53.1±4.9 (SD) years) with arterial hypertension (duration of hypertensions 9.7±7.8 (SD) years) were examined, including 99 patients (32M/67F, age 54.0±5.3 years) with the cardiac autonomic neuropathy (group 1) and 40 patients (16M/24F, age 52.6±4.7 years) without autonomic neuropathy (group 2) and control group consisting of 30 with arterial hypertension (duration of hypertensions 10.9±8.5 years) were studied by using Doppler echocardiography. LV ejection fraction (EF) and transmitral flow Doppler parameters (early peak diastolic-Emax and late peak diastolic velocity-Amax global LV isovolumic relaxation time-IVRT) were measured. Five standard cardiovascular reflex tests, proposed by Ewing, were performed in type 2 diabetic groups.
Results: LV ejection fraction (EF) and transmitral flow Doppler parameters (early peak diastolic-Emax and late peak diastolic velocity-Amax global LV isovolumic relaxation time-IVRT) did not differ between group type 2 diabetic patients without autonomic neuropathy and control group. The difference in the early peak diastolic-Emax (0.61±0.12 m/c) and late peak diastolic velocity-Amax (0.65±0.11 m/c) was statistically significant in the patients with type 2 diabetes with cardiac autonomic neuropathy as compared to the patients with type 2 diabetes without autonomic neuropathy (0.66±0,09 m/c vs 0.69±0.09 m/c) (P<0,05) and control group (0.71±0.16 m/c vs 0.69±0.14 m/c) (P<0.05). LV ejection fraction (EF) in patients with cardiac autonomic neuropathy (63.4±7.6%) was significantly lower than in control group (66.9±4.4%) (P<0.05) and did not differ with group without autonomic neuropathy (65.1±3.6%) (P>0.05). A significant positive correlation was found between global LV isovolumic relaxation time-IVRT and HbA1c (P=0.01) in group without autonomic neuropathy. A significant correlation was recorded between the early peak diastolic-Emax and levels insulin (P=0.01) and HOMA-IR (P=0.032) in patients with cardiac autonomic neuropathy.
Conclusion: Early subclinical left ventricular dysfunction was correlated in type 2 diabetic patients without cardiac autonomic neuropathy with hyperglycemia and in patient with cardiac autonomic neuropathy with hyperinsulinemia and insulin resistance.

1224

Differential injurious effects of antihyperlipidaemic agents, statins and fibrates on mitochondrial respiratory function

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Background and aims: Statins and fibrates are known to reduce the risk of vascular events in type 2 diabetic patients, but they are also known to possess the adverse effects on muscle and liver. Statins, a group of antihyperlipidemic agents which arrest the cholesterol biosynthesis by inhibiting HMG-CoA reductase, have recently been demonstrated to decrease in ubiquinone 10 (UQ10) level. This suggests that mitochondrial dysfunction is concerned in the toxicity mechanism for statins. The combination of the respiration-injurious effects with the obstruction of UQ10 biosynthesis, may prominently reinforce their mitochondria toxicity. The effects of statins on mitochondrial morphological structure and respiratory function were therefore examined in the present study using isolated rat liver mitochondria (MT) and submitochondrial particles (SMP).
Materials and methods: MT were isolated from liver homogenates of Wistar albino rats. SMP were prepared from MT. Mitochondrial structural alteration (swelling) was photometrically monitored by following the absorbance decrease at 520nm. Oxygen consumption was measured by using oxygen electrode. Respiratory activity was calculated from oxygraph data according to the method of Chance and Williams.

Results: Cerivastatin, fluvastatin, rosuvastatin and simvastatin were found to elicit cyclosporineA-insensitive swelling in mitochondria, inducing the permeability transition in the inner membranes. These statins were found to strongly detract both NAD- and succinate-linked respirations in MT. The ID50 values were lower than 50 μM. Statins were found not to directly interfere with any enzymes involving in the NADH- and succinate-linked respiratory chains in SMP. Fenofibrate and bezafibrate have been substantiated to inhibit the electron transport at the rotenon-site in NADH-linked respiratory chain. These show different modes between statins and fibrates. Pravastatin showed the weakest MT toxicity among statins tested in the present study.
Conclusion: These results suggest that mitochondria toxicity of statins should be considered when we treat with antihyperlipidemic agents.

1225

Fasting blood glucose levels and short-term beat-to-beat QT variability in subjects with normoglycaemia: is there a relationship?
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Background and aims: Determination of short-term QT-interval variability (QTV) is an intensively investigated new and non-invasive method for assessment of proarrhythmic risk. Several studies have demonstrated that temporal QTV is a more sensitive predictor of ventricular arrhythmias than conventional QT-measurement parameters. Enhanced QTV has been observed in patients with diabetes mellitus. The aim of the present study was to evaluate the possible connections between the QTV, the fasting blood glucose level and the autonomic function in subjects with normal carbohydrate metabolism according to the WHO criteria.
Materials and methods: 32 healthy subjects (age: 38.1±11.6 years, male/female ratio: 16/16, fasting blood glucose: 4.9±0.4 mmol/l [4.2-5.9 mmol/l], $HbA1_c$: 5.6±0.4%, BMI: 25.4±4.5 kg/m^2, blood pressure: 129/74±17.0/8.5 mmHg; mean±SD) were enrolled into the study. ECGs were recorded continuously for 5 min and all leads were acquired by an ECG signal processing system. After analogue-to-digital conversion, the data were stored on hard disk and analyzed off-line using SPEL Advanced Haemosys software (v3.0). The QT intervals were measured in 31 consecutive beats. To characterize the temporal instability of beat-to-beat repolarization, Poincare' plots of the QT intervals were constructed, where each QT value was plotted against its former value. QTV was calculated using the following formula: $QTV=\Sigma|QT_{n+1}-QT_n|/(30x\sqrt{2})$. Autonomic function was assessed by means of five standard cardiovascular reflex tests.
Results: In subjects with normal carbohydrate metabolism, the QTV (3.2±0.7 ms) exhibited positive correlation with the blood glucose ($r = 0.61$, $p < 0.0005$) and the $HbA1_c$ ($r = 0.60$, $p < 0.0005$) levels. There were no significant correlations betweeen the QTV values and the parameters of the autonomic tests.
Conclusion: Our data suggest a close relationship between higher fasting plasma glucose levels within the normal range, and the enhanced short-term QTV in healthy subjects. This increase of temporal QT variability may be considered as an early indicator of the increased instability of cardiac repolarization.
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1226

Insulin resistance is associated with microangiopathy in type 1 diabetic patients treated with intensive insulin therapy from the onset of disease
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Background and aims: Chronic complications of diabetes still remain important clinical problem. Factors influencing development and progression of diabetic micro- and macroangiopathy are being searched. The aim of the study was to evaluate the relationship of indirect parameters of insulin resistance with risk of microangiopathy in patients with type 1 diabets, from the begining treated with intensive functional insulin therapy.
Materials and methods: The study group consisted of 81 patients (30 women, 51 men) in mean age 34±6.4 years with type 1 diabetes, with mean value of HbA1c (from years 1997-2007) 8.2±1.4 %, from the begining treated with intensive functional insulin therapy, who are under continous observation of the Department (for 10±1.5 years). Indirect parameters of insulin resistance were evaluated, such as: waist circumference, waist to hip ratio [WHR], body mass index [BMI], daily insulin requirement, gain of weight from the begining of the disease, lipid profile, estimated glucose disposal rate [eGDR], features of metabolic sydrome according to IDF definition. Moreover, the presence of chronic complications of diabetes (retinopathy, nephropathy, neuropathy) was assessed. Patients were divided into two groups depending on the presence or absence of any diabetic microangiopathy.
Results: In the group with microangiopathy (n=36; 44.4%) in comparison with patients without vascular complications of diabetes we found: higher weight before diabetes (77.3±17.0 vs 67.0±12.5 kg; p=0.008), larger waist circumference (88.9±11.7 vs 83.7±10.2 cm; p=0.036), higher WHR (0.90±0.08 vs 0.86±0.08; p=0.048), higher serum level of triglicerides (1.3±0.8 vs 0.9±0.3 mmol/l; p=0.002) and lower eGDR (7.17±2.4 vs 8.8±1.9 mg/kg/min; p=0.0019). Moreover, in patients with any diabetic complication features of metabolic syndrome according to IDF were stated more often [12 (33.3%) vs 4 (8.9%); p=0.006]. A significant relationship, adjusted for sex, age and duration of diabetes, between eGDR and the presence of diabetic microangiopathy was revealed [OR 0.65 (95%CI 0.49-0.86); p=0.0037].
Conclusion: The results clearly revealed that in patients with type 1 diabetes, from the begining treated with intensive functional insulin therapy, there is an independent influence of increased insulin resistance on the development of diabetic microangiopathy.

1227

Alpha-lipoic acid prevent neointimal hyperplasia via induction of Nur77-mediated apoptosis of vascular smooth muscle cells
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Background and aims: Restenosis, a main obstacle in the success of balloon angioplasty or stenting, is caused by neointimal hyperplasia due to increased proliferation and decreased apoptosis of vascular smooth muscle cells (VSMCs) and restenosis rate is higher particularly in diabetic patients. This study was undertaken to verify the effect of alpha-lipoic acid (ALA), a naturally occurring antioxidant, on VSMC apoptosis and neointimal hyperplasia.
Materials and methods: Pro-apoptotic effect of ALA was examined by FACS, caspase-3 activity and TUNEL assays in VSMCs. Induction and nuclear export of Nur77 by ALA was demonstrated by Immunofluorescence and Western blotting. We confirmed the anti-restenoic effect of ALA in balloon-injured rat carotid arteries.
Results: ALA induced apoptosis of VSMCs, which is associated with intrinsic mitochondrial pathway, as evidenced by increased Bax expression, cytochrome c release and caspase-3 activation. Besides enhancement of Nur77 (NR4A1; NGFI-B/TR3) expression, ALA promoted nuclear export and interaction of Nur77 with Bcl-2, which triggers a Nur77-mediated apoptosis. Down-regulation of Nur77 by siRNA diminished the pro-apoptotic effect of ALA. Furthermore, ALA increased p38 MAPK phosphorylation, and a p38 MAPK selective inhibitor, SB203580, reduced both ALA-induced apoptosis and nuclear export of Nur77 in VSMCs. In balloon-injured rat carotid arteries, ALA increased Nur77 expression and TUNEL-positive apoptotic cells, leading to inhibition of neointima formation.
Conclusion: These results demonstrate that ALA prevents neointimal hyperplasia through induction of VSMC apoptosis mediated by Nur77 induction and p38 MAPK-dependent nuclear export of Nur77. Our results suggest that ALA can be used as a promising drug to prevent the development of vascular restenosis after angioplasty or stenting.
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PS 114 Rodent models for experimental diabetes

1228

Glycaemic variability leads to impaired ischaemia-induced angiogenesis in diabetic mice

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Background and aims: There is a growing epidemiological evidence that glycemic variability is an adjunctive risk factor of diabetic vascular complications. However, neither a cause-effect relation between glucose instability and vascular dysfunction, nor the molecular bases of a such dysfunction are known. Aim of our study was to investigate the role of glycemic variability on vascular complications and to explore the molecular pathways modulated by glycemic "swings".
Materials and methods: We used two mouse models: glycemic variability and chronic hyperglycemia. After induction of diabetes in 60 C57BL/6 mouse with STZ, 30 mice received once daily basal insulin administration - insulin glargine, 0.5 UI, 8 am - plus 2 oral bolus of 33% glucose solution (0.7 ml, 10 am and 2 pm) [glycemic variability group] and 30 mice once daily basal insulin only [chronic hyperglycemia]. During the treatment (30 days), we measured glycemia 4 times daily (10 am, 12 am, 2 pm, 4 pm). 30 days after induction of diabetes, we studied neovascularization in the hindlimb ischemia model in chronic hyperglycemia group, in glycemic variability group and in untreated age-matched controls.
Results: Indices of glycemic variability (MAGE, CONGA, SD) derived from about 3600 per group glucose measurements resulted significantly different (MAGE, p=0.019; SD, p=0.001; CONGA2h, p=0.006), whereas the mean of glycemic values did not (p=0.066). Laser Doppler perfusion imaging was performed before, immediately after, and on days 7, 14, 21 and 28 after hindlimb ischemia. Mean blood flow in untreated mice 28 days after hindlimb surgery reached ≈ 90% of the preischemic flow. Perfusion recovery was significantly attenuated in chronic hyperglycemia group mice compared with untreated mice on postoperative days 7, 14, 21 and 28 (respectively p=0.021, p=0.023, p=0.018 and p=0.032). Interestingly, the recovery was significantly impaired in glycemic variability group mice compared with chronic hyperglycemia group mice on postoperative days 7, 14, 21 and 28 (respectively p=0.023, p=0.026, p=0.008 and p=0.002). In addition, histological analysis revealed that the capillary density in ischemic limb was significantly increased in chronic hyperglycemia group mice whereas no such increase was noted in glycemic variability group mice (p=0.031). Finally, immunostaining and western blot analysis revealed that VEGF pathway on postoperative day 7 was altered in glycemic variability group mice compared with chronic hyperglycemia group mice.
Conclusion: Our data indicate that glycemic variability, regardless of average blood glucose levels, causes a significant impairment of ischemia-induced angiogenesis in diabetes and that this impaired collateral vessels formation is dependent on altered VEGF pathway.

1229

Immunohistochemical investigating advanced glycation end-product and its receptor expression in STZ-induced diabetic rat intestine

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Background and aims: Gastrointestinal (GI) tract sensory motor disorders are common in patients with diabetes mellitus (DM); however, the pathogenesis is not well understood. Advanced glycation end products (AGEs) are produced during the development of DM. It is well known that AGEs relate to the morphological changes and biomechanical remodelling of arteries in DM. Therefore, the abnormal AGEs accumulation and its receptors (RAGEs) expression may also contribute to the diabetic GI dysfunction. Aims of the present study was to compare the AGE amount and RAGE expression in the mucosa, submucosa and muscle layers including ganglia (both myenteric and submucosal plexus) between normal and STZ-induced diabetic rat jejunum, ileum and colon.
Materials and methods: Twelve male Wistar rats weighing about 300 g were used in this study. Diabetes was induced in 6 rats by a single intraperitoneal injecting STZ (50 mg/kg) and survived for 56 days, and other 6 rats were served as normal control. Approval of the protocol was obtained from the Danish Committee for Animal Experimentation. The rats were anaesthetized with Hypnorm and Dormicum. The jejunal, ileal and colon segments were selected for study. The samples were fixed in 10% formalin and embedded in paraffin. Five-um thick sections were cut and AGE (N epsilon-(carboxymethyl)lysine, CML) and RAGE were detected by immunohistochemistry staining . T-test and Anova analysis were applied to statistically analyze the differences between two groups.
Results: The blood glucose concentration increased four- to fivefold in the diabetic rats (P<0.001). DM generated pronounced morphometric and histological changes; the thickness of the intestinal layers was pronounced increased during DM (P<0.01). Comparing with the normal control, the density of AGE staining was increased in the smooth muscle and epithelial cells of villi and crypt and villus of jejunal, ileal and colon segments in the diabetic rats (P<0.05), but it did not differ in the ganglia. The RAGE expression was increased in the epithelial cells of crypt and villus brush border in diabetic jejunum and ileum as well as in the ganglia (both myenteric and submucosal plexus) in all three segments of diabetic rats.
Conclusion: High density of AGE staining was found in smooth muscle, crypt and villus of diabetic intestine, and the expression of RAGE increased in ganglia, crypt and brush border of diabetic jejunum and ileum as well as in ganglia of diabetic colon. Accumulation of AGE in the villus and crypt as well as the increased expression of RAGE in brush border in diabetic jejunum and ileum may contribute to the disorders of digestion and absorption of intestine in DM. Accumulation of AGE in the muscle layers and increased expression of RAGE in the ganglia may contribute to the motor disorders of intestine and colon in DM.
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1230

ACE inhibition abolishes plaque formation in RAGE deficient diabetic apoE knockout mice

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Background and aims: Activation of the Receptor for Advanced Glycation End-products (RAGE) in diabetic vasculature is considered to be a key mediator of atherogenesis. RAGE deletion attenuates the development of atherosclerosis in the diabetic apoE knockout (KO) model of accelerated atherosclerosis. The renin angiotensin system also plays a pivotal role in diabetes associated plaque development. In the current study we have investigated the effect of additional ACE inhibitor therapy (quinapril) in diabetic RAGE/apoE double knockout (DKO) mice.
Materials and methods: ApoE KO and RAGE/apoE DKO mice were rendered diabetic with streptozotozin and followed for 20 weeks, at which time plaque accumulation (%) was assessed by *en face* analysis. Subgroups were treated with quinapril in drinking water (30 mg/kg).
Results: Diabetic RAGE/apoE DKO showed a significant reduction in plaque area (6.9± 1.4%, compared to diabetic apoE KO mice, 10.4 ± 0.8%, p<0.01). Quinapril treatment completely abolished plaque area to levels lower than those observed in non-diabetic apoE KO mice (0.6 ± 0.1, p< 0.01). This anti-atherosclerotic effect of quinapril in diabetic RAGE/apoE DKO mice was observed in all regional areas of the aorta and was independent of glycaemic and lipid parameters. However, quinapril treatment was associated with a significant reduction in blood pressure (84± 4 mm Hg compared to diabetic RAGE/apoE DKO mice (105 ± 6 mm Hg, p< 0.01).
Conclusion: The AGE/RAGE axis and the renin-angiotensin system may contribute to diabetes associated atherosclerosis via different pathways. Thus strategies that inhibit both pathways may exert superior vasculoprotection in diabetes accelerated atherosclerosis.
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1231

Changes of FGF-21 and it's receptors in high-fat diet apoE$^{-/-}$ mice

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Background and aims: Fibroblast growth factor-21(FGF-21) has recently been characterized as a potent metabolic regulator. It exerted glucose-lowering activity in diabetic animal models, and the effects were insulin independent. FGFs bind and activate alternatively spliced forms of four tyrosine kinase FGF receptors (FGFRs 1-4). The expression of FGFs and FGFRs and the ability of specific ligand-receptor pairs to actively signal are important factors regulating FGF activity in a variety of biological processes. In our study, we investigate the effects of high-fat diet induced insulin resistance (IR) on Fibroblast growth factor-21 (FGF-21) and it's receptors levels in ApoE$^{-/-}$ mice.
Materials and methods: Healthy male ApoE$^{-/-}$ mice, eight-week-old, were randomly divided into normal-chow diet fed group (NF group, n=20) and high-fat diet fed group (HF group, n=20) differently for 16 weeks. The insulin sensitivity was evaluated by hyperinsulinemic-euglycemic clamp technique combined with 3- [^{3}H] glucose as a tracer. The mRNA expressions of FGF-21, β-klotho and FGFR1-4 were measured by quantitative RT-PCR. FGF-21 protein levels were determined by western blot.
Results: In HF group, FBG, PIns, FFA, TC, TG, LDL-C and HDL-C were significantly higher than in NF group (all P<0.01). During the steady-state of clamp, PIns was significantly higher in HF group than NF group (P<0.01), and glucose infusion rate (GIR) in HF group was significantly decreased compared with NF group (30.2±3.1*vs.*44.6±3.0 mg·kg^{-1}·min^{-1}, P<0.01). In the end of insulin clamp, glucose disappearance rate (G_{Rd}) was significantly lower in HF group than NF groups (38.2±0.8*vs.*48.0±0.9 mg·kg^{-1}·min^{-1}, P<0.01). The FGF-21 mRNA expressions of hepatic and adipose tissues in HF group were both significantly increased compared with NC group (both P<0.01), and β-klotho levels were also increased (P<0.05). In HF group, FGFR1 mRNA expressions were higher than NF group in hepatic and adipose tissues (both P<0.01), and FGFR3 mRNA were increased too (P<0.01 and P<0.05, respectively). FGFR4 mRNA levels were significantly up-regulated in HF group than NF group in hepatic tissue (P<0.05) but there were no significant differences in liver. Plasma FGF-21 protein levels were elevated in HF group compared with NF group (P<0.01), and FGF-21 protein expression of hepatic and adipose tissues were also increased (both P<0.05).
Conclusion: There is an abnormality of glucose-lipid metabolism and insulin resistance in ApoE$^{-/-}$ mice fed by high-fat diet, and FGF-21-β-klotho-FGFR1 and FGF-21-β-klotho-FGFR3 maybe the principal modes on regulating glucose-lipid metabolism.
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1232

Gene expression profiling of sulfonylureas in endothelial cells and vascular effects in diabetic ApoE null mice

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Background and aims: Sulfonylurea drugs (SU) are widely used and remain a mainstay in the treatment of type 2 diabetes. Recent research suggests that different SU may have different cardiovascular risk profiles. SU stimulate insulin secretion by closing the ATP-sensitive potassium channel (K_{ATP}) subunit SUR1 in beta-cells. K_{ATP} channels in beta cells and muscle share the common pore forming subunit Kir6.2 but vary in their sulfonylurea receptors SUR1 and SUR2. Previous studies have shown that certain SU such as glibenclamide target both SUR1/2 subunits whereas gliclazide is SUR1 specific. To evaluate the potential different vascular effects of SU firstly we assessed the impact of SU exposure on endothelial cell gene expression using microarray analysis. Secondly, we compared the effects of two SU on plaque development in a model of diabetes associated atherosclerosis.
Materials and methods: Primary HUVEC cells were exposed to 1μM of gliclazide and glibenclamide for 24 hours. Control groups received vehicle. Each experiment was performed as three biological replicates. Gene expression profiles were analyzed using GeneChip ST1.0 arrays. For each drug, gene lists were generated using the following criteria: fold change > 2 and $p < 0.05$ (test vs. ctrl) and $p > 0.05$ for the other drug. Pathways were analyzed using Ingenuity Pathway Analysis. Significant biological functions and canonical pathways were identified using Fishers exact test.
Streptozotocin diabetic Apolipoprotein E (ApoE) knockout (KO) mice were untreated or treated with either glibenclamide or gliclazide for 20 weeks. After staining lipids with Sudan IV, plaque areas were quantitated by the *en face* method for arch, thoracic, and abdominal segments of the aorta.
Results: Several networks were identified for both drugs. The major categories of these networks related to molecular transport and cell signalling. Both SU increased the expression of NOS2 and comparison of the analyses for upregulated genes showed an increased significance for gliclazide over glibenclamide of canonical pathways relating to nitric oxide signalling in the cardiovascular system as well as pathways linked to PDE5, the target of sildenafil. Analyses of plaque areas in diabetic ApoE deficient mice treated with glibenclamide showed a significant increase in total plaque area compared to untreated and gliclazide treated mice (control [non-diabetic], n=7, 1.6 ± 0.5%; untreated diabetic, n=8, 9.0 ± 1.5%; diabetic + gliclazide, n=11, 10.8 ± 0.9%; diabetic + glibenclamide, 17.5 ± 2.7%*, $p < 0.02$ vs. other groups). The increase in plaque area seen in glibenclamide treated mice was not restricted to one aortic region. No difference in plaque area was observed between gliclazide and untreated diabetic ApoE KO mice.
Conclusion: This study demonstrates that SU affect gene expression in endothelial cells. The differences in gene expression between drugs were more pronounced than the similarities suggesting a differential profile among SU in endothelial cells. The differences between SU may be relevant to the disparate vascular effects seen *in vivo* in preclinical models of diabetic atherosclerosis with glibenclamide and gliclazide. These observations are in agreement with previous findings suggesting that gliclazide is a general scavenger of radicals and this may relate to differences in SUR1/2 specificities.

1233

Compensated and non-compensated insulin resistance induces differential cardiovascular damage in obese BATIRKO mice

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Background and aims: Endothelial dysfunction is one manifestation of the many changes induced in the arterial wall by the metabolic abnormalities accompanying insulin resistance and diabetes. Among key players of insulin resistance, insulin receptor (IR) gene plays a relevant role. We have previously generated an experimental model of inactivation of IR in brown adipose tissue (BATIRKO) using the expression of recombinase Cre under the uncoupling protein-1 promoter. Mice lacking IRs in this tissue show a decrease in intercaspular brown fat mass and a progressive decrease in glucose tolerance due to an insulin-secretion defect. The aim of this work was to study the differential cardiovascular damage in compensated and non-compensated obese BATIRKO mice showing insulin resistance.
Materials and methods: BATIRKO mice were fed on standard diet (CT group) or high-fat diet (Obese group) for 16 weeks.
Results: Obese BATIRKO mice show a significant increase of WAT/ body weight ratio, hyperlipidemia, fatty liver, glucose intolerance and insulin resistance. However, two groups of compensated insulin resistance (hyperinsulinemic) and non-compensated insulin resistance (normoinsulinemic) were observed in obese BATIRKO mice. Moreover, lipid accumulation in peripheral tissues such as liver, heart, skeletal muscle and aortic wall was much depending on the level of expression of uncoupling protein 2 (UCP-2) or 3 (UCP-3), which in fact turned out in developing secondary insulin resistance. Non-compensated obese BATIRKO mice, but not those compensated, have a reduced acethylcholine-induced relaxation, an increased of ICAM-1, MCP-1 and PAI-1 expression in aorta tissue as compared with controls. In addition, structural alterations of the vessel wall and accumulation of lipids within intima of the aortic arch were also observed in the group of non-compensated insulin resistance as compared with the groups of compensated insulin resistance or control mice.
Conclusion: These data suggest that BATIRKO mice under high-fat diet, remarkably those that non-compensate ongoing insulin resistance increasing insulin secretion, induced an endothelial dysfunction and a greater inflammatory activity and intimal thickening in the aortic arch.
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1234

Lutein and docosahexaenoic acid prevent cortex lipid peroxidation in streptozotocin-induced diabetic rat cerebral cortex

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Background and aims: The mechanisms underlying diabetic encephalopathy, are only partially understood. In this study, we examined whether docosahexaenoic acid (DHA) and lutein could attenuate the oxidative changes of the diabetic cerebral cortex in a rodent model of diabetes.
Materials and methods: Male, Wistar rats were used in the study. Diabetes was induced in animals by a single intraperitoneal injection of STZ (65 mg/kg) in 0.1 M citrate buffer, pH 4.5. Animals were treated with lutein or DHA for a period of twelve weeks. At the end of week 12, the rats were killed by cervical dislocation. One of the hemispheres was dissected and fixed, cryoprotected and cryosectioned. The other hemisphere was dissected; cortex was homogenized in prechilled 0.2 M potassium phosphate buffer, pH 7.0. This homogenate was used to assay GPx activity, GSH, malondialdehyde (MDA), and protein concentrations.
Results: The levels of malondialdehyde (MDA) were significantly increased and glutathione levels (GSH) and glutathione peroxidase activity (GPx) were decreased in diabetic rats. The number of 4- hydroxynonenal (4-HNE) positive cells was increased in the cortex of diabetic rats. Treatment with insulin, lutein or DHA and the combination of each antioxidant with insulin, significantly restored all markers concentrations mentioned above, as well as the increase in 4-HNE inmunofluorescence. We combined 4-HNE immunofluorescence with NeuN (Neuronal Nuclei) staining. The NeuN staining demonstrated extensive overlap with the 4-HNE staining in the cortex from diabetic rats.
Conclusion: Our findings demonstrate a clear participation of induced glucose oxidative stress in the diabetes-induced alterations in cerebral cortex, and we also demonstrate, in vivo, that the cells suffering oxidative stress are neurons. Lowering oxidative stress through the administration of different antioxidants may be beneficial for the central nervous tissue in diabetes.
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1235

Brain signalling properties of long-acting insulin analogue glargine and detemir for memory and spatial learning in mice

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Background and aims: Central insulin action plays a crucial role in spatial learning and memory in central nervous system. Thus, intranasal injection of insulin is known to ameliorate the cognitive function in human. However, the impact of long-acting human insulin analogues glargine and detemir on the brain cognitive function is unknown. Replacement of amino acids and altered binding properties in these insulin analogues might affect the functional properties in brain. The aim of the present study was to investigate the impact of glargine and detemir on the signalling properties for spatial learning and memory in mice.
Materials and methods: Intracerebroventricular (i.c.v.) injection of glargine, detemir, and human insulin was conducted in C57BL/6J, db/+m control and db/db diabetic mice. Insulin-induced phosphorylation of Akt, which is important for the neuronal function and synaptic plasticity in the hippocampus, cerebral cortex and hypothalamus, was examined by Western blotting analysis in fasting mice. The effect of i.c.v. injection of insulin analogues on spatial learning and memory was assessed by Morris water maze test.
Results: We have previously shown that glargine possesses similar signalling properties in the peripheral target tissues compared to human insulin, whereas the biological properties of detemir are different depending on the tissue and the concentration of albumin in the milieu. In the present study, i.c.v. injection of glargine, detemir, and human insulin (10 mU/mouse) induced similar time-course of phosphorylation of Akt at Ser^{473} and Thr^{308} residues peaking at 30 min in hypothalamus of db/+m mice. On the other hand, the Akt phosphorylation was peaked at 3 h after the i.c.v. injection and decreased thereafter in the hippocampus and cerebral cortex of the control mice. Interestingly, the degree of detemir-induced phosphorylation of Akt was smaller, whereas the effect of glargine was similar, compared to human insulin. In the hippocampus and cerebral cortex of db/db mice, the Akt phosphorylation lasts longer and is still remained at 6 h after i.c.v. injection of both insulin analogues. C57BL/6J mice were trained for 4 days to memorize the location of platform in Morris water maze test. Although glargine, detemir, and human insulin i.c.v. injected immediately after finishing the training (24 h before the probe trial) did not affect the spatial learning and memory, the i.c.v. injections just before the probe trial considerably enhanced the ability in mice. In addition, the ability of spatial learning and memory evaluated by probe trial 7 days after i.c.v. injection was differentially altered between insulin and insulin analogues. These alterations are not due to the change of motor activity in mice, because the swimming speed and distance during the probe trial were not changed by i.c.v. injection of these insulins.
Conclusion: These results suggest that glargine is an insulin analogue physiologically more similar to human insulin than detemir with regard to the signalling property in the hippocampus and cerebral cortex of mice. In addition, all three insulins appear to mainly promote the recall process, but not consolidation process, of memory in mice. Glargine and detemir may affect the cognitive function by influencing the memory recall process with different properties in the physiological and pathological state of diabetes.
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1236

Defective pancreas vascularisation and downregulation of soluble epoxyde hydrolase gene expression in various organs in Goto-Kakizaki (GK) foetuses

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Background and aims: We identified islet inflammation associated with microangiopathy in diabetic GK rats (a spontaneous model of T2D). Signs of islet microangiopathy are also present in prediabetic 1-week-old GK neonates (1-w-GK), together with islet oxidative stress (OS). In addition, GK rats, particularly females, show hypercholesterolemia. Both hyperglycemia and hypercholesterolemia trigger OS and have deleterious effects on vessels. Both also increase placental inflammation, which takes place even during normal pregnancy. Moreover, there is evidence for linking dysmetabolic conditions during pregnancy to hypertension, cardiovascular disease and diabetes in the offspring. This probably occurs via altered fœtal programming. Because endothelial signals are essential during islet development, vascular alterations might act during fœtal life to trigger T2D. In E21 GK fœtuses, β-cell mass is decreased by 80%. Our aims were to evaluate blood glucose and lipids, pancreas vascularisation and gene expression in various organs in E21 GK fœtuses, as compared to Wistar controls.
Materials and methods: 1) glycemia and lipid assays were done on sera; 2) expression of 7 genes, selected on the basis of previous studies conducted in 1-w- Wistar and GK islets, were evaluated by quantitative RT-PCR in umbilical cord, placenta, liver and pancreas; 3) pancreatic vascularisation was quantified after immunohistochemistry for nestin, a recognized endothelial marker.
Results: 1) systemic glucose, cholesterol and cholesterol/HDL ratio were significantly higher in GK fœtuses, with no difference in triglycerides, FFA and HDL levels; 2) while in 1-w-GK islets, we found a significant upregulation of genes encoding inflammatory molecules, such as caspase-1 (x9.0), IL-18 (x3.9), IL-15 (x1.4) and CXCL-1 (x3.9), the pancreatic expression of the 4 genes was variable from one E21 GK RNA preparation to another, being however more often upregulated. The same trend toward upregulation of inflammatory genes was observed in the placenta, while it was less marked in liver and umbilical cord; the expression of 2 genes encoding proangiogenic factors, neuropilin-1 and neuropeptide Y, which was significantly downregulated in 1-w-GK islets (x0.6 and x0.1, respectively), was not decreased in most E21 GK organs; nevertheless, gene encoding soluble epoxyde hydrolase (sEH), which inhibits the catabolism of epoxyeicosatrienoic acids (EETs), was found to be downregulated by 70-90 % in E21 GK fœtal organs; sEH, by increasing levels of EETs, would augment their vasodilatator, antiinflammatory, antioxidative and proangiogenic effects; 3) pancreatic vascularisation was decreased by 60 % in E21 GK fœtuses.
Conclusion: E21 GK fœtuses are hyperglycemic and hypercholesterolemic. Their reduced β-cell mass is associated with decreased pancreatic vascular-

sation, which might participate to T2D pathophysiology. The strong under-expression of sEH in various GK fœtal organs suggests a very early defence mechanism in GK rats, probably linked to maternal OS induced by carbohydrate and/or lipid disturbances.
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1237

The motility of jejunum and ileum is changed in STZ-induced diabetic rats *in vitro*
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Background and aims: The symptoms related to gastrointestinal tract dysfunctions, such as diarrhoea and constipation, are common in patients with diabetes mellitus. Both intestinal hypo-motility and hyper-motility were reported in the diabetic patients and animals. Aim of the present study was to compare spontaneous, flow-induced and distension-induced contractions *in vitro* in the jejunal and ileal segments between normal and diabetic rats.
Materials and methods: Twenty-eight male Wistar rats weighing about 300 g were used in the study. Approval of the protocol was obtained from the Danish Committee for Animal Experimentation. The diabetes was induced by a single intraperitoneal injecting STZ (50 mg/kg) in 18 rats and survived for 28 and 56 days (9 rats in each group), and 10 normal rats were served as control. The rats were anaesthetized with Hypnorm and Dormicum. The motility experiments of intestinal segments were carried out in the organ bath containing Krebs solution aerated with a gas mixture of 95% O_2-5% CO_2 at pH 7.4, temperature 37 C. The pressure and outer diameter of intestinal segments were synchronically recorded during spontaneous contraction, flow (0.6 ml/min)-induced contraction with outlet open to 0, 1.5, 3 and 4.5 cmH_2O pressures and ramp distension up to pressure 10 cmH_2O with outlet closed. The frequency and amplitude of pressure and diameter changes of contraction were analyzed. The differences between the groups were statistically analyzed using t-test and Anova analysis.
Results: The blood glucose concentration increased four- to fivefold and the intestinal wall layer thickness pronounced increased in the diabetic rats ($P<0.001$). The changes of pressure and diameter can be measured during the intestinal contraction; however the diameter changes reflect the motility pattern better. For spontaneous, flow-induced and distension-induced contractions, the frequency did not differ between 28 days diabetic rats and normal rats ($P>0.05$); however the frequency was lower in 56 days diabetic rats than that in normal rats ($P<0.05$). The amplitude was higher in the intestinal segments of diabetic rats both for 28 and 56 days than that in the normal rats ($P<0.05$). During ramp distension, the threshold of pressure to induce phasic contraction did not differ between 28 days diabetic rats and normal rats ($P>0.05$); however the threshold of pressure to induce phasic contraction was lower in 56 days diabetic rats than that in normal rats ($P<0.05$). The diameter changes to induce phasic contraction was lower in diabetic rats both for 28 and 56 days than that in normal rats ($P<0.05$ and 0.01).
Conclusion: The motility patterns of jejunum and ileum differed between STZ-induced diabetic rats and normal controls. The diabetic intestine seems more sensitive to the flow and distension. These may contribute to the intestinal dysfunction, such as diarrhea seen in diabetics. Future studies should investigate the mechanism of intestinal motor disorder in diabetes, such as investigating advanced non-enzymatic glycation end products accumulation and its receptors expression at diabetic intestinal tissues and the enteric nerve function combined with the intestinal motility in the diabetic animal models.
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PS 115 New therapeutic options

1238

When it comes to platelet, hypoglycaemic drugs are not all the same. Antiplatelet treatment and pharmacological treatment for type 2 diabetes
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Background and aims: The use of antiplatet therapy for secondary prevention in patients who have experienced a cardiovascular event is well established. Diabetes has been found to be associated with ASA resistance. Since ASA resistance is associated with a higher risk of cardiovascular events, this phenomenon could be responsible for the reduced efficacy of antiplatelet therapy in diabetes suggested by some studies. Aim of this study is the identification of clinical and laboratory predictors of resistance to ASA among type 2 diabetic patients in primary prevention.
Materials and methods: A consecutive series of 80 patients were enrolled, provided that they satisfied the following criteria: 1)Established diagnosis of type 2 diabetes; 2)Current treatment with at least 100 mg ASA in the last three months; 3)Absence of other antiaggregant (ticlopidine, clopidogrel) or anticoagulant therapy; 4)Informed consent. All patients were assessed for post-treatment residual platelet reactivity (RPR) in platelet-rich plasma by 10 microM adenosine 5'-diphosphate (ADP), 1 mM arachidonic acid (AA) and 2 microg/ml collagen-induced platelet aggregation and in whole blood by the PFA-100 system. Patients were considered ASA resistant if AA levels were> 20% and ADP> 70%.
Results: Patients with ASA resistance showed a higher fasting plasma glucose, Erythrocyte Sedimentation Rate, and von Willeband factor, and a higher prevalence of renal insufficiency; furthermore, patients resistant to ASA showed a lower prevalence of treatment with statins and metformin, and a higher prevalence of insulin treatment. The association between metformin treatment and ASA sensitivity retained statistical significance at multivariate analysis, after adjusting for age, sex, and chronic renal insufficiency, whereas insulin therapy was associated with ASA resistance after adjusting for the same confounders .
Conclusion: This is the first report suggesting that hypoglycemic treatment could modulate sensitivity to antiaggregant therapy in type 2 diabetic patients.

1239

Effect of perindopril therapy on expression of genes involved in pathogenesis of chronic complications in patients with type 1 diabetes mellitus
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Background and aims: Study has focused on perindopril therapy effect on genes which products are involved in pathogenesis of chronic complications in patients with diabetes mellitus.
Materials and methods: 25 patients (14 males/11 females) with type 1 diabetes mellitus were enrolled in this study, mean age was 46±11 years, mean duration of disease was 14±6 years. Perindopril therapy started newly or after wash out period in dose 4 mg per day. Gene expression study has been carried out in time 0, 3 and 6 months after initiation. RNA has been extracted from monocytes of blood using automatic isolator (MagNA Pure Compact). Microfluid cards TLDA (TaqMan Low Density Array) and 7900HT Fast Real-Time PCR System have been used to parallel determination of gene expression in 90 studied genes.
Results: Modifications in expression level of many genes has been observed after 6 months of the study. Products of affected genes may play role in pathogenesis of both macroangiopathy [genes for endothelin 1 (ET1), fibronectin 1 (FN1), interleukin 4 (IL4), interleukin 10 (IL10), macrophage scavenger receptor 1 (MSR1), inducible NO synthase (NOS2), oxidized LDL receptor 1 (OLR1)] and microangiopathy [genes for caldesmon (CALD1), fibroblast growth factor 1 (FGF1), elongation factor 1 (EGF1), engulfment and

cell motility 1 factor (ELMO1), MAP kinase 2 (MKNK2), metaloproteinase 1 and 9 (MMP1, MMP9), inducibile cyklooxygenase 2 (PTGS2), AGE receptor (RAGE), transforming growth factor 1 (TGFB1)] and also in insulin resistence pathogenesis [membrane glycoprotein called plasma cell antigen 1 (ENPP1), estrogen receptor 1 (ESR1), PPAR γ (PPARG)]. Changes expressed as multiples of gene expression level together with significance level *p* are presented in brackets below. Gene expression increased in ELMO1 (1,5 times (T), *p<0,05*), ESR1(1,8 T, *p<0,01*), MSR1 (1,9 T, *p<0,01*), OLR1 (2,6 T, *p<0,05*) and more significantly in NOS2 (17 T, *p<0,01*), whereas decreased in CALD1 (-1,4 T, *p<0,05*), FGF1 (-1,6 T, *p<0,05*), EDN1 (-1,2 T, *p<0,05*), EGF (-2,1 T, *p<0,05*), ENPP1 (-1,9 T, *p<0,05*), FGF1 (-2,1 T, *p<0,05*), FN1 (-1,2 T, *p<0,05*), IL4 (-3 T, *p<0,05*), IL10 (-3,5 T, *p<0,01*), MKNK2 (-1,8 T, *p<0,05*), MMP1 (-2,1 T, *p<0,05*), MMP9 (-2,4 T, *p<0,05*), PTGS2 (-2 T, *p<0,05*), RAGE (-5 T, *p<0,05*), TGFB1 (-1,5 T, *p<0,05*) and more significantly in PPARG (-21 T, *p<0,01*). Surprisingly we didn´t find any modifications in expression level of genes for cytoadhesive molecules (ICAM-1, VCAM, PECAM-1, selectins), VEGF, PAI-1, genes for antioxidant enzymes.

Conclusion: The results of this study show that renin-angiotensin system blockade may positively affect various processes in the pathogenesis of chronic complications in Type 1 diabetes.

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1240

Tetramethylpyrazine protects oxidative damage and mitochondrial dysfunction in C2C12 muscle cells

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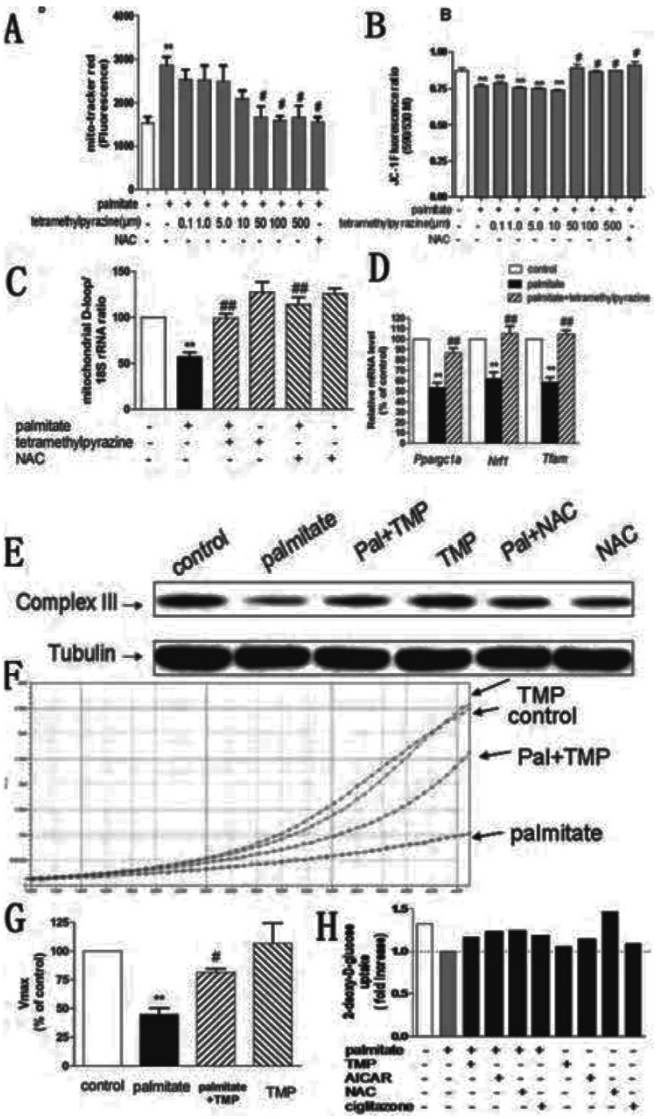
Background and aims: We previously reported "Qing Huo Yi Hao" (QHYH) a prescribed traditional Chinese medicine preparation could decrease urinary microalbumin excretion in type 2 diabetic patients and have an antioxidant effects on oxidative stress induced by high glucose in endothelial cells. TMP (tetramethylpyrazine) is one of the active components of QHYH, we had demonstrated it displayed comparable antioxidant and endothelial protective effects as QHYH by reverse downregulation of Akt and eNOS phosphorylation and reduction of NO generation in high glucose treated bEnd.3 cells. One of the metabolic features of diabetes or insulin resistance in skeletal muscle is an impairment of glucose utilization and insulin sensitivity that has been related to the oxidative stress induced induced by elevated plasma free fatty acids (FFA). The present study was designed to investigate whether TMP (tetramethylpyrazine) also improves free fatty acid induced muscle cell damage by reducing oxidative stress through the pathways that increase of mitochondrial biogenesis and mitochondrial fatty acid oxidation activity.

Materials and methods: Mouse C2C12 myoblasts was cultured and differentiated to C2C12 muscle cells and oxidative damage was induced by palmitate(0.2mmol/l) for 72h. Mitochondrial sources of reactive oxygen species(ROS) was measured by the MitoTracker Red method.The mitochondrial membrane potential was assessed by using the JC-1 dyes methods. mitochondrial DNA was quantified by real time PCR. The BD oxygen Biosensor system was used to detect the oxygen consumption as an indication of mitochondrial respiration activity. We also use western blot to detect the expression of mitochondrial respiratory chain complex III as an index of mitochondrial function. Uptake of 2-deoxyglucose by C2C12 myotubes was measured using glucose 3H labeled method.

Results: TMP level ranging from 50-500uM could significantly reduce palmitate induced ROS production from mitochondrial(Fig1A), and also significantly improve the mitochondrial membrane potential(Fig1B).The mitochondrial D-loop mRNA expression which reflecte the mitochondrial DNA synthesis was also significantly increased by TMP comparing with control(Fig1C).The downregulation of some key signal molecules such as PGC1α,NRF1 and Tfam that regulate the mitochondrial function and ROS production were also significantly reversed by TMP in palmitated induced state(Fig1D). The expression of mitochondrial respiratory chain complex III was upregulated by TMP compared with the palmitate induced state(Fig1E). For cell respiration the TMP could significantly reverse the reduction of oxygen consumption induced with palmitate(fig1F-G).For glucose uptake there seemed a trends that TMP might increase the uptake of glucose(fig1H).

Conclusion: Tetramethylpyrazine protects palmitate-induced oxidative damage and improves mitochondrial dysfunction in C2C12 muscle cells.



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1241

Sodium tungstate inhibits early atherothrombotic pathways in human endothelial cells

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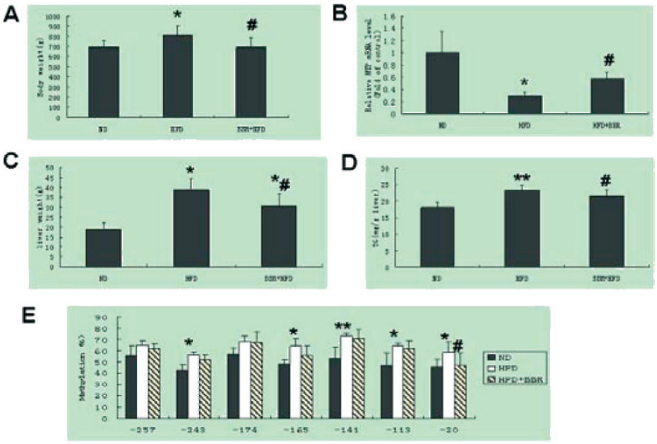
Background and aims: Visceral abdominal obesity is a major cardiovascular risk factor. Inflammatory endothelial injury initiates and sustains atherothrombosis. This study aims to investigate the therapeutic role of sodium tungstate in the activation of early atherothrombotic inflammatory mechanisms.

Materials and methods: We obtained omental adipose tissue from morbidly obese males, without other cardiometabolic risk factors (BMI: 40-45 kg/m2, n=6) undergoing bariatric surgery. Fat pads were separated in their stromal and adipocyte cell fractions. Resulting cell types and fat pads were incubated with nonfetal medium for 24h in order to obtain the secretome. Moreover half of this samples were also coincubated with sodium tungstate (100 um). 3 different conditioned media (CM) containing the secretome of the visceral fat pad or the visceral stromal or the adipocyte cell fraction were obtained. Studies were performed on human endothelial cells (EC) monolayer. EC were incubated for 24h with 100% of each CM. Expression of adhesion molecules in EC were determined by immunogold labelling and quantitative real time PCR.

Results: Recent results from sodium tungstate treated obese patients (data not shown) indicate that sodium tungstate presents antiinflammatory and endothelial protective properties (as determined by CRP decrease and no changes in endotelial circulant cells). Exposure of EC to the CM secreted by the visceral fat pad, adipocyte and stromal cellular fraction induces a significant expression of adhesion molecules (VCAM-1, ICAM-1). EC exposed to the secretome (CM) of sodium tungstate treated samples presents a significant (OR: 2,68; p< 0,05) reduction in the expression of the named adhesion molecules at protein and at gene level (VCAM-1 and ICAM-1: decrease of 32 and 27% respectively for fat pads; 20 and 27% for stroma; 18 and 30 % for adipocytes). No effect was observed in EC treated directly with sodium tungstate and in nontreated cells.
Conclusion: These results indicate that sodium tungstate inhibits in human endothelial cells the activation of early atherothrombotic inflammatory pathways through changes in the secretion pattern of inflammatory cytokines from the adipose obese tissue. This opens for sodium tungstate, previously studied for his antiobesity actions new therapeutical perspectives in the complementary prevention of cardiometabolic risk.
Supported by: grants from M.E.C., M.S.C. (SAF2006-07382);CIBERDEM (CB07/08/0009); Instituto de Salud Carlos III



Supported by: STCSM

1242

BBR reduces hepatic fat content in the rats of NAFLD by decreasing the methylation of MTP promoter

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Background and aims: Berberine (BBR) can reduce body weight, lower serum triglyceride(TG) and cholesterol, and improve insulin sentivity in db/db mice, high-fat-fed hamsters and patients with type 2 diabetes. There is an undisputed link between high-fat diet and the development of NAFLD, and downregulation of hepatic microsomal triglyceride transfer protein (MTP) activity is associated with NAFLD. But the mechanisms of the high-fat diet(environmental factor) regulation of MTP expression in liver still remains unclear. The interaction between genes and environment could involve epigenetic factors such as DNA methylation, and MTP promoter contains rich-GpC, which is susceptible to DNA methylation. Here we investigated whether hepatic MTP transcription is effected by the methylation of hepatic MTP promoter in NAFLD rats induced by high-fat diet and whether BBR reduces hepatic fat content by decreasing the methylation of MTP promoter.
Materials and methods: Twenty-four male Sprague -Dawley(SD) rats were divided at random into two groups. The rats in the control group(normal diet ND n=8) and in the NAFLD model (high-fat diet HFD n=16) were fed ad libitum with normal chow and high-fat diet(18% lard, 10% egg yolk powder, 2% cholesterol and 0.2% Sodium Cholate)respectively. After 8 weeks, Rats in the HFD group were divided randomly into two groups, one of which were treated with berberine (BBR) orally at 200mg/(kg d)(n=8)and the other with placebo(n=8), for sixteen weeks. On the last day of the experiment, all of the rats were euthanized after overnight fast. Hepatic TG content and mRNA level of hepatic MTP were measured in three groups. The CpG methylation status was determined by bisulphite direct sequencing of the PCR amplificates of MTP promoter.
Results: The body weights of rats with high-fat diet were greater than those with normal diet(p<0.05, Fig1A). The livers of the SD rats fed the high-fat diet showed pronounced hepatic steatosis. While MTP mRNA was reduced by 70%(p<0.002,Fig1B) in livers from rats in the HFD group, high-fat feeding caused a 2-fold increase in hepatic weight(p<0.05,Fig1C) and 1.3-fold increase in liver TG content(p<0.001,Fig1D). Treatment of high-fat feeding rats with BBR for 16 weeks reduced liver weight by 21%(p<0.05,Fig1C) and hepatic TG by 14% (p<0.01, Fig1D), with a 1.9-fold increase in hepatic MTP mRNA(p<0.05, Fig1B). DNA methylation level of MTP promoter (five out of seven CpG islands) of high-fat diet rats was increased in liver compared with normal diet group (p<0.05, Fig1E), while treated with BBR, the methylation level was decreased in almost all CpG sites of MTP promoter, especially in -20 CpG site, by up to 12%(p<0.05) compared with placebo in high-fat diet rats (Fig1E).
Conclusion: DNA methylation of MTP promoter are likely to be involved in the pathogenesis of high-fat diet induced NAFLD and BBR reduces hepatic fat content partially by decreasing the methylation of MTP promoter.

1243

Effects of prescription omega-3-acid ethyl ester vs olive oil on macro- and microvascular function in subjects with type 2 diabetes mellitus

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Background: Recent evidence supports protective effects of omega-3-acid ethyl esters on endothelial function in populations at risk. We investigated the effect of omega-3-acid ethyl ester (eicosapentaenoic acid - EPA- and docosahexaenoic acid - DHA) on postprandial vascular function in subjects with type 2 diabetes mellitus (T2DM).
Methods: We conducted a double-blind, placebo-controlled, randomized, cross-over study in 34 subjects with T2DM who received 2g/d purified EPA and DHA, or olive oil (placebo) capsules as add-on to their usual therapy and diet for 6 weeks. Macrovascular function was assessed by ultrasound measurements of flow mediated dilatation (FMD) of the brachial artery. Microvascular function was assessed using a Laser - Doppler method. We estimated the blood flow as area under the curve (AUC) following reactive hyperemia. At the beginning and the end of each 6 weeks period, investigations were performed in the fasting state (0h) as well as 2, 4 and 6 hours following a high-fat meal (600 kcal).
Results: Baseline vascular function (fasting state) did not change with EPA/DHA or placebo treatment. FMD following EPA/DHA treatment remained unchanged: 4.91±0.57% (0h), 4.26±0.51% (2h) (p=NS), 4.31±0.46% (4h) (p=NS) and 4.81±0.52% (6h) and following placebo treatment (olive oil) decreased significantly: 5.39±0.53% (0h), 4.11±0.56% (2h) (p<0.05), 3.56±0.52% (4h) (p<0.001 vs. fasting state, p<0.05 vs. EPA/DHA) respectively 4.57±0.44% (6h)
The microvascular function described by the AUC after reactive hyperemia increased postprandially following EPA/DHA (all measurements are expressed in arbitrary units - AU): 5724±617 AU (0h), 7124±506 AU (2h) (p=0.012), 6511±630 AU (4h), 6232±665 AU (6h). The AUC remained unchanged postprandially following olive oil: 7325±688 AU (0h), 7315±607 AU (2h), 7159±535 AU (4h), 7538±664 AU (6h).
Conclusion: In subjects with diabetes mellitus, a 6 weeks treatment with EPA/DHA preserves the FMD (macrovascular function) while olive oil deteriorates it. Concerning the microvascular function, EPA/DHA for 6 weeks improved blood flow following reactive hyperemia after the test meal. These observations suggest protective cardiovascular effects of omega 3 fatty acids vs. olive oil in this population.
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1244

Analogue versus human insulin therapy improves postmeal glucose and cardiac function in patients with type 2 diabetes with intensive conventional insulin therapy

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Background and aims: Compared to human insulins (HI), analogue insulins (AI) lower postprandial glucose levels more effectively also in type 2 diabetes (T2D). The importance of postprandial glucose control has been shown predominantly for cardiovascular risk and sparsely for myocardial dysfunction. Accordingly, it is not known whether therapy with AI can improve myocardial dysfunction in T2D, which is known for its high prevalence and prognostic relevance. This prospective, randomized, open long-term study tested the hypothesis, that intensive conventional insulin therapy (ICT) with AI vs. HI improves postprandial glucose control associated with improved myocardial function.
Materials and methods: For 24 months, 104 T2D patients were seen at 3 monthly visits to adapt antidiabetic ICT treatment for the target fasting glucose ≤110 mg/dl and postmeal glucose ≤150 mg/dl in these two randomised groups: AI (insulin detemir + insulin aspart, n=59) and HI (NPH-insulin and regular human insulin, n=45). Systolic (S') and diastolic myocardial function (E') were assessed by tissue Doppler before and two hours after a standardized test meal, a pure carbohydrate continental type of breakfast (48g carbohydrates). Both groups were comparable with regard to demographics, cardiovascular risk factors, concomitant medication, cardiac function and metabolic control at baseline.
Results: After 24 months, HbA1c decreased in AI from 7.0±1.5 to 6.5±0.7%, p<0.02) and in HI from 7.6±1.8 to 6.5±0.8%, p<0.001. Postprandial serum glucose was reduced (p<0.001) by 38±81 mg/dl in AI but not significantly by 8±57 mg/dl in HI (p<0.04 compared to AI). Fasting serum glucose was reduced by 16±48 mg/dl in AI (p<0.02) and by 15±43 mg/dl in HI (p<0.003). In AI, S' increased significantly from 7.4±1.1 to 7.7±1.1 cm/s (p<0.04) as did E' (7.8±1.4 to 8.1±1.4 cm/s, p<0.04) associated with a parallel increase post- meal (p<0.002). In HI, however, fasting S' remained unchanged (from 7.6±1.0 to 7.7±1.2 cm/s) and so did fasting E' (from 8.2±1.7 to 8.2±2.0 cm/s) similar to the postprandial values.
Conclusion: In patients with T2D**,** 24 months ICT-therapy with AI significantly improved postprandial glucose control associated with improved cardiac function compared to ICT with HI. Given the prognostic relevance of myocardial dysfunction in T2D, this association should be taken into consideration when selecting an insulin regimen for people with T2D.

1245

Rationale and design of the Trial Evaluating Cardiovascular Outcomes with Sitagliptin (TECOS)

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The purpose of TECOS is to evaluate the potential impact of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on cardiovascular outcomes and clinical safety in a multinational, randomized, double-blind, placebo-controlled trial. TECOS is a pragmatic, academically run trial that will recruit approximately 14,000 patients with type 2 diabetes who are at least 50 years old, have documented vascular disease, and who have an HbA_{1c} betwen 6.5% and 8% on stable doses of any one or two of three oral antihyperglycemic agents (metformin, sulfonylurea, pioglitazone). A minimum of 2000 patients will be on metformin monotherapy and 2000 patients on pioglitazone (alone or in combination). Randomization will be 1:1 to the addition of double-blind sitagliptin (100 mg/day) or matching placebo to a patient's existing diabetes care regimen in a usual care setting, with the aim of achieving glycemic equipoise in the two groups. Patients with moderate, but not severe, renal insufficiency can be included but will be given reduced doses of sitagliptin. Patient accrual, which began in December 2008, will take 2 years in around 30 countries. The primary endpoint will be the time to the first occurrence of a composite cardiovascular outcome (cardiovascular death, nonfatal myocardial infarction, nonfatal stroke or hospitalization for unstable angina). Cardiovascular events will be adjudicated by an independent committee, blinded to study therapy. In this non-inferiority trial, 1300 confirmed primary endpoints are needed to provide 90% power to yield the upper limit of the adjusted 95% CI for a hazard ratio <1.20 at a one-sided alpha level of 0.025. Follow up will be four monthly in the first year, then twice yearly for a minimum of four years or until 1300 primary endpoints have occurred. TECOS will assess the impact of sitagliptin on cardiovascular outcomes when used in addition to usual diabetes care.
Supported by: Merck & Co., Inc.

1246

Anti-inflammatory effects of cilostazole on vascular smooth muscle in *in vitro* and *in vivo*

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Cilostazol is classified as an antiplatelet agent because it inhibits platelet aggregation induced by collagen, 5'-adenosine diphosphate (ADP), epinephrine, and arachidonic acid. Since receiving approval in the United States for the treatment of intermittent claudication in 1999, cilostazol continues to demonstrate promise in the treatment of cardiovascular disorders. We recently reported that cilistazol restored endothelial function in diabetic rats (OLETF), showing one of the mechanisms for cilostazol to protect endothelial cells against inflammatory stimuli that cilostazol inhibits the cytokine-induced expression of pro-inflammatory and adhesion molecule genes by suppressing NF-κB activity via AMP-activated protein kinase (AMPK) activation, not via the cyclic AMP (cAMP)/ protein kinase A (PKA) pathway using cultured vascular endothelial cells. To show that this mechanism works in *in vivo* vascular vessels, we examined aortas with cilostazol-treated rats whether AMPK is associated with the protective effect of cilostazol. We found that AMPK is highly expressed in the aorta from cilostazol-treated rats compared with control rats and that AMPK expression is strongly induced in aortic smooth muscle cell layers in cilostazol-treated rats. We thus investigated whether cilostazol exerts anti-inflammatory effects on those cultured vascular smooth muscle cells (VSMC). Cilostazol was observed to activate AMPK and its downstream target, acetyl-CoA carboxylase, in VSMC. Phosphorylation of AMPK with cilostazol was not affected by co-treatment with an adenylate cyclase inhibitor, SQ 22536, and a cell-permeable cAMP analogue, pCTP-cAMP, did not induce AMPK phosphorylation and had no effect on cilostazol-induced AMPK phosphorylation, suggesting that cilostazol-induced AMPK activation occurs through a signalling pathway independent of cyclic AMP. Cilostazol also dose-dependently inhibited tumor necrosis factor alpha (TNF)-induced NF-κB activation and TNF-induced IκB kinase activity. Furthermore, cilostazol attenuated the TNF-induced promoter activation of iNOS gene and iNOS gene expression, resulting in reduction of NO production. Transduction with dominant-negative AMPK abolished the suppressive effect of cilostazol on iNOS promoter activity and mRNA expression and NO production in VSMC. Furthermore, cilostazol treatment of the LPS-treated rats reduced the induction of iNOS expression in the aorta. In the light of these findings, we suggest that cilostazole plays anti-inflammatory roles on vascular smooth muscle cells *via* induction and activation of AMPK.
Supported partly by a grant from the Japan Private School Promotion Foundation

PS 116 Statins

1247

Circulating neopterin and monocyte chemoattractant protein-1 are responsive to statin therapy but are poor predictors of cardiovascular events in type 2 diabetes in CARDS

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Background and aims: Neopterin is produced by stimulated macrophages and monocyte chemoattractant protein-1 (MCP-1) is a chemokine involved in immune cell recruitment. Both have been suggested as useful biomarkers of cardiovascular disease (CVD). Data on whether they are useful in those with diabetes and whether they are responsive to statin therapy are scarce. We assessed whether circulating levels of neopterin and MCP-1 predict future CVD events and respond to atorvastatin 10 mg daily in diabetes in the randomised trial the Collaborative Atorvastatin Diabetes Study (CARDS).
Materials and methods: We used a nested case control design sampling all those incident cases of CVD (n=210) in CARDS in whom a valid serum sample was available (n=198) and randomly selecting three controls per case from those who completed the trial without a CVD event, stratified by treatment allocation. The median follow up duration in the trial was 3.9 years. Neopterin was measured in ng/mL using an ELISA method. MCP-1 was measured in pg/ml using the Human Cytokine LINCO Plex kit. Analysis was by Cox regression and logistic regression with adjustment for treatment allocation and relevant covariates.
Results: Baseline median (interquartile range) of neopterin was 2.5ng/ml (1.9-3.4). At one year follow up neopterin had increased significantly more in the placebo group (median increase of +0.8 ng/ml) than in the Atorvastatin group (median increase +0.4 ng/ml p=0.01 for the treatment associated difference). Baseline median (interquartile range) of MCP-1 was 134 pg/mL (90-192). At one year follow up MCP-1 had risen very slightly in the placebo group (median change of 0.7 units) but on average had fallen in the Atorvastatin group (median fall -11 pg/ml p=0.015 for the treatment associated difference). However baseline levels of neopterin did not predict CVD (Hazard ratio for a doubling in neopterin adjusted for age sex and trreatment allocation= 0.99 95% CI 0.78-1.25, p=0.90) and neither did levels of MCP-1 (Hazard ratio per doubling in MCP-1 = 1.05 95% CI 0.9-1.2, p=0.60) whether adjusted for other risk factors or not. Neither were on treatment neopterin or MCP-1 levels predictive of events.
Conclusion: Atorvastatin reduces neopterin and MCP-1 levels in those with diabetes consistent with a reduction in macrophage activation. However our data do not support the use of circulating neopterin or MCP-1 as surrogate markers of CVD events in diabetes trials.
The CARDS trial was funded by Pfizer Ltd, Diabetes UK and the UK NHS R&D

1248

Efficacy of fenofibric acid in combination with atorvastatin in patients with type 2 diabetes mellitus and mixed dyslipidaemia

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Background and aims: Patients with Type 2 diabetes (T2DM) are at high risk for coronary heart disease and frequently have mixed dyslipidemia (high triglycerides, low HDL-C and elevated LDL-C) which may be refractory to single-agent lipid-altering therapy. This preplanned analysis of the subgroup of patients with T2DM and mixed dyslipidemia enrolled into a multicenter, double-blind, controlled phase 3 study evaluates the efficacy of the combination of fenofibric acid (FA) and atorvastatin (A) on multiple lipid parameters.
Materials and methods: A total of 613 patients with LDL-C ≥ 130 mg/dL (3.36 mmol/L), triglycerides ≥150 mg/dL (1.70 mmol/L) and HDL-C < 40 mg/dL (1.03 mmol/L) for men (< 50 mg/dL [1.29 mmol/L] for women) were randomized to either FA 135 mg, A (20, 40 or 80 mg) or combination therapy (FA + either A20 or A40 mg) and treated for 12 weeks. A total of 147 (25.5%) of the 576 patients included in the primary efficacy analysis had T2DM. The primary efficacy comparisons were mean percentage change in HDL-C and TG (FA+A v. A), and LDL-C (FA+A v. FA). Secondary endpoints included mean percentage change in non-HDL-C and apolipoprotein B (FA+A v. A).
Results: FA+A combination therapy resulted in numerically greater improvements in TG and HDL-C than the corresponding atorvastatin monotherapies and greater improvement in LDL-C than FA monotherapy in patients with T2DM (Table). After 12 weeks of treatment, FA+A resulted in mean values of LDL-C, TG and HDL-C that were within or close to ADA-recommended target values. All treatments were generally well tolerated, and no case of rhabdomyolysis was reported.
Conclusion: The combination of FA+A resulted in more comprehensive improvement of multiple lipid parameters than either monotherapy for patients with T2DM and mixed dyslipidemia.

Mean % change from baseline to final visit in lipid parameters in patients with T2DM

Lipid Parameter	FA (135 mg)	A (20 mg)	FA+A (135/ 20 mg)	*P*-Value	A (40 mg)	FA+A (135/ 40 mg)	*P*-Value
HDL-C	n = 22	n = 25	n = 26	0.12	n = 25	n = 21	0.07
BL mean	37.9	39.0	38.3		38.8	38.1	
Final mean	45.4	38.9	42.5		38.8	42.6	
Mean % Δ	+19.6	+2.0	+11.5		+1.5	+13.3	
(SE)	(4.66)	(4.37)	(4.29)		(4.37)	(4.77)	
Triglycerides	n = 26	n = 26	n = 28	<0.001	n = 28	n = 26	0.003
BL mean	281.3	258.8	296.0		271.1	294.5	
Final mean	197.8	246.8	140.0		221.9	154.5	
Mean % Δ	-28.3	-10.8	-45.5		-14.9	-44.0	
(SE)	(6.97)	(7.00)	(6.73)		(6.73)	(6.98)	
LDL-C	n = 24	n = 26	n = 26	<0.001	n = 26	n = 23	<0.001
BL mean	162.1	161.9	154.7		155.4	165.4	
Final mean	145.0	91.6	102.1		86.3	100.7	
Mean % Δ	-7.2	-42.2	-30.7		-44.4	-33.8	
(SE)	(4.14)	(3.98)	(3.99)		(3.98)	(4.25)	
Non-HDL-C	n = 22	n = 26	n = 26	0.23	n = 25	n = 21	0.86
BL mean	220.2	214.7	218.8		216.4	227.1	
Final mean	178.2	140.7	127.6		125.5	129.6	
Mean % Δ	-16.8	-33.8	-40.8		-42.5	-41.3	
(SE)	(4.44)	(4.10)	(4.10)		(4.17)	(4.56)	
Apolipoprotein B	n = 26	n = 27	n = 28	0.60	n = 27	n = 26	0.92
BL mean	143.3	143.7	144.8		139.7	152.0	
Final mean	123.4	95.5	91.8		88.9	94.0	
Mean % Δ	-11.1	-33.0	-35.4		-36.6	-36.1	
(SE)	(3.24)	(3.18)	(3.13)		(3.19)	(3.27)	

Means are mg/dL. BL = baseline, SE = standard error
P-values are for FA+A vs A, except for LDL-C, which is FA+A vs FA.

Abbott sponsored and conducted this clinical study

1249

Atorvastatin treatment affects the relationships of LDL and non-HDL cholesterol with ApoB in type 2 diabetes mellitus: modification by triglycerides and CETP

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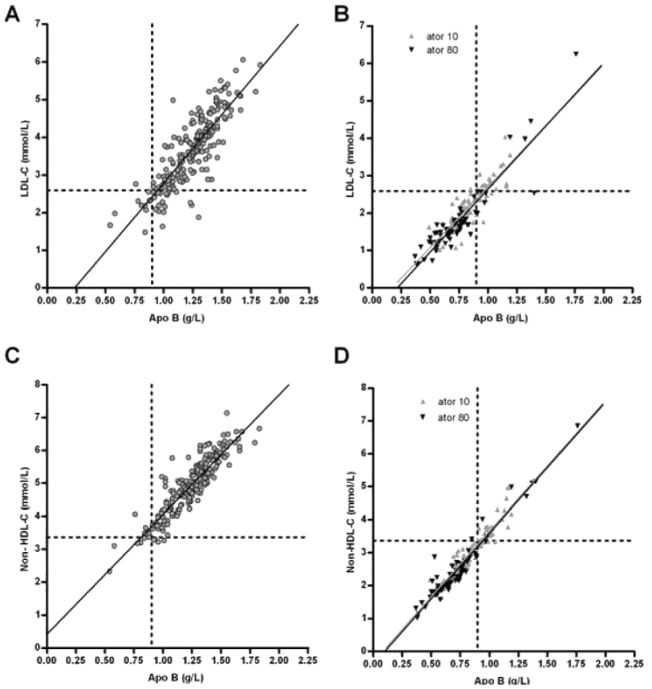
Aims and hypothesis: Current guidelines propose non-HDL-cholesterol (non-HDLC) and apolipoprotein (apo) B as treatment targets in addition to LDL-cholesterol (LDLC). The extent to which statin therapy affects the relationships of LDLC and non-HDLC with apo B was examined in type 2 diabetes.
Methods: Analyses were performed in the DALI cohort (217 moderately hypertriglyceridaemic type 2 diabetic patients). Sixty-one placebo- and 135 atorvastatin-treated patients completed 30 weeks of follow-up. LDLC was calculated by the Friedewald formula. Apo B was assayed by immunoturbidimetry. Cholesteryl ester transfer protein (CETP) mass was measured by ELISA.
Results: Baseline fasting LDLC of 2.42 mmol/l and non-HDLC of 3.69 mmol/l corresponded to the apo B guideline target of 0.90 g/L. During atorvastatin

(10 and 80 mg daily), the LDLC target was achieved more frequently than the apo B target, and lower LDLC (2.38 and 2.29 mmol/l) and non-HDLC (3.24 and 3.19 mmol/l) concentrations corresponded to this apo B goal. Decreases in LDLC during atorvastatin treatment were negatively ($p < 0.001$), but decreases in non-HDLC were positively related to changes in triglycerides ($p < 0.001$), independently from decreases in apo B ($p < 0.001$ for all). Decreases in LDLC and non-HDLC were positively associated with decreases in CETP mass ($p < 0.001$).

Conclusion and interpretation: Atorvastatin leads to a shift in the relationships of fasting LDLC and non-HDLC with apo B in type 2 diabetes towards lower LDLC and non-HDLC levels that correspond to an apo B guideline target of 0.90 g/l. Triglycerides and CETP modify these relationships.



Legends to Figure 1
Changes in the relationships of non-high density lipoprotein cholesterol (non-HDLC) and low density lipoprotein cholesterol (LDLC) with apolipopoprotein B (apo B) during atorvastatin treatment in type 2 diabetic patients.
A: LDLC vs. apo B at baseline.
B: LDLC vs. apo B during treatment with atorvastatin 10 mg daily (ator 10) and atorvastatin 80 mg daily (ator 80). Grey line: regression line of data during ator10; black line: regression line of data during ator 80.
C: Non-HDLC vs. apo B at baseline.
D: Non-HDLC vs. apo B during treatment with atorvastatin 10 mg daily (ator 10) and atorvastatin 80 mg daily (ator 80). Grey line; regression line of data during ator 10; black line: regression line of data during ator 80.

The original DALI study was partly supported by an unrestricted grant of Parke-Davis, The Netherlands.

1250

The relationship between HbA_{1c} and cardiovascular disease in patients with Japanese hypercholesterolaemia and effect of low dose pravastatin

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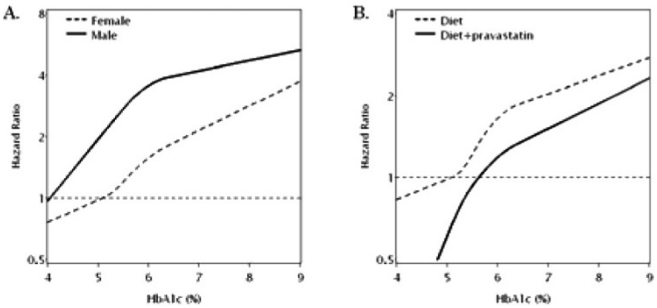
Background and aims: The relationship between HbA1c and cardiovascular risk in patients with hypercholesterolemia is not well understood because of sparse data. The aim of the present study was to investigate the relationship between HbA1c and future development of cardiovascular disease in patients with hypercholesterolemia, and to evaluate the effect of pravastatin in a post-hoc analysis of the large-scale primary prevention study with pravastatin (The Management of Elevated cholesterol in the primary prevention Group of Adult Japanese (MEGA) Study).

Material and methods: The MEGA Study sought to evaluate the effect of low-dose pravastatin (10-20 mg/day, approved dose in Japan) on primary prevention of cardiovascular disease in a total of 7,832 Japanese patients (male, 40 to 70 years; female, post-menopause to 70 years) with mild to moderate hyperlipidemia without cardiovascular disease. Patients were randomized to diet alone or diet + pravastatin and followed for more than 5 years. A total of 4,002 patients with baseline and follow-up HbA1c levels were analyzed. The relationship between HbA1c and the risk of cardiovascular disease was evaluated according to sex and treatment arm, using multivariate Cox proportional hazards model with a restricted quadratic spline based on three knots for HbA1c quartiles (5.2, 5.5, 6.4 %).

Results: The spline curve in 4,002 patients continuously rose to around an HbA1c of 6%, and thereafter the curve rose more gently. cardiovascular disease risk was approximately 2-times higher in men than in women, and the shape of the curves were similar for men and women (Figure A). The curve in the diet group was consistently lower compared with that in the diet + pravastatin group, regardless of the HbA1c level (Figure B).

Conclusion: A continuous increase, up to an HbA1c of 6%, in cardiovascular disease risk was found, and thereafter a more gentle increase, in Japanese men and women with hypercholesterolemia. The beneficial effect of pravastatin is achieved regardless of the HbA1c level.

Figure



We are grateful to the MEGA Study Group for the use of their study data

1251

Atorvastatin lowers remnant-like particle cholesterol without affecting LDL size in type 2 diabetes mellitus: relevance for non-HDL cholesterol and apoB targets

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Background and aims: The extent to which atorvastatin treatment affects LDL size, LDL subfractions levels and remnant-like particle cholesterol (RLP-C) was determined in Type 2 diabetic patients. We also compared LDL size and RLP-C in relation to American Diabetes Association guideline cut-off values for LDL cholesterol, non-HDL cholesterol and apolipoprotein (apo) B.

Materials and methods: Changes in RLP-C (immuno-separation technique; Japan Immunoresearch Laboratories, Takasaki, Japan) and LDL size (polyacrylamide gradient gel electrophoresis) were determined in fasting plasma from Type 2 diabetic patients after 30 weeks follow-up in response to atorvastatin (10 mg daily, n=65; 80 mg daily, n=62) and placebo (n=58). In 74 participants, LDL subfractions were measured by density gradient ultracentrifugation using a six-step, discontinuous salt gradient. The patients participated in The DALI (Diabetes Atorvastatin Lipid Intervention) study, which is a randomized placebo-controlled multicentre trial that has been performed in 3 outpatient university clinics.

Results: Atorvastatin decreased LDL cholesterol, non-HDL cholesterol, triglycerides, apo B and apo E ($P < 0.001$ for each dose). Atorvastatin also lowered RLP-C ($P < 0.001$ for each dose), but LDL peak particle size remained unaffected (Table). All LDL subfractions decreased after atorvastatin ($P < 0.001$ for each dose) without a preferential shift towards LDL_3. RLP-C was lower in those patients achieving the non-HDL cholesterol (0.20 (0.17-0.30) mmol/L vs 0.35 (0.22-0.45) mmol/L, $P < 0.01$) or the apo B guideline targets (0.20 (0.17-0.30) mmol/L vs 0.39 (0.20-0.45) mmol/L, $P < 0.01$), but the LDL cut-off value failed to discriminate between lower and higher RLP-C concentrations (0.20 (0.17-0.34) mmol/L vs, 0.29 (0.20-0.40) mmol/L, $P > 0.10$).

Table (abstract 1251): Remnant like particle cholesterol (RLP-C) concentration, LDL size pattern, LDL particle size and at baseline and after placebo or atorvastatin treatment (10 mg or 80 mg daily) in type 2 diabetic patients. Data in mean ± SD or in median (interquartile range). LDL size pattern distribution by Chi-square analysis: * baseline: $P = 0.68$; **follow-up: $P = 0.63$. ***$P < 0.001$ compared to baseline. *$P < 0.01$ and **$P < 0.001$ compared to baseline. §P-value for between group difference in change by ANOVA.

	Placebo(N = 58)		**Atorvastatin 10 mg** (N= 65)		**Atorvastatin 80 mg**(N= 62)		§***P*-value**
	Baseline	Follow-up	Baseline	Follow-up	Baseline	**Follow-up**	
RLP-C(mmol/L)	0.45 (0.30-0.64)	0.37 (0.28-0.49)	0.43 (0.33-0.70)	0.25 (0.17-0.37)***	0.47 (0.32-0.64)	0.21 (0.17-0.33)***	< 0.001
LDLparticle size pattern (A/B)	41/17*	41/17**	42/23*	50/15**	38/24*	42/20**	
LDLparticle size (nm)	25.90±0.72	25.94±0.62	25.87±0.75	26.01±0.71	25.76±0.75	25.92±0.73	0.63

Conclusion: In Type 2 diabetic patients usual and high dose atorvastatin lower fasting RLP-C without affecting LDL size and changing LDL subfractions towards more boyant LDL. The proposed guideline cut-off levels for non-HDL cholesterol and apo B may be superior to the LDL cholesterol target in discriminating between higher and lower RLP-C levels.

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1252

Long-term ezetimibe/simvastatin (E/S) + extended release niacin (N) treatment in hyperlipidaemic patients with diabetes and metabolic syndrome

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Background and aims: Guidelines recommend combination therapy to achieve optimal LDL-C lowering and broader lipid regulation in high-risk patients such as those with diabetes (DM) and metabolic syndrome (MS). We assessed the efficacy and safety of combination *E/S+N* in hyperlipidemic patients with DM or MS.

Methods: This analysis of a double-blind, randomized 64-week study in hyperlipidemic patients assessed the effect of *E/S* (10/20 mg) +*N* (to 2 g) or *E/S* (10/20 mg) treatment for 64 weeks on lipid parameters in subjects with DM, with MS without DM (MS/nonDM), or without DM or MS (nonDM/nonMS). Safety issues including fasting glucose (FG) and new onset DM were monitored.

Results: *E/S+N* improved HDL-C, non-HDL-C, TG, ApoB (see table), ApoAI and lipid ratio levels more than did *E/S* in all subgroups. Reductions in LDL-C and hsCRP were similar for *E/S+N* and *E/S* in both DM and MS subgroups, but *E/S+N* had a greater effect than *E/S* in the nonDM/nonMS group. Changes in total cholesterol were comparable for both treatments in all groups. FG trended higher for *E/S+N*, peaked at 8 weeks above baseline (1.2 and 0.6 mmol/L [21.2 and 11.2 mg/dL] for *E/S+N* vs *E/S* respectively), then gradually declined to pretreatment levels at 64 weeks for both treatments in DM patients. New onset DM, due mostly to 2 consecutive FG increases ≥7.0 mmol/L (≥126mg/dL), was higher in the MS/nonDM than nonMS/nonDM group for either treatment (see table) and occurred predominantly during the first 24 weeks.

Conclusion: *E/S+N* improved lipid/lipoprotein profiles and was generally well tolerated in hyperlipidemic patients with DM or MS during 64 weeks. Increases in FG occurred more often in patients with DM and new onset DM in those with MS. These effects, observed mainly during the first 6 months of therapy, abated with time. These results indicate that triple combination *E/S+N* is a treatment option for dyslipidemia in patients with DM and MS, but requires monitoring of glucose changes.

Parameter	**DM**		**MS/nonDM**		**NonDM/nonMS**	
	E/S **n=29**	***E/S+N*** **n=55**	***E/S*** **n=93**	***E/S+N*** **n=141**	***E/S*** **n=84**	***E/S+N*** **n=171**
HDL-C						
% change†	8.6	30.9	9.4	30.7	8.7	30.4
95% CI	1.2,16.0	25.5, 36.3	5.1,13.6	27.2, 34.1	4.3, 13.2	27.3, 33.6
TG						
% change‡	-27.3	-50.3	-32.9	-47.5	-18.5	-38.4
95% CI	-38.5,-16.1	-56.7,-43.9	-39.1,-26.8	-52.3,-42.7	-25.6,-11.3	-42.2,-34.5
Non-HDL-C						
% change†	-48.9	-53.6	-45.3	-50.2	-43.7	-53.8
95% CI	-55.4,-42.3	-58.4,-48.8	-49.0,-41.5	-53.2,-47.1	-47.6,-39.7	-56.6,-51.0
LDL-C						
% change†	-55.3	-55.7	-49.8	-52.4	-46.7	-54.7
95% CI	-62.1,-48.4	-60.7,-50.7	-53.7,-45.9	-55.6,-49.2	-50.8,-42.6	-57.6,-51.9
ApoB						
% change†	-44.0	-48.0	-41.4	-46.8	-38.7	-48.0
95% CI	-50.7,-37.4	-52.7,-43.4	-45.0,-37.7	-49.8,-43.7	-42.5,-34.9	-50.8,-45.3
New Onset DM						
n/N (%)§	NA	NA	7*/119 (5.9)	22**/239 (9.2)	0/108 (0.0)	6***/326 (1.8)

Subgroup analysis of primary efficacy population = all patients randomized to ***E/S+N*** or ***E/S*** who continued the study from 24 wks and had a baseline and at least one on-treatment measurement at 64 wks. E/S = ezetimibe/simvastatin (10/20mg); N = extended-release niacin (2g); DM=diabetes; MS=metabolic syndrome; FG= fasting glucose; †LS mean for within-treatment change from baseline based on ANOVA model with terms for treatment, DM/MS category, baseline LDL-C, baseline TG, gender, and treatment by DM/MS category interaction; ‡ Hodges-Lehmann estimate of median difference; §Defined as diagnosis of DM, 2 consecutive increases FG ≥7.0 mmol/L (≥126mg/dL) or initiation of antidiabetic medication; n/N = # of patients with new onset DM/# of patients in the subgroup; NA=not applicable; *2/7, **21/22, ***4/6 patients diagnosed at 24 wks.

1253

Influence of metabolic syndrome factors and insulin resistance on ezetimibe/simvastatin and atorvastatin treatment efficacy in patients with metabolic syndrome and moderately high/high coronary heart disease risk

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Background and aims: Metabolic syndrome (MS) affects about 25% of the US population. MS associated factors (abdominal obesity, low HDL-C; and elevated triglycerides, blood pressure and fasting glucose) as well as insulin resistance (IR) may be associated with increased coronary heart disease (CHD) risk. This study assessed the influence of these factors on % change from

baseline low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), non-HDL-C, total cholesterol (C), very low density lipoprotein cholesterol (VLDL-C), triglycerides (TG), apolipoprotein (Apo) B, Apo AI, high sensitivity C-reactive protein (hs-CRP), and lipoprotein ratios (total C:HDL-C, LDL-C:HDL-C, Apo B:Apo AI, non-HDL-C:HDL-C).

Materials and methods: Post-hoc analysis of a multicenter, 6-week, double-blind, randomized, parallel group study in over 1000 hypercholesterolemic MS patients with moderately high (MHR) or high (HR) CHD risk, treated with ezetimibe (E) 10mg/simvastatin (S) 20mg compared to atorvastatin (A)10mg or A20mg, or E10/S40mg vs. A40mg. Percent attainment of LDL-C < 1.8 mmol/L (70 mg/dL) for HR patients with atherosclerotic vascular disease (AVD) or LDL-C < 2.6 mmol/L (100 mg/dL) for MHR and HR patients without AVD was also evaluated.

Results: E/S and A efficacy were generally consistent across MS factor and IR subgroups. E/S produced greater incremental percent reductions in LDL-C (Table), non-HDL-C, Apo B, total C, and lipoprotein ratios for all subgroups and larger % increases in HDL-C and Apo AI for all but non-obese and HDL-C ≥ 40 mg/dL subgroups compared to A. TG, VLDL-C, and hs-CRP results were more variable. E/S produced greater attainment of LDL-C <1.8 mmol/L (70 mg/dL) and < 2.6 mmol/L (100mg/dL) than A for all treatment comparisons across all subgroups, except non-obese HR patients with AVD (data not shown - some sample size limitations).

Conclusion: These results suggest that efficacy of E/S and A are generally well maintained across MS and IR factors, with E/S > A for most treatment differences. Observed effects of E/S vs. A may be useful in evaluating treatment options for MS patients with moderately high/high CHD risk.

Table. Percent Change from Baseline LDL-C

Factor		n	A10*	A20*	E/S20*	A40*	E/S40*
Obesity† males [females]	< 102 cm (40in) [< 89 cm (35in)]	20-36	-37.1 (-43.0, -31.3)	-36.6 (-42.4, -30.8)	-45.7 (-52.2, -39.3)	-48.2 (-55.9, -40.4)	-46.2 (-53.1, -39.2)
	≥ 102 cm (40in) [≥ 89 cm (35in)]	177-196	-36.2 (-38.9, -33.5)	-39.7 (-42.3, -37.0)	-50.1 (-52.7, -47.6)	-45.7 (-48.2, -43.1)	-55.0 (-57.5, -52.4)
TG	< 1.7mmol/L (150 mg/dL)	62-77	-34.7 (-39.0, -30.4)	-38.6 (-42.7, -34.5)	-50.6 (-54.6, -46.6)	-44.9 (-49.3, -40.4)	-54.7 (-59.1, -50.3)
	≥ 1.7 mmol/L (150 mg/dL)	142-155	-37.2 (-40.1, -34.3)	-39.8 (-42.7, -36.8)	-49.1 (-52.0, -46.1)	-46.4 (-49.3, -43.6)	-53.6 (-56.5, -50.8)
HDL-C males [females]	≥ 1.0 mmol/L (40 mg/dL) [≥ 1.3 mmol/L (50 mg/dL)]	82-99	-35.6 (-39.3, -31.9)	-40.8 (-44.4, -37.2)	-49.9 (-53.4, -46.3)	-47.6 (-51.5, -43.7)	-53.5 (-57.4, -49.7)
	< 1.0 mmol/L (40 mg/dL) [<1.3 mmol/L (50 mg/dL)]	119-135	-37.1 (-40.3, -33.9)	-38.2 (-41.5, -35.0)	-49.4 (-52.5, -46.2)	-45.0 (-48.1, -42.0)	-54.2 (-57.3, -51.2)
SBP [DBP]	< 130 [< 85] mmHg	11-21	-32.8 (-43.3, -22.3)	-40.8 (-48.5, -33.2)	-50.6 (-60.0, -41.3)	-49.7 (-57.8, -41.7)	-52.1 (-59.9, -44.3)
	≥ 130 [≥ 85] mmHg	194-205	-36.7 (-39.1, -34.2)	-39.2 (-41.7, -36.7)	-49.5 (-52.0, -47.1)	-45.6 (-48.2, -43.1)	-54.1 (-56.7, -51.6)
FG	< 5.6 mmol/L (100 mg/dL)	52-75	-37.4 (-41.7, -33.2)	-40.1 (-44.5, -35.8)	-47.0 (-51.0, -42.9)	-47.1 (-52.0, -42.3)	-54.7 (-59.0, -50.4)
	≥ 5.6 mmol/L (100 mg/dL)	144-165	-36.0 (-38.9, -33.1)	-39.0 (-41.9, -36.1)	-50.9 (-53.9, -48.0)	-45.6 (-48.4, -42.8)	-53.6 (-56.4, -50.7)
HOMA-IR	< 2.72	57-81	-39.5 (-43.6, -35.4)	-38.2 (-42.3, -34.1)	-50.5 (-54.4, -46.7)	-46.6 (-50.7, -42.5)	-54.7 (-59.3, -50.0)
	2.72 - 4.81	68-78	-34.2 (-38.3, -30.1)	-39.8 (-44.0, -35.6)	-50.1 (-54.3, -45.9)	-45.7 (-49.8, -41.6)	-54.5 (-58.5, -50.6)
	>4.81	69-79	-35.4 (-39.6, -31.3)	-40.1 (-44.3, -35.9)	-47.8 (-51.9, -43.6)	-45.8 (-50.0, -41.6)	-52.9 (-56.9, -49.0)

*values are percent change from baseline LDL-C (95% confidence interval)
†Obesity = waist circumference
A10/20/40=atorvastatin10/20/40mg; E=ezetimibe 10mg; FG=fasting glucose; HDL-C=high density lipoproprotein cholesterol; HOMA-IR=Homeostasis Model Assessment of Insulin Resistance; n=number of patients in subgroup-range for all treatments; S20/40=simvastatin 20/40mg; SBP (DBP)=systolic blood pressure (diastolic blood pressure); TG=triglyceride

1254

Effects of low-dose, long-term fluvastatin treatment on oxidative/antioxidative parameters in the pancreas and heart from streptozotocin-induced diabetic rats

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Background and aims: Statins (HMG-CoA reductase inhibitors) exert many pleiotropic effects in addition to lowering cholesterol levels. Oxidative stress is universal in diabetes, being ultimately involved with the development complications. Among various statins, fluvastatin is thougt to be the most powerful antioxidant. This study was designed to investigate cholesterol-independent antioxidative effect of chronic fluvastatin treatment in diabetic rats.

Materials and methods: Wistar male rats were made diabetic by streptozotosin (STZ: 55 mg/kg, ip). Some of them and control rats (D and C groups) were treated orally for 6 months with fluvastatin (2mg/kg/day, p.o), starting one week after STZ injection (CF and DF groups) (preventive study). In pancreas and heart homogenates, the oxidative stress markers, superoxide dismutase (SOD) and catalase (CAT) enzyme activities, malon dialdehit (MDA), protein carbonyl content (PCC) (by spectrophotometric method) and 3-nitrotyrosine (3-NT) levels (by ELISA) were measured.

Results: Body weight, hyperglycemia and dyslipidemia in D groups remained unchanged after fluvastatin treatment (p>.05, ANOVA, n:8-10 each group). Oxidative stress parameters studied in pancreas and heart tissues of diabetic animals were mostly alleviated by fluvastatin treatment (Table:Enzymes: U / mg protein; MDA, PCC: nmol / mg protein, 3-NT: mol/mg protein. Data± SEM, Sign. ≠ vs. C; * vs. D; ANOVA test, p<.05, n:5 each,).

Conclusion: Fluvastatin used in a dose too low to alter the lipid profile seems to be beneficial to reduce oxidative stress seen in diabetic state. We can speculate that early and long-term fluvastatin therapy in diabetes may lead to a progressive reduction of organ injury.

Effects of Fluvastatin treatment on oxidative stress parameters in the groups.

Groups/ Parameters	C	CF	D	DF
SOD Pancreas	0.098±0.022	0.152±0.020≠	0.178±0.020≠	0.181±0.021≠
SOD Heart	0.075±0.011	0.077±0.010	0.089±0.005	0.089±0.005
CAT Pancreas	312.87±24.66	306.64±13.73	436.57±28.34≠	339.62±12.11*
CAT Heart	34.58±2.80	36.23±1.95	40.45±2.38≠	39.93±1.36*
3-NT Pancreas	83.38±8.99	101.73±8.46	121.21±11.95≠	74.13±4.13*
3-NT Heart	82.13±2.81	73.56±6.05≠	96.66±4.21≠	60.98±3.28*≠
MDA Pancreas	0.485±0.029	0.385±0.039≠	0.705±0.083≠	0.472±0.033*
MDA Heart	1.802±0.202	2.503±0.418	0.310±0.3462≠	2.117±0.177*
PCC Pancreas	23.07±3.34	31.18±6.19	35.90±2.67≠	29.93±1.41*≠
PCC Heart	12.48±1.31	14.42±2.73	19.75±1.98≠	16.12±1.2*≠

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PS 117 New therapeutic options

1255

Role of PPARα in human white adipocyte metabolism

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Background and aims: Understanding of adipose tissue metabolism is important to prevent the development of obesity-associated diabetic complications. PPARs are key regulators of lipid metabolism. Among them, PPARα is predominantly expressed in tissues with a high rate of fatty acid oxidation and is known for its role in the regulation of genes involved in lipid catabolism in the liver. However, the role of PPARα in human adipocyte metabolism remains elusive. We previously showed that the activation of PPARα by its agonist GW7647 leads to enhanced expression and activity of glycerol kinase (GyK) in human white adipocytes. This probably results from a direct activation of GyK transcription by PPARα. This result suggested that PPARα could promote an energy dissipating fatty acid cycling between lipolysis and triglyceride synthesis. In order to investigate the involvement of PPARα in human white adipocyte metabolism, we compared the effects of GW7647 and of rosiglitazone, a PPARγ agonist, by a transcriptomic analysis, and we identified 80 genes differentially regulated by PPARα and PPARγ. DNA microarray data were confirmed by quantitative RT-PCR as 21 of 26 genes were differentially regulated. Study of the functions of these genes showed that part of them is involved in glycolysis and β-oxidation pathways.
Results: We next showed that GW7647 increased mRNA expression of enzymes involved in mitochondrial uptake of acyl-CoA and β-oxidation (hydroxyacyl-coenzyme A deshydrogenase α/β: HADHA/B, carnitine palmitoyltransferase 1b: CPT1b). These genes are regulated to a lesser extent or identically by rosiglitazone. Moreover, the PPARα but not the PPARγ agonist increased two fold palmitate oxidation in human white adipocytes. By silencing PPARα expression with a siRNA, we were able to confirm that palmitate oxidation was PPARα-dependent. In addition, GW7647 downregulates the expression of many enzymes of the glycolytic pathway (aldolase, glyceraldehyde-3-phosphate dehydrogenase, enolase, pyruvate kinase) and of a major regulatory protein: pyruvate deshydrogenase kinase 4 (PDK4). These genes are regulated to a lesser extent by the PPARγ agonist. This variation of gene expression is concomitant with: 1) an increase of pdk4 protein expression and 2) a decrease of glycolysis shown by reduced pyruvate and lactate accumulation in the culture medium.
Conclusion: In human white adipocytes, activation of PPARα leads to a shift in energy fuel selection. The alteration of gene expression by the PPARα agonist GW7647 is associated with a concomitant higher fatty acid oxidation level and a lower glucose utilization level, which are not observed with the PPARγ agonist. We are currently investigating the involvement of PPARα using an antagonist of the nuclear receptor.The stimulation of fatty acid consumption combined with dissipation of energy due to PPARα-induced fatty acid cycling could be envisioned as a mean to limit systemic fatty acid release, which is a crucial step in the development of insulin resistance associated with type 2 diabetes.

1256

Serum concentrations of novel metabolic regulator FGF19 in patients with type 2 diabetes: the influence of acute hyperinsulinaemia and PPAR-α agonist treatment

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Background and aims: Experimental studies have identified fibroblast growth factor-19 (FGF19) as a novel endocrine and paracrine regulator with multiple effects on metabolic processes and energy homeostasis. At present, little is known about its role and regulation of its circulating levels in humans. The aim of our study was to measure serum FGF19 concentrations in patients with obesity and type 2 diabetes mellitus (T2DM) and healthy subjects (C) and to assess the changes of its circulating levels after pharmacological interventions.
Materials and methods: We measured biochemical parameters, serum FGF19, adiponectin, leptin and insulin levels by commercial ELISA and RIA kits in 21 T2DM patients and 29 control subjects. The interventions were acute hyperinsulinemia during isoglycemic-hyperinsulinemic clamp and 3-months treatment with PPAR-alpha agonist fenofibrate.
Results: Baseline serum FGF19 levels did not significantly differ between T2DM and C groups (189.24 ± 30.9 vs. 202.20 ± 16.74 pg/ml, n.s.). In a combined population of T2DM and C subjects FGF19 levels significantly negatively correlated with glycemia (r= -0,46, p<0.005) and HOMA index (r= -0.45, p=0.005). No significant relationship was found between FGF19 and BMI, cholesterol, triglyceride, insulin and leptin levels, respectively. 3 months of fenofibrate treatment significantly reduced FGF19 levels in T2DM patients (194.58 ± 26.2 vs. 107.47 ± 25.0 pg/ml, p<0.05). Acute hyperinsulinemia during isoglycemic-hyperinsulinemic clamp decreased FGF19 levels in both healthy and T2DM subjects (214.5 ± 55.2 vs. 145.7 ± 19.9 and 198.6 ± 48.6 vs. 147.2 ± 24.7 pg/ml, respectively, n.s.). 3-months treatment with fenofibrate abolished insulin-induced decrease in FGF19 concentrations during isoglycemic-hyperinsulinemic clamp in T2DM group.
Conclusion: The presence of obesity and type 2 diabetes mellitus did not influence fasting serum FGF19 levels. On the contrary, both acute hyperinsulinemia and treatment with PPAR-alpha agonist fenofibrate significantly decreased FGF19 levels. We suggest that decrease of FGF19 levels may play a role in some of positive metabolic effects of PPAR-alpha activation.
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1257

WITHDRAWN

1258

Roles of peroxisome proliferator-activated receptor delta and 4-hydroxy-dodecadienal in the regulation of glucose transport in vascular endothelial cells

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Background and aims: Vascular endothelial cells protect their intracellular environment against deleterious effects of ambient high glucose concentrations by downregulating the rate of glucose uptake subsequent to a reduction of total cell content and plasma membrane abundance of their principal glucose transporter GLUT-1. We have discovered that high glucose levels increase the expression of the arachidonic acid metabolizing enzyme 12-lipoxygenase (12-LO) and enhance the biosynthesis of its product 12-HpETE in vascular endothelial cells (VEC). In addition, the protein calreticulin, whose expression is also increased in these cells, interacts with and destabilizes GLUT-1 mRNA, leading to a decreased expression of the transporter. The present study has aimed at ascertaining whether the peroxidation product of 12-HpETE, namely 4-hydroxy-dodecadienal (4-HDDE) activates a peroxisome-proliferator-activated receptor (PPAR) family member to induce calreticulin expression and destabilize GLUT-1 mRNA.
Materials and methods: Naïve primary cultures of bovine aortic EC or cultures expressing an ectopic hPPAR isotype (α, γ or δ) and a PPAR-response element (PPRE) driven-luciferase reporter construct were incubated at normal or high glucose levels and treated with various PPAR agonists or 4-HDDE. The rate of glucose transport, luciferase activity, mRNA and protein expression as well as the subcellular distribution of GLUT-1 and calreticulin mRNA and protein levels were determined. In addition, the amount of 4-HDDE secreted from VEC exposed to normal or high glucose was measured by HPLC.
Results: This study shows that hyperglycemia-induced production of 12-HpETE results in its excessive peroxidation to generate augmented amounts of 4-HDDE. When added to normoglycemic VEC, both 4-HDDE, at non-toxic concentrations, or the PPARδ selective agonist GW501516 mimicked high glucose-induced downregulation of glucose transport and reduced GLUT-1 mRNA and protein content while increasing calreticulin expression. 4-HDDE induced PPRE-luciferase reporter activity only in VEC that ectopically expressed hPPARδ, but not hPPARα, hPPARγ1 or hPPARγ2. In addition, partial silencing of PPARδ expression with siRNA reversed high glucose induced down and upregulation of GLUT-1 and calreticulin expression, respectively. Finally, while pharmacological inhibition of 12-LO with baicalein prevented high glucose-dependent downregulation of the glucose

transport system the presence of 4-HDDE or GW501516 restored this down-regulatory capacity.
Conclusion: This study shows that 4-HDDE is a potent physiological agonist of PPARδ. The generation of 4-HDDE is augmented in VEC under high glucose conditions due to an increased availability and peroxidation of 12-HpETE. This peroxidation product activates PPARδ, which in turn increases the expression of calreticulin that destabilizes GLUT-1 mRNA in VEC.
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1259

Preclinical safety of INT131, a selective PPARγ modulator (SPPARM), is differentiated from PPAR full agonists in chronic rodent and monkey studies
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Background and aims: PPARγ is a validated clinical target for addressing insulin resistance, a key etiologic factor in Type 2 Diabetes Mellitus (T2DM). However, full activation of PPARγ by agents such as the thiazolidenediones (TZDs) also causes weight gain, fluid retention, CHF, and increased bone fracture risk. These effects are also observed in preclinical species, which have been predictive of clinical experience for full PPARγ agonists, including rosiglitazone and pioglitazone. In fact, cardiovascular mortality has limited full agonist exposure in chronic preclinical studies.
INT131 is a potent, novel, non-TZD selective PPARγ modulator (SPARRM) designed to minimize or eliminate the typical side effects of the full PPARγ agonists while retaining the potent effects on glucose metabolism and insulin sensitization. Pharmacology models of diabetes and a Phase 2a clinical study in T2DM have demonstrated a lack of significant weight gain and fluid retention at doses of INT131 providing similar or better anti-diabetic efficacy to rosiglitazone and pioglitazone. Six month rat and monkey studies of INT131 have shown no significant fluid retention, weight gain, cardiac effects or adipose marrow replacement at very high multiples (>400 fold for rat ,and >200 fold for monkey) of the Phase 2a exposure of INT131 that provided comparable efficacy to high dose TZDs.
Materials and methods: The safety profile of INT131 was characterized in long term studies in rodents and monkey with special attention to known cardiac and other full PPARγ agonist effects. Monkey: Groups of 4 male and female cynomolgus monkeys were administered 0, 0.3, 1.0, or 3.0 mg/kg/day INT131 for 52 weeks, with additional groups allowed a 4 week post-dosing period. A comprehensive assessment of safety was conducted. Rodent: Groups of 60 male and 60 female rats and mice were administered vehicle or one of 4 doses of INT131 (range 0.3-300 mg/kg/day) for up to 104 weeks. A safety assessment was conducted. All three studies included additional evaluations to characterize toxicity of PPAR ligands designated in FDA Draft Guidance for Diabetes Drugs.
Results: INT131 was well tolerated as determined by lack of significant effect on clinical observations, survival, weight, and food intake at anticipated exposures greater than ~130 (mouse, 2 years), ~100 (rat, 2 years), and ~40 (monkey, 1 year) fold over the Phase 2a exposure of INT131 giving similar efficacy to high dose TZDs.
Conclusion: Available nonclinical and clinical data support the potential of INT131 to function as a SPPARM to provide potent insulin sensitization and glucose reduction separate from the undesirable effects of full-agonist TZDs such as weight gain, adiposity and adipose marrow replacement, fluid retention, and CHF. Findings of interest for PPARγ ligands will be discussed, particularly those involving the cardiovascular system.

1260

Effect of adjunct metformin treatment on levels of plasma lipids in patients with type 1 diabetes
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Background: In addition to its glucose-lowering effect, metformin treatment has been suggested to improve lipidaemia in patients with type-2 diabetes. In contrast, in patients with type-1 diabetes (T1DM), information about the effect of metformin treatment on lipidaemia is limited. Here we report the effect of a one-year treatment with metformin versus placebo on plasma lipids in T1DM patients and persistent poor glycaemic control.
Methods: One hundred T1DM patients and HaemoglobinA_{1c} ≥8.5% during the year before enrolment entered a one-month run-in on placebo treatment. Thereafter, patients were randomised (baseline) to treatment with either metformin (1000 mg twice-daily) or placebo for 12 months (double-masked). Patients continued ongoing insulin therapy and their usual outpatient clinical care. Outcomes were assessed at baseline and after one year.
Results: After one year, in those patients who did not start or stop statin therapy during the trial, metformin treatment significantly reduced total and LDL cholesterol by approximately 0.3 mmol/l as compared to placebo (p=0.021 and p=0.018, respectively). Adjustment for statin use or known CVD did not change conclusions. In statin users (metformin: n=22; placebo: n=13), metformin significantly lowered levels of LDL and non-HDL cholesterol by approximately 0.5 mmol/l compared to placebo (adjusted for changes in statin dose or agent: p=0.048 and p=0.033, respectively). HaemoglobinA_{1c} (previously reported) was not significant different between treatments.
Conclusion: In patients with poorly controlled T1DM, at similar glycaemic levels, adjunct metformin therapy during one year significantly lowered levels of pro-atherogenic cholesterolaemia independent of statin therapy.
Supported by: the Danish Diabetes Association

1261

Dalcetrapib safety and tolerability in high-risk patients with type 2 diabetes mellitus and/or metabolic syndrome
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Background and aims: Individuals with type 2 diabetes mellitus (T2DM) and metabolic syndrome (MetSyn) are at markedly increased cardiovascular risk, even following intensive statin therapy. Low HDL cholesterol levels, characteristic of T2DM and MetSyn, constitute an independent cardiovascular risk factor. Dalcetrapib, a selective inhibitor of cholesteryl ester transfer protein (CETP) activity, effectively raises HDL cholesterol. This analysis evaluated the safety and tolerability of dalcetrapib 900 mg daily in patients with dyslipidaemia, CHD or CHD risk equivalents with and without T2DM and/or MetSyn (T2DM/MetSyn).
Materials and methods: Evaluation of the safety and tolerability profile of dalcetrapib 900 mg was evaluated in patients with T2DM/MetSyn (as defined by NCEP ATPIII guidelines) by *post hoc* analysis of a placebo-controlled Phase II trial of 24 weeks duration with 24-week extension.
Results: Overall, 107 patients with T2DM/MetSyn received dalcetrapib (n=74) or placebo (n=33) and 28 patients without T2DM/MetSyn received dalcetrapib (n=15) or placebo (n=13) for 24 weeks. Patients were of mean age 59-64 years and predominantly male (73-93%) and white (76-93%). In the T2DM/MetSyn group, the number of patients reporting ≥1 adverse event (AE) by week 24 was 61 (82%) in the dalcetrapib group and 27 (82%) in the placebo group. In patients without T2DM/MetSyn, AEs were reported in 13 (87%) and 10 (77%) patients, respectively. Most AEs were mild to moderate in intensity and considered unrelated to study drug. AEs occurring most commonly (≥10% of patients) in those with T2DM/MetSyn administered dalcetrapib or placebo were (dalcetrapib/placebo); upper respiratory tract infection (URTI [15%/9%]) and diarrhoea (14%/12%). Patients without T2DM/MetSyn showed a similar profile, commonly reported AEs (≥10%) being (dalcetrapib/placebo); URTI (13%/23%), diarrhoea (20%/8%), flatulence (7%/23%) and nasopharyngitis (7%/15%). There was no effect of treatment on blood pressure. Serious AEs (SAEs) were few. In patients with T2DM/MetSyn administered dalcetrapib or placebo, SAEs occurred in only 6 (8%) and 2 (6%) of patients, respectively. In those without T2DM/MetSyn, SAEs were reported in 3 (20%) and 2 (15%) of patients. No SAE was considered related to study treatment. The reported tolerability profile was generally sustained for up to 48 weeks. Dalcetrapib had no effect on fasting glucose or HbA_{1C}.
Conclusion: This analysis showed that dalcetrapib 900 mg was well tolerated in high-risk patients with T2DM and/or MetSyn after exposure for 24 weeks.
Supported by: F. Hoffmann-La Roche Ltd

1262

Dalcetrapib in high-risk patients with type 2 diabetes mellitus and/or metabolic syndrome

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Background and aims: Even with statin therapy, type 2 diabetes mellitus (T2DM) markedly increases cardiovascular (CV) risk, accounting for 70-80% of deaths. Low HDL cholesterol (HDL-C) levels often occur with T2DM and metabolic syndrome (MetSyn), and constitute an independent CV risk factor. Dalcetrapib is a selective inhibitor of cholesteryl ester transfer protein (CETP) activity that significantly raises HDL-C levels in healthy subjects and in patients with hypercholesterolaemia. This analysis evaluated the efficacy of dalcetrapib in patients with dyslipidaemia, CHD or CHD risk equivalents with and without T2DM and/or MetSyn (T2DM/MetSyn).
Materials and methods: *Post hoc* analysis of dalcetrapib 600 mg (dose used in the Phase III **dal**-OUTCOMES trial) in a total of 488 study participants, with most (~60%) having T2DM/MetSyn. Data were derived from 4 placebo-controlled Phase II trials (of up to 12 weeks' duration).
Results: Participants were predominantly male (72-88%), white or caucasian (85-95%) with a mean age 54-60 years. Overall, 296 patients with T2DM/MetSyn received dalcetrapib 600 mg (n=124) or placebo (n=172); 192 without T2DM/MetSyn received dalcetrapib 600 mg (n=90) or placebo (n=102) for 4 weeks. Mean percent changes (SE) from baseline at 4 weeks for key parameters are shown in the Table. Both groups showed similarly significant increases in HDL-C and apolipoprotein (Apo) A-I and a significant reduction in CETP activity. There was little or no change in LDL cholesterol (LDL-C) or ApoB in either patient group. Total cholesterol (TC) was significantly increased in patients with T2DM/MetSyn, presumably reflecting the increase in HDL-C. TC was also increased in patients without T2DM/MetSyn but did not reach significance. There was no significant change in triglyceride level in either patient group.
Conclusion: Dalcetrapib 600 mg had comparable lipid efficacy in patients with and without T2DM and/or MetSyn.

Parameter	With T2DM/ MetSyn	*P* (vs placebo)	Without T2DM/ MetSyn	*P* (vs placebo)
HDL-C	+24.0 (1.5)	<0.0001	+26.4 (1.8)	<0.0001
ApoA-I	+10.2 (1.1)	<0.0001	+10.7 (1.2)	<0.0001
LDL-C	-2.8 (2.2)	ns	-2.4 (2.6)	ns
ApoB	+4.4 (1.5)	<0.05	-3.1 (1.7)	ns
ApoB:ApoA-I	-4.5 (1.6)	ns	-11.8 (1.8)	<0.0001
TC	+6.6 (1.2)	<0.0001	+2.7 (1.5)	ns
CETP activity	-11.1 (3.1)	<0.0001	-10.0 (3.6)	<0.0001
Triglyceride	+0.42 (3.0)	ns	-8.2 (3.5)	ns

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PS 118 Fatty liver

1263

Non-alcoholic fatty liver disease increases the risk of cardiovascular disease in Korean type 2 diabetic patients with metabolic syndrome

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Background and aims: The metabolic syndrome (MS) is characterized as a cluster of risk factors that includes dyslipidemia, hypertension, glucose intolerance and central obesity. This syndrome increases the risk of cardiovascular disease. Type 2 diabetes is often associated with non-alcoholic fatty liver disease (NAFLD). Patient with NAFLD may be also at greater risk for CVD than those without NAFLD, and the relationship of NAFLD and metabolic syndrome is increasingly recognized. The aim of this study is to determine the association of NAFLD and the cardiovascular disease between type 2 diabetic patients with and without metabolic syndrome.
Materials and methods: Four hundred thirty type 2 diabetic patients (M: 226, F: 204; mean age: 60.92 ± 12.43 years) were recruited. All subjects underwent assessment for diabetes duration, the degree of obesity and cardiovascular risk factors, and measured fasting plasma glucose, fasting immunoreactive insulin, HbA_{1C}, fasting plasma C-peptide, fasting free fatty acid, high sensitivity C-reactive protein and lipid profiles. NAFLD was assessed by patient history and liver ultrasound. Metabolic syndrome was defined based on NCEP-ATP III criteria. The previous or current coronary heart disease (myocardial infarction, angina, or revascularization) was assessed. The patients were categorized as four groups according to the presence of metabolic syndrome and NAFLD; MS (-) and NAFLD (-), MS (-) and NAFLD (+), MS (+) and NAFLD (-), and MS (+) and NAFLD (+).
Results: The prevalence of coronary heart disease (CHD) in type 2 diabetic patients with MS was higher than that in patients without MS (44.8% vs. 31.2%, $P < 0.05$). The prevalence of CHD in type 2 diabetic patients with NAFLD was higher than that in patients without NAFLD (47.8% vs. 34.5%, $P < 0.01$). The type 2 diabetic patients with MS (-) and NAFLD (+) had the risk of CHD comparable to the risk of patients with MS (-) and NAFLD (-) (35.9% vs. 26.3%, P=0.364). However, the risk of CHD in patients with MS was significantly increased by the presence of NAFLD (51.6% vs. 34.1%, P = 0.001). In type 2 diabetic patients with MS (+) and NAFLD (-), diabetes duration ($r = 0.340$, $P < 0.001$), systolic blood pressure ($r = 0.197$, $P < 0.05$), and diastolic hypertension ($r = 0.333$, $P < 0.001$) were positively associated with CHD. Apo A1 was negatively associated with CHD ($r = -0.294$, $P = 0.001$). On a multivariate analysis, diabetes duration was independently associated with CHD. In type 2 diabetic patients with MS (+) and NAFLD (+), the number of components of MS ($r = 0.247$, $P < 0.001$), body mass index ($r = 0.153$, $P < 0.05$), and systolic blood pressure ($r = 0.145$, $P < 0.05$) were positively associated with CHD. HDL-cholesterol was negatively associated with CHD ($r = -0.209$, $P = 0.01$). On a multivariate analysis, the number of components of MS was independently associated with CHD.
Conclusion: This study suggested that the presence of NAFLD increased the risk of cardiovascular disease in type 2 diabetic patients with metabolic syndrome, but not the presence of NAFLD in patients without metabolic syndrome.

1264

Glucose intolerance in non-alcoholic fatty liver disease (NAFLD) subjects contributes to cardiovascular risk through increased subclinical inflammation rather than endothelial dysfunction

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is associated with insulin resistance and glucose intolerance. However, whether a diagnosis of NAFLD can help identify apparently healthy subjects with an increased cardiovascular risk is still unclear. The study aimed at assessing endothelial function and subclinical inflammation in subjects with NAFLD and no previously known glucose intolerance or cardiovascular disease.
Materials and methods: Study group was 46 subjects (mean age 41.5±10.0 years, BMI 30.1±8.6 kg/m2) with biopsy-confirmed NAFLD. The controls

were age- and gender-matched 13 healthy persons. All subjects had oral glucose tolerance test (OGTT) performed. Plasma lipids, insulin resistance (adiponectin, HOMA index), endothelial function (sVCAM, sICAM, sE-selectin, endothelin-1, thrombomodulin) and subclinical inflammation markers (IL-6, IL-1β) were assessed in a fasting state in all subjects.
Results: NAFLD subjects had significantly lower plasma adiponectin (2990±2883 vs 6680±4328 ng/ml, p<0.001), HDL-cholesterol (47.3±11.6 vs 59.7±14.7 mg/dl, p<0.001) and higher plasma triglycerides (240±222 vs 102±58 mg/dl, p<0.05), sVCAM (1341±833 vs 857±312 ng/ml, p<0.05), sE-selectin (60.9±32.1 vs 30.2±20.8 ng/ml, p<0.001), and thrombomodulin (1.9±0.8 vs 1.1±0.5 ng/ml, p<0.001) than controls. Upon OGTT fourteen (30%) NAFLD subjects presented with abnormal glucose tolerance (IFG and/or IGT) and 32 had normal plasma glucose. NAFLD subjects with glucose intolerance were significantly more insulin resistant than NAFLD persons with normal glucose levels (HOMA 4.2±2.2 vs 2.7±1.7, p<0.05), had similar level of endothelial dysfunction, however they had significantly greater plasma interleukin 1β levels (25.8±15.6 vs 11.3±16.5 pg/ml, p<0.05). No differences in subclinical inflammation markers were noted between NAFLD subjects with normal plasma glucose and controls.
Conclusion: In conclusion, NAFLD is generally associated with increased vascular risk due to prevalent endothelial dysfunction, which however is not related to glucose intolerance or insulin resistance. Once - whatever mild - glucose abnormalities have developed, they worsen vascular risk in NAFLD subjects by increasing subclinical inflammation.
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1265

Non-alcoholic and alcoholic fatty liver disease - two diseases of affluence associated with the metabolic syndrome and type 2 diabetes. The FIN-D2D survey
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Background: Non-alcoholic fatty liver disease (NAFLD) is known to be associated with the metabolic syndrome (MetS) and abnormal glucose tolerance. Whether alcoholic fatty liver disease (AFLD) is associated with similar metabolic abnormalities has not been examined in a population-based study.
Aims: To assess the prevalences of NAFLD and AFLD, and to examine to what extent these conditions are associated with MetS and abnormal glucose tolerance.
Materials and methods: Features of insulin resistance, components of the MetS, glucose tolerance status by an OGTT, liver function tests (LFTs), and daily alcohol consumption were assessed in a population-based sample of 2766 subjects aged 45-74 years from Central Finland.
Results: Subjects with NAFLD and AFLD were equally obese and had similar fasting and insulin concentrations. Alcohol consumption averaged 4±5 and 32±21 g/day in subjects with NAFLD and AFLD. The prevalences of NAFLD and AFLD were 21% and 7%. Thus, of subjects with elevated LFTs, 75% had NAFLD. The MetS was more prevalent in AFLD (73%) than in NAFLD (70%, p=0.028), and type 2 diabetes was similarly prevalent in NAFLD and AFLD (24-25%). The MetS and type 2 diabetes were more prevalent in subjects with NAFLD or AFLD compared to subjects with normal LFTs (53% and 14%, p<0.0001 for both).
Conclusion: The prevalence of NAFLD is 3-fold higher than that of AFLD. The prevalences of MetS and type 2 diabetes are, however, significantly increased in both NAFLD and AFLD compared to subjects with normal LFTs. Subjects with AFLD are thus similarly metabolically unhealthy as subjects with NAFLD.
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1266

Non-alcoholic fatty liver disease in patients with chronic plaque psoriasis
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Background and aims: Non-alcoholic fatty liver disease (NAFLD) and chronic plaque psoriasis are both associated with the metabolic syndrome and increased risk of incident cardiovascular disease. Although it is likely that both entities may coexist in a given patient, however, there is currently a lack of information on the association between NAFLD and psoriasis. We assessed the frequency and characteristics of NAFLD in patients with chronic plaque psoriasis.
Materials and methods: We recruited 130 consecutive patients with chronic plaque psoriasis and 260 healthy controls, who were matched for age, sex and body mass index. NAFLD was diagnosed by abdominal ultrasound after excluding other secondary causes of chronic liver disease (alcohol abuse, viral hepatitis, and current use of potentially hepato-toxic medications, including also methotrexate or tumor necrosis factor antagonists).
Results: The frequency of NAFLD was remarkably greater in psoriasis patients than in matched controls (47% vs. 28%; p<0.0001). Patients with psoriasis and NAFLD (n=61) were more likely to have the metabolic syndrome (according to the Adult Panel Treatment III definition) and had higher plasma C-reactive protein levels and greater severity of psoriasis according to the Psoriasis Area and Severity Index (PASI) score (14.2±12.6 vs. 9.6±7.4; p<0.01) than those with psoriasis alone (n=69). In multivariable stepwise regression analysis, NAFLD was associated with higher PASI score independently of age, sex, BMI, psoriasis duration and daily alcohol consumption.
Conclusion: NAFLD is frequent in patients with chronic plaque psoriasis - affecting up to nearly half of these patients - and is strongly associated with the psoriasis disease severity. Early recognition of NAFLD by radiological imaging tests in this group of patients is warranted.

1267

Liver fat associates with postprandial triglyceride-enrichment of HDL particles and endothelial dysfunction in men with type 2 diabetes and men with the metabolic syndrome
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Background and aims: Liver fat accumulation is common in type 2 diabetes (T2DM), and closely related with features of the metabolic syndrome (MetS) and increased risk of cardiovascular disease (CVD). Liver fat associates with postprandial hypertriglyceridemia, thus potentially contributing to postprandial triglyceride-enrichment of HDL (HDL-TG), and subsequent enhanced HDL particle clearance and HDL dysfunction. Low HDL cholesterol is an independent CVD risk factor and also a feature of the MetS.
Materials and methods: We assessed postprandial HDL composition changes and their association with liver fat and endothelial function, before and following 3 consecutive meals, given as breakfast (t=0h), lunch (t=4h) and diner (t=8h) during a 16h period in 12 men with uncomplicated T2DM (mean±SE: age 55±1 yrs; BMI 32±1 kg/m^2; HbA1c 7.2±0.3%), 12 normoglycemic men with the MetS, and 12 age-matched controls. Blood sampling was performed before each meal, and 12h and 16h after breakfast. Flow-mediated dilation (FMD) of the brachial artery was measured by ultrasound before each blood collection. After ultracentrifugation, TG, cholesterol, and protein content were determined in HDL. Liver fat content was measured by proton magnetic resonance-spectroscopy.
Results: Plasma TG increased significantly after the meals (P<0.001 in all groups compared to baseline), and remained elevated after diner in T2DM and MetS men only. Fasting HDL-TG was highest in T2DM, relative to MetS and controls (147.5±16, 108.3±13, 78.3±8 nmol/g, respectively; P=0.002), and increased postprandially in all groups (P<0.001). FMD correlated inversely with HDL-TG, especially after diner (r=-0.57, P<0.001). Liver fat content was highest in T2DM compared to MetS and controls (24±6, 11±3, 7±2%, P<0.02, respectively) and associated with HDL-TG-content after 3 meals (r=0.64, P<0.001), after adjustment for age, metabolic state, TG and waist.
Conclusion: We conclude that in men with T2DM and the MetS, who had the highest liver fat content, exposure to 3 consecutive meals produced exaggerated HDL-TG enrichment, which was closely associated with endothelial dysfunction.

1268

ER stress contributes to apoptosis induced by elevated fatty acids in HepG2 liver cells

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Background and aims: Chronic elevation in plasma free fatty acid (FFA) levels is commonly associated with impaired insulin-mediated glucose uptake, and often coexists with obesity and type 2 diabetes. Studies have shown that obesity causes endoplasmic reticulum (ER) stress and, in turn, insulin resistance in liver cells. In this study, we aimed to examine the effects of FFAs on ER stress and apoptosis in the human liver HepG2 cell line and to investigate whether attenuation of ER stress could protect HepG2 cells from FFA-induced apoptosis.
Materials and methods: Human liver carcinoma HepG2 cells were exposed to varying concentrations of Sodium Palmitate (saturated fatty acid, NaPa) or Sodium Oleate (unsaturated fatty acid, NaOl) for 24 h and MTT assay was conducted to measure cell viability. Apoptosis was analyzed with flow cytometry using Annexin V surface staining and PI staining. ER homeostasis of HepG2 cells was monitored over a time course of fatty acid treatment using markers of the ER stress response pathway, including GRP78/Bip and phosphorylation of eIF2α by Western Blot. Other markers of the ER stress response pathway, including IRE1α, CHOP and Bip were measured by real time RT-PCR. An expression plasmid encoding for the ER chaperone Bip (pBip) was transfected into HepG2 cells to attenuate ER stress. Small interfering RNA siCHOP was used to knockdown the expression of CHOP in HepG2. ER stress related markers, as well as apoptosis-related markers (Bax, Bcl-2 and cleaved caspase 3) were measured by Western Blotting.
Results: The saturated fatty acid NaPa led to cytotoxicity in HepG2 cells in a dose-dependent pattern; NaPa induced significant apoptosis in HepG2 cells as measured with cell cytometry. In contrast, the unsaturated fatty acid NaOl was not cytotoxic to HepG2 cells. Incubation of HepG2 cells with NaPa (0.45 mM) was associated with the induction of ER stress as indicated by increased phosphorylation of eIF2α, as well as upregulation of IRE1α and CHOP. Bip expression levels were slightly down regulated 6 h after NaPa (0.45mM) treatment. In contrast, NaOl did not induce ER stress in HepG2 cells. Overexpression of Bip attenuated NaPa-induced ER stress and led to a significant reduction in NaPa-mediated apoptosis, indicating a requirement of ER stress for lipotoxicity in liver cells. However, siRNA-mediated reduction of CHOP did not protect against NaPa-induced apoptosis.
Conclusion: These data demonstrate that the saturated fatty acid, palmitate induces ER stress and apoptosis in HepG2 cells, whereas the unsaturated fatty acid, oleate, does not. The data indicate that while ER stress makes a necessary contribution to palmitate cytotoxicity, inhibition of CHOP alone is not sufficient to prevent palmitate-induced apoptosis.

1269

Abnormal hepatic energy homeostasis in type 2 diabetes

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Background and aims: Increased hepatocellular lipids relate to insulin resistance and are typical for type 2 diabetic patients (T2DM). Steatosis and T2DM have been further associated with impaired muscular ATP turnover indicating reduced mitochondrial fitness. Thus, we tested the hypothesis that hepatic energy metabolism could be impaired even in metabolically well controlled T2DM.
Materials and methods: We measured hepatic lipid volume fraction (HLVF) and absolute concentrations of γATP, inorganic phosphate (Pi), phosphomono- and diesters (PME, PDE) using noninvasive $^{1}H/^{31}P$ magnetic resonance spectroscopy in T2DM (58±6 years, 27±3 kg/m^2), age- and BMI-matched (mCON, 61±4 years, 26±4 kg/m^2) and young lean humans (yCON, 25±3 years, 22±2 kg/m^2, p<0.005, p<0.05 vs. T2DM and mCON). Insulin-mediated whole body glucose disposal (M) and endogenous glucose production (iEGP) were assessed during euglycemic-hyperinsulinemic clamps.
Results: T2DM had 26% and 23% lower γATP (1.68±0.11; 2.26±0.20; 2.20±0.09 mmol.L^{-1}; p<0.05). Further they had 28% and 31% lower Pi than mCON and yCON (0.96±0.06; 1.33±0.13; 1.41±0.07 mmol.L^{-1}; p<0.05). PME, PDE and liver transaminases did not differ between groups. HLVF was not different between T2DM and mCON, but higher (p=0.002) than in yCON. T2DM had 13-fold higher iEGP than mCON (p<0.05). Even after adjustment for HLVF, hepatic ATP and Pi related negatively to hepatic insulin sensitivity (iEGP) (r=-0.665, p=0.010, r=-0.680, p=0.007) but not to whole body insulin sensitivity.
Conclusion: These data suggest that impaired hepatic energy metabolism and insulin resistance could precede the development of steatosis in T2DM.
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PS 119 New insights into lipid and lipoprotein function

1270

Preβ and αHDL kinetics measured by a stable isotopic and two step electrophoresis technique

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Background and aims: Apolipoprotein A-I (apoA-I), the main apolipoprotein of HDL is mainly secreted by the liver. Once secreted, apoA-I acquires lipid to form nascent discoidal preβHDL which are converted into spherical αHDL particles by gaining lipid. Current understanding of HDL metabolism is limited due to technical difficulties in measuring the kinetics of HDL subclasses. Traditionally, HDL is separated by ultracentrifugation (1.063<d<1.21 g/ml), however, this process may damage the integrity of certain HDL particles leading to the dissociation of apoA-I. The aim of this study was to develop a method to separate and measure the kinetics of preβHDL and αHDL apoA-I following isotopic labelling techniques.
Materials and methods: Following an overnight fast, four healthy subjects (3F+1M) undertook a 9 hour primed-constant infusion of 1-^{13}C-leucine with blood samples taken hourly. A novel two-step electrophoresis technique was developed and optimised to isolate apoA-I from αHDL and preβHDL subclasses. Briefly, preβHDL and αHDL in plasma were separated by agarose gel electrophoresis. The separated subclasses were then quantitatively isolated from the agarose and the apoA-I from each subclass isolated by SDS-PAGE. HDL_2 and HDL_3 subclasses from the same plasma samples were isolated by sequential ultracentrifugation and apoA-I separated by SDS-PAGE. ApoA-I bands were excised and hydrolyzed with 6M HCl. Amino acids were purified by cation exchange chromatography and derivatised for GC-MS. The isotopic enrichment of ^{13}C-leucine in apoA-I was measured by GC-MS in negative chemical ionisation (NCI) mode.
Results: For the four subjects, mean plasma cholesterol was 4.28 (SEM 0.23) mmol/L, triglyceride 0.62 (0.04) mmol/L, HDL cholesterol 1.17 (0.13) mmol/L and total plasma apoA-I was 1.43 (0.1) mg/ml. The fractional clearance rate (FCR) of preβHDL apoA-I was significantly higher than that of αHDL, 0.190 (0.022) vs 0.152 (0.017) pools/day respectively (*P*=0.039). There was also a significant difference between HDL2 and HDL3 FCR, 0.162 (0.018) vs 0.144 (0.017) pools/day respectively (*P*=0.003). There was no significant difference in HDL2 and HDL3 production rate (PR); 4.069 (0.610) vs 2.838 (0.374) mg/kg/day respectively.
Conclusion: This is the first time that αHDL and preβHDL have been separated and the kinetics measured using this technique. The results indicate αHDL and preβHDL kinetics can be measured by this methodology and that this technique is sensitive enough to measure significant differences in the kinetics of HDL2 and HDL3 particles. This methodology can be used to measure HDL subclass kinetics in type 2 diabetes and metabolic syndrome.

1271

Apolipoprotein C-I concentration is increased in type 2 diabetes

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Background and aims: Apolipoprotein C-I (ApoC-I) is bound to Very Low Density Lipoproteins (VLDL) and High Density Lipoproteins (HDL). ApoC-I has been shown in animal models to inhibit Lipoprotein Lipase and to promote VLDL production. Mice overexpressing human ApoC-I, present moderate hypercholesterolemia and hypertriglyceridemia. Furthermore, ApoC-I has also been shown to promote inflammation and atherosclerosis. So far, ApoC-I has never been studied in type 2 diabetes (DT2). This prompted us to study ApoC-I in patients with type 2 diabetes.
Materials and methods: ApoC1 concentration was measured by a specific immunoassay (ELISA) in total plasma in 30 patients with type 2 diabetes and in 32 normolipidemic controls. Moreover the distribution of ApoC-I among lipoproteins has been studied by direct measurement of ApoC-I in the HDL fraction and determination of VLDL-ApoC-I by the difference between total Apo C-I and HDL-ApoC-I.
Results: Plasma total ApoC-I (112.8 ± 36.3 vs 89.3 ± 29.0 mg/L, p=0.007) and VLDL-ApoC-I (40.7 ± 25.3 vs 23.4 ±14.3 mg/L, p=0.002) levels were significantly higher in DT2 patients. HDL-Apo C-I level (72.1 ± 21 vs 64.9 ± 19.1 mg/L, p=0.179) was also increased in the DT2 group but not significantly. In the control group, ApoC-I was mainly bound to HDL (74.2%). In patients with DT2, ApoC-I was significantly less bound to HDL (65.9% vs 74.2%, p=0.014) and more bound to VLDL (34.1% vs 24.9%, p=0.007). In unvaried analysis, total-ApoC-I was positively correlated with total cholesterol (p=0.001), LDL-C (p=0.022), triglycerides (TG) (p<0.0001), fasting glycaemia (p=0.015), BMI (p=0.001). In multivariate analysis, total-ApoC-I was correlated with TG (p<0.0001) and LDL-C (p=0.0248), but not with HDL-C, age, BMI, sex, fasting glycemia.
Conclusion: ApoC-I concentration in plasma is increased in type 2 diabetic patients. This rise is mainly due to an increase in VLDL-Apo C-I. Apo C-I level is independently associated with triglycerides and LDL, suggesting important cross-links between ApoC-I and lipoprotein metabolism. Further studies are needed to evaluate the consequences of the increase of total ApoC-I and VLDL-ApoC-I on lipoprotein metabolism and the vascular wall, in type 2 diabetes.

1272

Isosteviol elevates plasma HDL-cholesterol level - a potential new drug for the treatment of coronary heart disease

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Background and aims: The risk of coronary heart disease (CHD) is increased 2-4 times in type 2 diabetes (T2DM). With the epidemic growth in T2DM in recent years CHD is becoming a global problem of great importance. The last 20 years have witnessed dramatic reductions in cardiovascular risk using statins to lower LDL-Cholesterol. Epidemiological studies have identified low levels of HDL-Cholesterol, a common feature of T2DM, to be an independent determinant of CHD. In preliminary studies with isosteviol (ISV), a diterpene glycoside with proven anti-diabetic and anti-hypertensive capabilities, we found indications that it may possess beneficial effects on lipid levels. The aims of the study is to investigate the impact of ISV on the lipid profile of fat fed hyperlipidaemic Wistar rats.
Materials and methods: 44 normal wistar rats at the age of 6 weeks and a bodyweight of about 230 g were studied. Rats were randomized to 4 groups, with 11 in each. Group 1 and 2 were fed with standard chow diet ± ISV (0.03 g/kg/day). Group 3 and 4 were fed with a high cholesterol containing diet (1.63 % g cholesterol, 0.41 % g Cholic acid, 16.3 % g sunflower oil) ± ISV (0.03 g/kg/day). ISV had a purity of 99.4 %. The treatment period was 20 weeks. The fasting total plasma cholesterol level and the HDL-cholesterol were measured by an enzymatic colorimetric test, the "cholesterol CHOD - PAP" method, using commercially available test kits. For statistical analysis were used student t-tests and two way ANOVA-tests.
Results: The plasma HDL-cholesterol level increased by 9% for group 1 (- ISV) (Δ 0.13 ± 0.04 (SEM) mmol/l; p<0.004), and by 14% for group 2 (+ ISV) (Δ -0.17 ± 0.04 (SEM) mmol/l; p<0.002) from start to end of the study. The fasting total plasma cholesterol level increased by 29% for group 1 (-ISV) (Δ 0.39 ± 0.06 (SEM) mmol/l; p<0.0001) and increased by 4% for group 2 (+ ISV) (Δ 0.07 ± 0.06 mmol/l; p<0.267). The plasma HDL-cholesterol level increased by 42% in group 3 (- ISV) (Δ 0.49 ± 0.15 (SEM) mmol/l; p<0.011), and by 88% in group 4 (+ ISV) (Δ 1.11 ± 0.26 (SEM) mmol/l; p<0.002). The fasting total plasma cholesterol level increased by 145% in group 3 (- ISV) (Δ 2.2 ± 0.14 (SEM) mmol/l; p<0.0001), and by 175% in group 4 (+ ISV) (Δ 2.77 ± 0.38 mmol/l; p<0.0001). No deaths were observed for group 2 and 4 being treated with ISV, whereas two deaths occurred in group 3 on high fat diet and no ISV. The body weight was reduced by 16% during the 20 weeks for group 2 (chow diet + ISV) compared to group 1, (chow diet control).
Conclusion: ISV in addition to anti-hyperglycaemic and blood pressure lowering effects also seems to possess a putative role as HDL-Cholesterol enhancer with the potential to effectively counteract the CHD risk.
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1273

Association of ApoB and ApoA1 with the insulinaemic status in prediabetic subjects

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Background and aims: Investigation of covariates of insulin resistance and secretory defect can be an important tool to get more insight on the interplay of genetic and environmental risk factors in the development of the diabetic state and its complications. The present study was aimed to explore the causal association of the atherosclerotic apolipoproteins (ApoB and ApoA1) and their ratio in the basic defects of pancreatic B cell dysfunction and insulin resistance in prediabetes (IGT and IFG together) and T2DM.
Materials and methods: Following standardized selection process a total number of 131 subjects consisting of 18 IFG, 56 IGT and 57 T2DM subjects were purposively recruited. Fifty-nine healthy subjects served as control. Glucose was estimated by glucose-oxidase, lipids by enzymatic colorimetric, insulin by enzyme linked immunosorbent assay (ELISA). Serum ApoB and ApoA1 were estimated by immuno-nephelometric method.
Results: Absolute insulin (μIU) level in IGT and T2DM groups was significantly higher compared to the controls (p<0.001-0.001). HOMA%B (mean±SD) was significantly lower in T2DM groups (p<0.001) and higher in IGT (p=0.008) compared to the controls although it is significantly lower in IFG (p=0.001). HOMA%S was significantly lower in IGT and T2DM group (p=0.001 and 0.002 respectively), but did not show any significant difference in IFG. Triglyceride level was significantly higher in all three groups (p=0.044 -<0.001) and total cholesterol only in T2DM group (p=0.001). Mean (±SD) ApoA1 value was 0.97±0.25, 1.05±0.27, 1.05±0.25 and 1.08±0.26 in the Control, IFG, IGT and T2DM groups respectively. Mean (±SD) ApoB value was 0.82±0.19, 0.91±0.34, 0.92±0.26 and 1.04±0.29 respectively. ApoA1 was significantly higher only in the T2DM group and ApoB was higher in IGT and T2DM group (p=0.026-<0.001). Neither ApoB nor ApoA1 showed any significant difference in the IFG group as compared to control. ApoB-ApoA1 ratio did not show significant difference among the groups. Correlation analyses revealed significant positive correlation for ApoB with both fasting and postprandial glucose in T2DM (p=0.006 and 0.040 respectively). In IGT group ApoB was positively correlated with absolute insulin (r=0.314, p=0.025) and HOMA%B (r=0.277, p=0.049) and negatively with HOMA%S (r=-0.312, p=0.026). In T2DM ApoB was negatively correlated with HOMA%B (r= -0.318, p=0.017). ApoB-ApoA1 ratio showed similar pattern of that of ApoB alone.
Conclusion: ApoB, but not ApoA1 or ApoB-ApoA1 ratio, seem to have a causal association with insulin resistance and elevation of ApoB is also modulated by obesity and atherogenic lipids. Since insulin resistance is a substantially modifiable factor through life style adjustment, the association of the atherogenic apolipoproteins with this defect can have a great public health consequence.
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1274

Genetic determinants of atherogenic dyslipidaemia in patients with type 2 diabetes mellitus

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Background and aims: Elevated triglycerides (TG) and reduced high-density lipoprotein cholesterol (HDL-cholesterol) levels are the main features of atherogenic dyslipidemia, which contributes to increased cardiovascular risk in patients with type 2 diabetes mellitus. The apolipoprotein A1 (apoA1) and A5 (apoA5) genes have been shown to play an important role in determination of plasma TG and HDL-cholesterol concentrations, but little is known whether polymorphic variants of these genes influence lipid disorders in type 2 diabetic patients. The aim of our study was to evaluate if there is an association between apoA1 and apoA5 gene polymorphisms and atherogenic dyslipidemia in patients with type 2 diabetes mellitus.
Materials and methods: We examined 225 patients with type 2 diabetes mellitus (161 females and 64 males, mean age 57±0,4 years), not taking lipid-lowering drugs. Serum lipids were evaluated by enzymatic method. G-75A and C83T apoA1, S19W and -1131T>C apoA5 gene polymorphisms were identified by polymerase chain reaction - restriction fragment length polymorphism method
Results: Atherogenic dyslipidemia was revealed in 115 patients (51,1%), 110 patients (48,9%) had normolipidemia (controls). The apoA5 S19W genotypes frequency was significantly higher in male patients with hypertriglyceridemia (TG above the 90th percentile for age and sex) than in patients with normolipidemia (p=0,032) and in female patients who had hypertriglyceridemia with low HDL-cholesterol level (HDL-cholesterol below the 10th percentile for age and sex) than in controls (p=0,013). The frequency of apoA1 genotypes with -75A allele was significantly higher in female patients who had hypertriglyceridemia with low HDL-cholesterol than in control patients (p=0,016). There was not significant difference in distribution of C83T apoA1 and -1131T>C apoA5 allele and genotype frequencies between patients with dyslipidemia and controls.
Conclusion: Our findings reveal an association between apoA1 and apoA5 gene variants and atherogenic dyslipidemia in patients with type 2 diabetes mellitus.

1275

Apoprotein A5 protects from atherosclerosis in mice

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Background and aims: Apoprotein A5 (apoA5) is a regulator of plasma triglycerides (TG) in patients with and without diabetes. Transgenic mice (apoA5tr) had decreased, and knock out mice 4fold increased TG. Based on human data it has been proposed that apoA5 may reduce coronary heart disease. In this study, a possible anti-atherogenic effect of apoA5 was investigated in different animal models.
Materials and methods: The apoA5tr was bred both onto the FVB-apoE deficient (apoEKO) background and on the FVB-LDL-receptor deficient (LDLRKO) backgrounds. At 22 - 26 weeks, lipoprotein profiles of individual mice were measured, and hearts and aortas were removed for quantification of atherosclerosis.
Results: ApoA5 was able to reduce atherosclerosis in both the apoEKO and the LDLRKO animal models by more than 50%. This finding was confirmed by en face preparation of whole aortas. In apoEKO mice, apoA5 dramatically reduced plasma VLDL TG by more than 70% in both genders. Here, lesion size correlated best with VLDL TG. Lipoprotein profile changes were lass pronounced on the LDLRKO background. As in wild type animals, turnover studies with different lipoprotein labels using apoE deficient VLDL showed a faster plasma TG and lipoprotein particle turnover. However, liver lipoprotein uptake was not significantly affected by apoA5. To explore the function of apoA5 for receptor mediated endocytosis at the cellular level, uptake of radiolabeled remnants in primary hepatocytes from WT and apoA5tr mice was measured. Here, preliminary data do not reveal significant differences.
Conclusion: It is concluded, that apoA5 is strongly atheroprotective in mice independent from the mouse model used.
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1276

Comparison of efficacy of pitavastatin and colestimide in Japanese patients with diabetes mellitus complicated by hyperlipidaemia and metabolic syndrome

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Background and aims: Metabolic syndrome refers to a syndrome in which risk factors for atheroclerosis such as hyperglycemia, insulin resistance, hyperlipidemia, hypertension, and other conditions have accumulated, and is associated with a very high risk of triggering of atherosclerosis. Hyper-LDL-cholesterolemia is known to be a strong risk factor for coronary artery disease independent of metabolic syndrome. Anion exchange resin preparations have recently been attracting close attention because they exert hypoglycemic effects in addition to lipid profile-improving effects. The present study was

undertaken to evaluate the effects of pitavastatin and colestimide (a drug stimulating bile acid excretion) in patients with diabetes mellitus complicated by hyperlipidemia and metabolic syndrome.
Materials and methods: Forty-eight Japanese patients with diabetes mellitus complicated by hyperlipidemia and metabolic syndrome were randomly assigned to either a 24-week pitavastatin (1.0mg/day) treatment group or a colestimide (3.0g/day) treatment group. There were no significant differences between the two groups in demographic parameters, blood glucose level, lipid profiles, oral drugs used, or other test data at baseline. Over the 24-week period of administration, systolic and diastolic blood pressure, BMI, triglyceride(TG), HDL- and LDL-cholesterol, AST, ALT, plasma glucose, HbA1c, IRI, and high sensitivity CRP were measured. Postprandial glucose levels were evaluated after 120 min in the 75gOGTT. Pulse wave velocity (PWV) were measured with an automatic electronic sphygmomanometer.
Results: Treatment with pitavastatin significantly reduced LDL-C and TG, while that with colestimide significantly reduced waist circumference, BMI, LDL-C, HbA1c, FPG, PPG, and HOMA-R. Furthermore, hs-CRP also exhibited significant reduction with colestimide treatment (586.3 ± 352.9 vs 1016.0 ± 1063.2 μg/dl, $p<0.05$). Percent improvement in LDL-C was significantly greater in the pitavastatin group than in the colestimide group. Compared to the pitavastatin group, the colestimide group exhibited significantly greater improvement in mean change of HbA1c (-9.0 vs 0.3%, $p<0.001$), FPG (-9.7 vs 1.3%, $p<0.01$), PPG (-15.2 vs 5.4%, $p<0.05$), HOMA-R (-19.3 vs 24.0%, $p<0.05$), IRI (-11.7 vs 19.4%, $p<0.05$), and BMI (-4.3 vs 1.0%, $p<0.01$).
Conclusion: Colestimide appeared to be useful in the management of Japanese patients with diabetes mellitus complicated by metabolic syndrome, since it alleviates obesity and insulin resistance in addition to exhibiting lipid profile-improving effects, and can thus improve markers of atherosclerosis.

1277

High fat diet and chronic ethanol consumption impair the expression of key molecules in AMP activated protein kinase pathway in rat cardiac muscle

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Background and aims: Our previous work has shown that chronic ethanol consumption impairs the mRNA expression of key factors of insulin-dependent pathway, for example, IR, IRS, GLUT4 etc. in the cardiac muscle in rats. Also long-term moderate ethanol consumption reversed the adverse effects of saturated fatty acid on GLUT4 expression in adipose tissues of rats. In the present study, we investigated the combined effects of saturated fatty acid and ethanol on several signal molecules in AMP activated protein kinase(AMPK) pathway in the cardiac muscle in rats, and thus probe into the possible mechanism of these effects on the glucose metabolism in the cardiac muscle.
Materials and methods: Forty-eight male Wistar rats, randomly divided into four groups: normal diet control group (C), high fat diet group (HF), ethanol group (E) and high fat diet plus ethanol group (HF+E). The HF diet consisted of 59 % fat from lard (a representative food full of saturated fatty acid in China) whereas the normal diet contained 10% fat, and the edible ethanol dosage was 5 g·kg-1·d-1. After treatment for 22 weeks, all animals were sacrificed and the left ventricles were quickly removed and saved for later analysis. The mRNA and protein levels were determined by RT-PCR and western blotting method respectively.
Results: Compared with the control group, all mRNA and protein expression of the selected signal molecules were decreased in treatment groups. The data are as follows in table 1.

Table 1. The expression decreases of signal molecules compared to group C

		group E	group HF	group HF+E
mRNA	AMPK α1	49.50 % *	41.06 % **	33.70 %
	AMPK α2	34.50 % *	21.29 % **	15.99 %
	MEF2A	57.68 % *	15.61 %	26.14 % **
	MEF2D	35.79 % **	20.32 %	35.81 % **
	GLUT4	41.00 % *	8.27 %	18.92 %
protein	AMPK α	37.72 %	36.59 %	41.74 % **
	P-AMPK α	33.91 %	11.58 %	15.31 % **
	MEF2	21.27 % **	29.82 % **	23.71 % **
	GLUT4	33.28 % *	18.50 %	30.42 % **

* P < 0. 01 , ** P < 0. 05

Either ethanol alone intaking (group E) or high fat diet alone (Group HF) significantly decreased the expression levels of key signal molecules in the AMPK/MEF2 pathway, while in group HF+E, the mRNA decreases in both AMPK α1 and AMPK α2 subunits were not so remarkable. The protein expression of key signal molecules in the AMPK/MEF2 pathway was correspondingly reduced compared to group C. However, the P-AMPKα activity level in group HF+E showed an obvious reversion relative to the impairment in group E. This is consistent with our former reports in adipose tissues and the study of Fisher H, that is, the moderate saturated fatty acid can partly neutralize the effect of ethanol to AMPK expression.
Conclusion: High fat diet and chronic ethanol consumption impair the expression of key molecules in AMPK pathway. Moderate saturated fatty acid plus chronic ethanol intaking presents, at least at a certain extent, a benefit effect on AMPK expression in rat cardiac muscle.
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1278

High FFA-induced proliferation and apoptosis in human umbilical vein endothelial cell partly through Wnt/β-catenin signal pathway

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Background and aims: Free fatty acids (FFA)-induced proliferation and apoptosis was studied in human umbilical vein endothelial cells (HUVECs).
Materials and methods: A recombinant adenovirus containing a RNAi cassette targeting the GSK-3β gene was produced and its silencing effect on GSK-3β gene was detected by Western blot analysis and immunohistochemistry assay in HUVECs. The effect of the RNAi on the protein level of β-catenin was explored by transfecting the RNAi adenovirus to inhibit the expression of GSK-3β protein. The subsequent effect on the Wnt/GSK-3β/β-catenin signal pathway and on proliferation and apoptosis of HUVECs cultured with FFAs, was analyzed by BrdU assay ,Annexin V-FITC /PI Apoptosis Detection Kit , and 4',6-diamidino-2- phenylindole(DAPI) in order to explore the possible connection between the signaling pathway and FFA-induced proliferation and apoptosis.
Results: The Western blot results showed that the expression of GSK-3β protein in HUVECs could be inhibited efficiently by the RNAi adenovirus, and that the protein level of β-catenin was increased by RNAi adenovirus transfection. The results of the BrdU assay suggested that knockdown of GSK-3β with the RNAi adenovirus may stimulate the proliferation of HUVECs. Apoptosis was observed in HUVECs exposed to FFAs (0.75 mmol/L) for 72 h, and this effect could be partly reversed when interfering with the RNAi adenovirus.
Conclusion: It may be concluded that the RNAi adenovirus specific to GSK-3β may partly protect HUVECs from apoptosis induced by FFAs. Up-regulation of the Wnt/β-catenin signal pathway can partly reverse FFA-induced apoptosis in HUVECs.
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1279

Adiponectin is an independent predictor of apolipoprotein B/A1 ratio in different grades of glucose intolerance

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Background and aims: Recent studies have reported that Apolipoprotein B/A1 ratio is a better predictor of atherosclerotic vascular disease compared to LDL-C. The aim of this study was to assess the association of serum Apolipoprotein B/A1 ratio with insulin resistance and PWV in patients with different grades of glucose intolerance
Materials and methods: According to glucose tolerance, NGT without metabolic syndrome group (N=229), IFG group (subjects with fasting plasma glucose level of between 100-125 mg/Dl, N=658), type 2 diabetes group(N=381) were divided. To minimize the possible confounding effects of glucose-lowering pharmacological treatment, included diabetic patients were not treated with insulin or thiozolidinedione, and taking lipid lowering medication(fibrate or statin) were excluded. BMI, WC, and serum concentration of apolipoprotein B, apolipoprotein A1, glucose, lipids (triglycerides,

LDL cholesterol, HDL cholesterol, and total cholesterol) were measured. Insulin resistance was estimated by the insulin resistance index of homeostasis model assessment (HOMA-IR) & serum adiponectin. PWV was evaluated to assess arterial stiffness

Results: The subjects were divided 3 groups according to glucose tolerance(NGT, IFG and T2DM group), there were significant differences in metabolic parameters among the groups, and WC, insulin, adiponectin, HOMA-IR, aortic and peripheral PWV, Apo B/A1 ratio which increased sequentially with glucose intolerance. Apo B/A1 ratio significantly correlated with total cholesterol (NGT,IFG,T2DM: γ=0.33, γ=0.21, γ=0.38, all P<0.01), TG (γ=0.28, γ=0.22, γ=0.29, all P<0.01), LDL-C (γ=0.47, γ=0.29, γ=0.39, all P<0.01), HDL-C (γ= -0.46, γ= -0.28, γ= -0.34, all P<0.01), adiponectin (γ= -0.35, γ= -0.28, γ= -0.21, all P<0.01), HOMA-IR (γ=0.20, γ=0.09, γ=0.08 , all P<0.05). Multple regression analysis showed that Apo B/A1 ratio was significantly associated with total cholesterol (β=0.50, β=0.18, β=0.27, all P<0.01), LDL-C (β=0.44, β=0.22, β=0.18, all P<0.01), HDL-C (β= -0.59 β= -0.32, β= -0.29, all P<0.01), and adiponectin (β= -0.11, β= -0.09, β= -0.13, all P<0.05)

Conclusion: The apo B/A1 ratio is significantly associated with insulin resistance according to glucose intolerance and serum adiponectin is an important independent factor associated with Apo B/A1 ratio in Koreans.

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1280

High prevalence of persistent lipid abnormalities in statin treated patients with sedentary lifestyle in Europe and Canada: results of the Dyslipidemia International Study

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Background and aims: Statins are widely used for treatment of dyslipidemia. Many patients on statin however do not reach their lipid targets. This analysis of patients from DYSIS investigated whether patients with sedentary lifestyle (SL) differ in cardiovascular disease risk factor profile (RFP) and LDL-C target achievement from patients without this risk factor. Sedentary lifestyle was considered if the patient was reported not to conduct usual physical activity (i.e. a minimum of walking 20-30 minutes 3-4 days a week or equivalent).

Materials and methods: The data originate from a large cross-sectional study in 12 countries (Europe, Canada) done in 2008 that comprise data from clinical examination as well as latest lipid values from consecutively recruited outpatients ≥45 years on chronic statin therapy. To evaluate the role of SL a stepwise logistic regression analysis adjusting for patient characteristics and lipid lowering therapy was performed.

Results: Prevalence of RFP (e.g. diabetes mellitus, metabolic syndrome, obesity, and other comorbidities) was higher in patients with SL compared to those without SL as well as LDL-C not at goal, low HDL-C, and elevated triglycerides (TG) according to ESC Guidelines. The results identified SL as independent predictor of 'LDL-C not at goal' (OR 1.19, 95%-CI 1.11-1.26).

Conclusion: Patients with SL have an unfavourable RFP and a worse lipid profile than those without. A majority of statin treated patients with SL and comorbidities in this cohort was not at lipid goal and/or had abnormal levels of HDL-C and triglycerides. These results prompt to improve life style and a more intensive and comprehensive lipid management in these patients.

	Patients with sedentary lifestyle N=10,384 (49.0%)	Patients without sedentary lifestyle N=10,801 (51.0%)
Age (years, ± SD)	66.1 ±10.2	65.5 ±9.6 [t]
Female [%]	44.2	39.1*
Parental history of diabetes	31.0	26.9*
BMI ≥ 30 mg/m² [%]	39.4	27.5*
Diabetes mellitus[%]	43.4	41.7*
Metabolic syndrome (ATP III) [%]	56.4	43.3*
Current smoker [%]	17.2	13.0*
Hypertension [%]	78.0	35.3*
Ischemic heart disease [%]	35.7	36.8[§]
Heart failure [%]	11.3	7.0*
≤10 mg/day Simvastatin equivalent [%]	11.5	12.9 [tt]
20-40 mg/day Simvastatin equivalent [%]	76.1	75.9[§]
≥80 mg/day Simvastatin equivalent [%]	12.4	11.2 [tt]
Ezetimibe [%]	10.1	9.6[§]
LDL-C not at goal [%] (1)	50.7	47.0*
Low HDL-C [%] (2)	31.0	25.7*
Elevated TG [%] (3)	53.2	43.7*

(1) LDL-C ≥100 / 115 mg/dL (high-risk and low-risk patients respectively)
(2) HDL-C < 40 mg/dL (male); < 45 mg/dL (female)
(3) TG > 150 mg/dL
* p<.0001, [t] p<.001, [tt] p<.01, [§] not statistically significant

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PS 120 Lipids and metabolism

1281

Hypertriglyceridaemic waist phenotype is associated with increased severity of coronary artery disease both in type 2 diabetes and nondiabetic patients

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Background and aims: It has been previously shown that the presence of hypertriglyceridemic waist (HTgW) phenotype (waist girth ≥90 cm in men and ≥85 cm in women, and plasma triglyceride (Tg) concentration of ≥2.0 mmol/L) could identify a high-risk patient with excess visceral adipose tissue who is at high risk for development of coronary artery disease (CAD). However, the relationship between the presence of HTgW phenotype and related metabolic parameters (insulin sensitivity (IS) and cholesterol subfraction levels) and the severity of coronary artery disease (CAD) expressed as the number of stenotic coronary arteries (SCAs) in patients with Type 2 diabetes (T2D) has not yet been elucidated. Therefore, this study was aimed to analyze the (a) severity of CAD, (b) IS levels, (c) lipoprotein subfraction levels and (d) LDL particle size in the following groups of patients referred to and underwent coronary angiography for suspected CAD: (a) T2D with HTgW (group A, n=28); (b) T2D without HTgW (group B, n=12); (c) nondiabetics with HTgW (group C, n=22); (d) nondiabetics without HTgW (group D, n=38).
Materials and methods: CAD was angiographically verified in each patient included in this study and defined as a stenosis with narrowing of the lumen >50% with respect to the pre-stenotic segment in the main coronary blood vessels. The severity of CAD was evaluated based on the number of SCAs and defined as single vessel CAD (one SCA) or multivessel CAD (at least two SCAs or the stenosis of the left main artery). Insulin sensitivity levels (Si) was determined by minimal model analysis, total cholesterol (Ch), HDL-Ch, LDL-Ch and Tg levels by enzymatic methods and the LDL particle diameter by using gradient gel electrophoresis.
Results: We found that in both T2D and nondiabetic patients significantly higher percentage of patients with HTgW phenotype had multivessel CAD in comparison to patients without this phenotype (group A vs B: 83.3% vs 37.5%, p<0.05; group C vs D: 64.3% vs 33.3%; p<0.05). Also, Si values were lower in group A vs B (A: 1.47+/-0.34; B:1.91+/-0.03 $min^{-1}/mU/lx10^4$; p<0.05) and in group C vs D (C: 3.86+/-0.66; D: 6.24+/-1.12 $min^{-1}/mU/lx10^4$; p<0.05). Simultaneously, in T2D patients we found significantly lower level of HDL-Ch in group A vs B (0.92+/-0.07 vs 1.21+/-0.07 mmol/l; p<0.05) while we could not find significant differences in total, LDL-Ch and the mean LDL particle size levels between these groups. In contrast, in nondiabetic patients we did not find differences in HDL-Ch levels, while we found significantly higher levels of total Ch and LDL-Ch and smaller mean LDL particle size in group C vs D (26.02+/-0.18 vs 26.89+/-0.18 nm; p<0.01).
Conclusion: Our results signify that both in T2D and nondiabetic patients presence of HTgW phenotype is strongly associated with multivessel CAD as a more severe type of CAD. Also, the data signify that this association might be based on increased insulin resistance both in T2D and nondiabetics as well as on different lipid abnormalities involving primarily reverse cholesterol metabolism in T2D and impairments in the structure of LDL with accumulation of small dense LDL particles in nondiabetic subjects.

1282

The higher burden of small low-density lipoprotein particles is associated with profound changes in serum testosterone concentration in male adolescents

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Background: Males are at higher cardiovascular risk than females from a young age. Dyslipidaemia, including a higher burden related to small low-density lipoprotein (LDL), plays an important role in precipitating atherosclerosis in both males and females. It is important to clarify which factors are related to these sex differences in cardiovascular risks from childhood, especially in males. We investigated the sex differences of atherogenic lipoprotein burden and independent predictors of LDL particle size in children and adolescents.
Methods: We measured the concentrations of total testosterone, sex hormone-binding globulin (SHBG), estradiol (E2), adiponectin, total cholesterol, triglyceride (TG), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), and LDL particle size in 135 children and adolescents (67 boys, 68 girls).
Results: Serum testosterone concentration was significantly negatively correlated with LDL particle size (r = -0.336, p = 0.016) in male adolescents, but oestrogen and LDL particle size were not related. Higher TG and lower HDL-C concentrations were independently correlated with LDL particle size in both children and adolescents. After excluding the lipid profile in the multivariate analysis, BMI (β = -0.311, p = 0.006) in children and testosterone concentration (β = -0.336, p = 0.016) in adolescents were independently associated with LDL particle size. After mid-puberty, LDL particle size declined markedly in male adolescents.
Conclusion: Serum testosterone concentration correlated significantly with LDL particle size in male adolescents, especially after mid-puberty. The prominent decrease in LDL particle size associated with the increase in testosterone concentration in males might explain why males are more likely to display atherogenic dyslipidaemia from adolescence.

1283

Elevated circulating NEFA concentration are related to cardiac dysfunction of obese rats

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Background and aims: Obesity is associated with cardiac hypertrophy and abnormal cardiac function, the mechanism of which is still not quite clear. Elevated circulating NEFA level may play an important role in the development of heart dysfunction. The aim of the study is to explore the effects of decreasing blood NEFA concentration on cardiac function in obese rats.
Materials and methods: 4 week aged male S-D rats were divided into three groups: control group(NC, n=16) fed normal chow, obese group(OB, n=16)and fenofibrate group (OB+F, n=16) fed high fat chow. Four weeks later, Fenofibrate(20mg/kg/d) was given to the rats in OB+F group by oral gavage. AT the end of 8 weeks and 16 weeks the maximum velocity of myocardial contraction ($+dP/dt_{max}$) and relaxation ($-dP/dt_{max}$) were tested by physiological polygraph. The concentrations of triglyceride (TG) and NEFA both in blood and in left ventricular were measured. The expressions of NF-κB and iNOS in myocardial cell were visualized by immunohistochemical

Table 1 (abstract 1283) The cardiac function and TG and NEFA concentrations in blood and myocardium

		NC		OB		OB+F	
		8w(n=8)	16w(n=8)	8w(n=8)	16w(n=8)	8w(n=8)	16w(n=8)
Weight (g)		369.5±41.3	425.9±24.1	423.9±43.9*	510.8±41.8**	411.3±26.2	534.1±60.1
TG	blood(mmol/l)	0.76±0.17	0.83±0.17	1.32±0.35*	1.57±0.37**	0.87±0.34#	1.00±0.39#
	Myocardium(mmol/g)	0.68±0.16	0.87±0.22	1.23±0.35*	1.48±0.36*	0.95±0.28#	0.99±0.31#
NEFA	blood(μmol/l)	221.9±75.9	360.7±91.8	679.3±157.6*	1247.4±227.9**	467.9±124.9#	501.4±188.3#
	myocardium(μmol/g)	433.8±146.4	518.4±151.9	682.9±201.1*	744.7±213.5*	521.0±120.5	614.8±240.3
$+dp/dt_{max}$ (mmHg/s)		4334±427	3787±544	3444±416*	2965±320*	3546±752	3448±647
$-dp/dt_{max}$ (mmHg/s)		-4174±462	-3564±440	-3267±538*	-2815±391*	-3388±667	-3223±522

* P <0.05, ** P <0.01, vs the NC group; #: P<0.05, ##: P<0.01, vs the OB group.

methods and semi-quantified by morphometric image analysis (integrated optical density, IOD)
Results: The OB group rats developed obesity and left ventricular hypertrophy. The TG and NEFA levels either in myocardial homogenate or blood were increased and the values for $+dP/dt_{max}$ and $-dP/dt_{max}$ were lower (table 1) in OB group. Fenofibrate intervention improved lipids profile and cardiac function. The expressions of NF-κB and iNOS in myocardium were higher in OB group (IOD: NFκB: 32000, vs. 55176, P<0.05; iNOS: 24949, vs. 68315, P<0.01), which was partly restored in OB+F group (IOD: NFκB: 30741; iNOS: 39525). Intramyocardial lipids deposition was associated with plasma TG and NEFA concentrations. The values of $+dp/dt_{max}$ and $-dp/dt_{max}$ were negatively correlated with the intramyocardial TG and NEFA concentration and the score of NF-κB, iNOS.
Conclusion: Elevated circulating TG and NEFC levels are associated with ectopic lipid accumulation in myocardium, which may play an important role in the development of heart dysfunction through triggering an inflammatory signaling cascade.
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1284

Prevalence of lipid abnormalities before and after introduction of lipid modifying therapy among Swedish patients with type 2 diabetes and/or coronary heart disease (PRIMULA Sweden)
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Background and aims: Recent data on the extent of lipid abnormailities and treatments used among adults with dyslipidemia are limited; particularly among those with coronary heart disease (CHD) or type 2 diabetes mellitus (T2DM). We therefore examined the prevalence of lipid abnormalities and utilization of lipid modifying therapies (LMT) to determine any gaps in therapy in a Swedish longitudinal assessment of CHD and T2DM patients.
Materials and methods: This retrospective longitudinal study is based on primary health care electronic medical records (EMR) from the county of Uppsala, Sweden. We identified patients at least 35 years of age, experiencing CHD only (CHD without T2DM), CHD+ T2DM and T2DM only (T2DM without CHD), who initiated a LMT between May 1994-June 2007, continued treatment for 1 year and had at least 1 lipid abnormality prior to initiation of LMT. Patients selected were also required to have a complete lipid panel consisting of low-density lipoprotein cholesterol (LDL-C), high- density lipoprotein cholesterol (HDL-C) and triglycerides (TG), 13 months pre (baseline) and 1-year post (follow-up) therapy initiation. Target/normal levels for LDL-C, HDL-C and TG were specified as per NCEP ATP III Guidelines.
Results: Sample consisted of CHD only (n=337), CHD+T2DM (n=95) and T2DM only (n=1,033) patients. Mean age of the above 3 groups was 74, 75 and 67 years respectively with 57%, 60% and 54% men in the respective groups. At baseline, 91% of patients with CHD exhibited elevated LDL-C compared to 82% of those with CHD+ T2DM and 90% of those with T2DM. Low HDL-C was observed among 30%, 46% and 42% of patients with CHD, CHD+ T2DM and T2DM respectively while 31% of patients with CHD, 41% with CHD+ T2DM and 52% with T2DM had elevated TG levels. Prior to therapy, 39%, 46% and 57% of patients with CHD, CHD+T2DM and T2DM respectively experienced elevated LDL-C coupled with low HDL-C and/or elevated TG. Over 90% of patients utilized statin monotherapy. Post therapy breakdown for lipid abnormalities is shown in the table.

Lipid Abnormality Profile by Risk Status

	CHD only (n=337) (1)	CHD+T2DM (n=95) (2)	T2DM only (n=1,033) (3)	p value (1) vs. (2)	p value (1) vs. (3)	p value (2) vs. (3)
Elevated LDL-C	59%	38%	51%	<0.01	<0.05	<0.05
Low HDL-C	22%	39%	41%	0.001	<0.01	0.677
Elevated TG	18%	24%	36%	0.162	<0.01	<0.05
Elevated LDL-C + low HDL-C and/ or elevated TG	18%	17%	28%	0.828	<0.01	<0.05

Conclusion: In this longitudinal study of Swedish high CV risk patients primarily treated with statins, improvement in LDL-C level post LMT was substantial: at least one out of three patients achieved LDL-C goal, approximately additional 13-17% achieved normal TG levels while improvement in HDL-C level was small (up to 8% among CHD+ T2DM patients). Post therapy patients with CHD had significantly higher percentage with elevated LDL-C and significantly less with low HDL-C and elevated TG compared to CHD+ T2DM and T2DM groups. Despite LMT, over a quarter of T2DM patients and up to one in five patients from the other two groups, continued to exhibit elevated LDL-C coupled with low HDL-C and/or elevated TG and could potentially benefit from higher doses of statin or as recommended in the guidelines from adding another therapy to statins.

1285

High-density lipoprotein cholesterol effects of pioglitazone compared with non-TZD oral medications in a US managed care cohort
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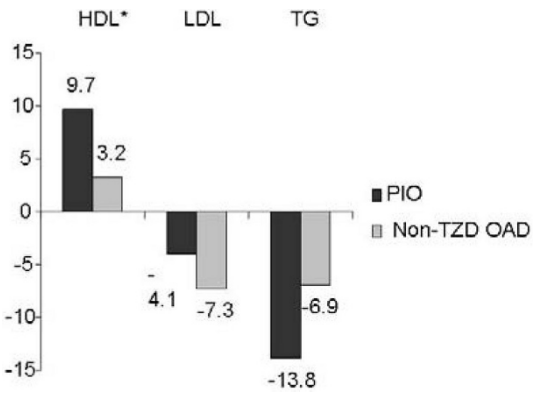
Background and aims: Decreased high-density lipoprotein cholesterol (HDL-C) is characteristic of diabetic dyslipidemia and a risk factor for cardiovascular disease among patients with type 2 diabetes mellitus (T2DM). Nearly half of patients with T2DM in a primary care cohort had HDL-C values below normal, suggesting that strategies to raise HDL-C are not incorporated into treatment regimens. In randomized controlled clinical trials, pioglitazone (PIO), an oral antidiabetic (OAD) agent, has consistently raised HDL-C and lowered triglycerides (TG). To further explore the lipid effects of PIO, a retrospective claims analysis in a large database of United States privately-insured enrollees was conducted.
Materials and methods: The Integrated Healthcare Information Services database (01/01/00-03/31/07), a database of US managed care population was used. Analysis inclusion criteria were new-onset T2DM patients treated with PIO or non-TZD OAD monotherapy regimens. Patients using insulin were excluded. Patients were required to have at least one HDL-C, a low-density lipoprotein cholesterol (LDL-C), and TG lab value at baseline and another value during 12 months follow-up.
Results: Both treatment groups were similar in age (55 years). There was a higher percentage of women in the non-TZD OAD (47.2%) than the PIO (35.2%) group. Baseline lipid values were (mg/dL): LDL-C 117.2, TG 229.3, and HDL-C 43.2 for PIO and LDL-C 120.2, TG 220.8, HDL-C 44.4 for non-TZD OAD. While HDL-C was increased from baseline in both groups over 12 months, the magnitude of increase was greater with PIO (P<0.0001 Figure). Both PIO and non-TZD OAD treatment were associated with reductions of LDL-C and TG. After age and gender adjustments, HDL-C increases remained significant for both groups and between groups. Among patients who took statins prior to OAD treatment, HDL-C increased by 10.0% in the PIO group and 2.8% in the non-TZD group, while LDL-C decreased 1.5% and TG decreased 13.8% from baseline in the PIO group and 4.8% and 4.9%, respectively, in the non-TZD group.
Conclusion: PIO is associated with increased HDL-C and decreased TG levels compared with baseline and non-TZD OAD treatment in this database, a finding consistent with data from clinical trials and independent of statin use. Further analyses are planned to assess the economic benefit of these improvements.
Figure: Percent Change in HDL-C, LDL-C, and TG in Patients Treated with Pioglitazone or non-TZD OAD for 12 Months
* P<.0001 PIO vs. OAD



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1286

Beneficial effects of a high-fat diet on plasma HDL concentration and macrophage cholesteropl efflux in a murine model of metabolic syndrome

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Background and aims: The metabolic syndrome (MS) is a cluster of abnormalities conferring increased risk of type II diabetes and cardiovascular diseases. We generated transgenic mice overexpressing human apo AII (hapo A-II), a major HDL apolipoprotein, which present three MS traits: postprandial hypertriglyceridemia, low plasma HDL levels and glucose intolerance. In this study our first aim was to investigate whether the low plasma HDL levels originate from decreased formation by macrophages. Macrophages in the arterial wall accumulate cholesterol following uptake of oxidized LDL (a non-regulatable process), and are thus implicated in initial steps of atherosclerosis. The cholesterol load of macrophages decreases through cholesterol efflux, stimulated by ABCA1 (ATP Binding Cassette Transporter AI), and resulting in formation of nascent HDL. Our second aim was to study the effects of a moderate high-fat (HF) diet on plasma HDL concentration and on HDL formation by macrophages.
Materials and methods: Hapo A-II transgenic mice and control C57BL/6 mice were fed for 2 months either low-fat chow diet or HF diet (20% coconut oil containg 48% lauric acid). Plasma lipoproteins were analyzed after ultracentrifugation and FPLC. Mouse peritoneal macrophages (MPM) were isolated from transgenic and control mice on both diets, and primary cultures were performed. MPM were loaded with cholesterol by incubation with acetyl-LDL and [3H] cholesterol, and treated with cAMP to upregulate ABCA1 expression. Efflux of [3H] cholesterol was measured in the absence or presence of acceptors (lipid-free apo A-I or apo A-II in equimolar concentrations). Nascent HDL were detected in the media by bi-dimensional electrophoresis.
Results: Under HF feeding, plasma HDL and apo A-I levels increased 2.5 times in transgenic mice, whereas their hypertriglyceridemia persisted.
MPM from the four groups of mice (hapo AII-transgenic and control mice, under chow or HF diet) displayed similar cholesterol efflux capacities, both in the absence of acceptors (passive efflux) and in the presence of apo A-I or apo A-II (ABCA1-mediated efflux). Of note, apo A-I and apo A-II promoted cholesterol efflux to similar extents. Nascent HDL were formed with apo A-I and apo A-II. MPM from chow and fat-fed transgenic and control mice displayed comparable capacities to synthesize nascent HDL.
Conclusion: The low plasma HDL of our murine model for MS is not due to impaired HDL synthesis by macrophages. Interestingly, the lauric acid-rich moderate HF diet improved the MS phenotype of transgenic mice by markedly increasing HDL, a protective factor against atherosclerosis. Moreover, this diet allowed an efficient cholesterol efflux from MPM, as well as an adequate HDL synthesis.
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PS 121 Endothelial dysfunction

1287

The effect of hypoxia and hyperglycaemia on migration of microvascular endothelial cells

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Background and aims: Type 1 and 2 diabetes mellitus lead to micro and macrovascular angiopathies which lead to morbidity and mortality. Furthermore, delayed wound healing is one of the leading concerns of the microvascular complications of diabetes. Hyperglycaemia and hypoxia characterise a wound environment of a person with diabetes. The aim of this work was to determine the combined effect of hypoxia and hyperglycaemia on endothelial cell migration.
Materials and methods: The radial migration assay was employed to assess the effect of hypoxia and hyperglycaemia on human endothelial cell migration. This assay was carried out using human microvascular endothelial cells of adult dermis (HMVECad). The migration was studied under normal oxygen (20%) tension (normoxia) and below normal oxygen (5%) tension (hypoxia), in either 5mmol/l or 20mmol/l D-glucose and in the presence of an anti-proliferative agent hydroxyurea (5mmol/l). HMVECad were seeded into circular rings of 4mm diameter in 6 well plates and after 5 hours of incubation, rings were removed and wells were incubated in the test conditions outlined above. Migration distance was measured by recording the radii of a defined area of cells at 0 h, 24 h and 48 h following incubation.
Results: The results were obtained by measuring 20 radii from each of 3 monolayers (n=60) within each test group and are presented in the table. The results are presented as net migration, which was defined as the difference between the measurements of 24 h or 48 h with those of 0 h. This experiment was repeated on two further separate occasions and found to be reproducible. The data was analysed using a paired *t* test (2-tailed).Migration of HMVECad was found to be significantly ($p<0.001$) higher in hypoxic conditions at 24 h and 48 h compared with normoxic conditions. High glucose concentration significantly ($p<0.001$) reduced the migration of cells at 24 h and 48 h compared to the cells incubated in 5mmol/l glucose at the same time point. Combining high glucose concentration with hypoxia attenuated ($p<0.001$) the migration of cells compared to hypoxic cells in 5mmol/l glucose. In the presence of hydroxyurea, the same relative increase with hypoxia ($p<0.001$) and decrease with high glucose concentration ($p<0.001$) were evident at 24 h.
Conclusion: Hypoxia and hyperglycaemia had opposing effect on microvascular endothelial cell migration. Hypoxia combined with high glucose decreased migration of cells in comparison with hypoxia alone. Future study will involve investigation of the molecular mechanisms underpinning the changes observed in endothelial cell migration and proliferation as a result of oxygen and glucose concentration.

Net migration of HMVECad (mean ± SEM) (in μm)

Treatment	without hydroxyurea		with hydroxyurea
	24 h	48 h	24 h
20% oxygen 5mmol/l glucose	95.74 ± 1.26	140.21 ± 1.32	87.30 ± 1.79
20% oxygen 20mmol/l glucose	56.44 ± 1.32	105.67 ± 0.89	76.53 ± 0.98
5% oxygen 5mmol/l glucose	117.55 ± 1.44	173.32 ± 1.31	114.62 ± 1.26
5% oxygen 20mmol/l glucose	81.50 ± 1.24	126.22 ± 0.86	72.61 ± 1.36

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1288

Fenofibrate suppresses microvascular inflammation and apoptosis through AMP-activated protein kinase activation

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FIELD (Fenofibrate Intervention & Event Lowerig in Diabetes) showed that treatment with fenofibrate in individuals with type 2 diabetes mellitus not only reduced nonfatal coronary events but also reduced the need for laser treatment for diabetic retinopathy and inhibited the progress of diabetic nephropathy. However, the mechanism of the effect of fenofibrate on the microangiopathy is not yet clear. We here investigated the mechanism of microvascular protection by fenofibrate using human glomerular microvascular endothelial cells (HGMEC). Treatment of HGMEC with fenofibrate resulted in transient activation of AMP-activated protein kinase (AMPK), as monitored by phosphorylation of AMPK and its down-stream target, acetyl-CoA carboxylase. Fenofibrate caused phosphorylation of Akt and eNOS, leading to increased production of NO, and also caused inhibition of advanced glycated end-product (AGE)-induced NF-kB activation, leading to suppression of expression of adhesion molecule genes. Significant decreases in eNOS activity and NO production in response to fenofibrate were observed in cells treated with AMPK siRNA or with compound C, a pharmacological inhibitor of AMPK. The attenuation of fenofibrate-induced inhibition of NF-kB activation was observed in cells treated with AMPK siRNA or with compound C. Furthermore, fenofibrate dose-dependently suppressed apoptosis under high glucose culture condition, detected by the ELISA method of cell death detection and morphological assessment. Treatment with compound C abolished the suppressive effect of fenofibrate on HGMEC apoptosis. Our findings suggest that the beneficial effects of fenofibarate on microvascular endothelial cells might be attributed to the induction of AMPK activation beyond its lipid-lowering actions.

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1289

Cardiac microvascular dysfunction in diabetes and insulin treatment: role of glucose-induced PKC-βII activity

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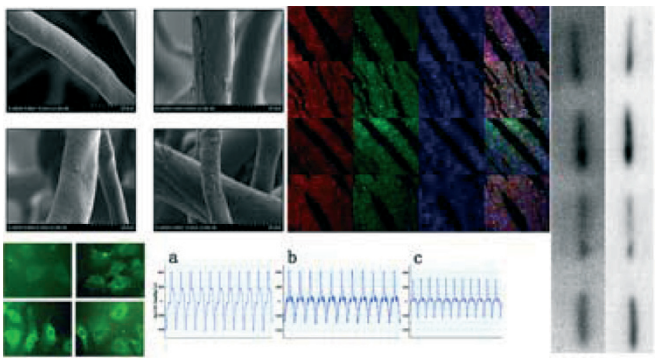
Background and aims: Multiple lines of evidence support the viewpoint that diabetes mellitus is an independent risk factor for cardiovascular disease. Activity of the protein kinase C (PKC) family has been well documented in diabetic myocardium for its numerous biological responses. However, little attention is addressed on PKC-βIIin cardiac microvascular dysfunction, which takes a significant role in the pathophysiological cascade of diabetic cardiomyopathy.
Materials and methods: In the animal experiment, normal Sprague-Dawley rat, streptozotocin-induced diabetic rat model,insulin-treated and physiological saline-treated diabetic rat were administrated with a serial of evaluations for 4 weeks natural course including pressure measurements, angiogenesis and permeability observations under electron microscope, histopathologic analysis for cardiac microvascular endothelium cell with CD31, terminal dUTP nick end-labeling (TUNEL), and Western blotting for PKC-βII. On the part of cell research, cardiac microvascular endothelial cells (CMECs) in three different mediums (normal medium, high-glucose concentration medium, insulin-stimulated and physiological saline-stimulated high-glucose medium) were investigated with MTT, apoptosis, quantitative permeability assessment via Vitro Vascular Permeability Assay Kit, and detailed analysis for PKC-βII.
Results: 1. Accompanied with more active expression of PKC-βII and higher apoptosis rate in diabetic model, either increased microvascular permeability or pathological angiogenesis is observed, and which is attenuated in certain extent while receiving insulin treatment. 2. At the same moment, accordant results from cell research were obtained. Compared with normal group, CMECs in high-glucose medium are demonstrated with poor proliferation, more notable apoptosis, increased permeability of cell monolayer, and augmented PKC-βII expression. As far as insulin-stimulated group concerned, it poses a midst performance between normal group and high-glucose group.
Conclusion: Increased PKC-βII activity has been implicated responsible for the pathogenesis of cardiac microvascular dysfunction in diabetes and elevated glucose is sufficient to induce these effects. Moreover, PKC-βII is indicated to occupy an important position in the whole process of insulin treatment. A thorough understanding of the pathophysiology of cardiac microvascular insufficiency and the function of PKC-βII in diabetes also leads to more efficacious suggestions for therapeutic strategies.



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1290

Elucidation of the molecular mechanisms involved in endothelial dysfunction induced by hyperglycaemia via the activation of the hexosamine pathway

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Background and aims: Despite intense research the molecular processes by which diabetes promotes atherosclerosis are not yet fully understood . One unexplored possibility is that hyperglycemia, acting through the glucosamine (GlcNAc) pathway may induce endothelial dysfunction promoting the misfolding of proteins in the endoplasmic reticulum (ER) and consequently ER stress. The aim of this study is to test this hypothesis and to analyze the functional consequences in endothelial cells.
Materials and methods: Human umbilical vein endothelial cells (HUVECs) were cultured in ECM medium at 37°C with 5% CO_2. Time-course experiments were carried out treating HUVECs for 24, 48, 72 and 96 hrs in the presence of 7.5mM GlcNAc. The non-metabolizable sugar mannitol was used as an osmotic control. Total RNA obtained from GlcNAc treated HUVECs and control cells were used to evaluate XBP-1 splicing and expression of BiP, Calnexin, IL-6, CRP, ICAM-1, VCAM-1 by RT-PCR. Total protein lysates were employed to assess ATF6 cleavage, Bip, Calnexin, CRP, IRE-1, pJNK and JNK levels by Western blot. All experiments were carried out in triplicate or quadruplicate, data are given as mean ± standard deviation (SD). Statistical differences were assessed by Student's t test as compared to control cells, a p value of 0.05 has been considered statistically significant.
Results: Initially, we demonstrated that GlcNAc induces ER stress assessing the expression levels of two key ER stress markers, the chaperones Bip and Calnexin. Bip mRNA levels increased by 43±20%($p \leq 0.01$), 20±14%($p \leq 0.05$), 36±10 ($p \leq 0.001$), 10±10%, while Bip protein levels increased by 40±30% ($p \leq 0.05$), 48±19% ($p \leq 0.05$), 67±10% ($p \leq 0.001$), 39±10% ($p \leq 0.05$) at 24, 48, 72 and 96 hrs respectively in GlcNAc treated HUVECs as compared to control cells. Calnexin transcription increased by 58±30% ($p \leq 0.05$), 132±42% ($p \leq 0.001$), 112±48% ($p \leq 0.01$), 386±100% ($p \leq 0.01$) and Calnexin protein levels by 50±20% ($p \leq 0.05$), 35±15% ($p \leq 0.05$), 50±30 ($p \leq 0.02$), 25±25% respectively after 24, 48, 72 and 96 hrs. Then to evaluate the impact of GlcNAc induced ER stress we investigated if the induction of ER stress was paralleled by an increase in the synthesis and release of proinflammatory cytokines and adhesion molecules. We observed that C reactive protein expression increased by 58±7% and 46±7% ($p \leq 0.05$) at mRNA and protein levels respectively in HUVECs treated with GlcNAc for 24hrs, IL-6 mRNA levels increased by 38±3%, while ICAM1 and VCAM-1 mRNA levels were increased by 36%±20 ($p \leq 0.09$) and 54%±10 ($p \leq 0.05$), respectively. We then proceeded to a detailed characterization of the activation of the different known ER stress pathways in GlcNAc treated HUVECs; we did not observe an increased cleavage and activation of the transcription factor ATF-6 or an increased alternative splicing of the transcription factor XBP-1, by contrast we detected a significant increase of IRE-1 levels ($p \leq 0.05$) as well as a significant increase in JNK phosphorylation ($p \leq 0.05$), suggesting that this may be the major pathway mediating ER stress induction in response to GlcNAc.

Conclusion: Accumulation of intracellular GlcNAc in endothelial cells, observed in diabetes, induces ER stress and promotes an inflammatory state. The increased cytokines expression can in turn exacerbate GlcNAc induced endothelial dysfunction, by independently promoting ER stress activation on one side and compromising insulin signalling on the other.
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1291

Acute administration of high polyphenol content chocolate improves endothelial function in type 2 diabetes even in the presence of hyperglycaemia
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Background and aims: Endothelial dysfunction has been shown to be a reliable marker of cardiovascular disease. Individuals with type 2 diabetes are known to have a higher risk of cardiovascular disease. It has been proposed that bioactive compounds in foods such as chocolate can increase nitric oxide levels; this has been associated with improved vascular health and improved ability of the endothelial tissue to manage mechanical and metabolic stressors.
Materials and methods: Ten subjects with well controlled type 2 diabetes (HbA1c 6.6±0.7%), treated with lifestyle or metformin were enrolled. Subjects following screening attended for 3 oral glucose tolerance tests (OGTT) which were 1 week apart. The study visits schedule started with subject acclimatisation to the room prior to a baseline assessment of endothelial function, measured by EndoPAT 2000 (Intamar, Israel) to give the Reactive Hyperaemia Index (RHI), then were given water alone or water plus either high polyphenol dark content chocolate (Acticoa, Barry Callebaut, Belgium) or a low polyphenol dark chocolate . The measurement was repeated at 60 minutes, immediately prior to starting the OGTT and at 180 minutes (timed at the end of the OGTT)
Results: Table 1: The RHI for each of the tests at baseline and at 60minutes

	0 minutes		60 minutes		
	Mean	SD	Mean	SD	p value Vs 0 minutes
Water	1.70	0.36	2.05	0.54	0.02
High Polyphenol Chocolate	1.68	0.47	2.32	0.81	0.01
Low Polyphenol Chocolate	1.87	0.53	1.98	0.53	0.6

When data is standardised at baseline, high polyphenol dark content chocolate showed a significant improvement in RHI compared to both low polyphenol dark content chocolate at 60 minutes (p= 0.048) and post OGTT (p =0.04) and water at 60 minutes (p= 0.028) and post OGTT (p= 0.045). In all groups the glucose challenge did not worsen the endothelial function.
Conclusion: High polyphenol dark content chocolate appears to improve endothelial function, which is sustained following a glucose challenge. High polyphenol dark content chocolate may contain bioactive compounds that increase RHI, which may be modulated through enhanced release of nitric oxide.
Supported by: Barry Callebaut, Belgium

PS 122 Oxidative stress

1292

The effect of glucose and glucose metabolites on calcium-induced mitochondrial permeability transition (MPT)
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Background and aims: Mitochondrial production of reactive oxygen species (ROS) due to upregulated glucose oxidation is supposed to play a crucial, unifying role in pathogenesis of long-term diabetic complications. We investigated the effect of glucose metabolites on the mitochondrial permeability transition pore (MPT) and mitochondrial ROS production.
Materials and methods: Mitochondria were isolated from rat liver. The inner membrane potential was measured by fluorescent probe JC-1, and mitochondrial swelling (MPT) was detected as light scatter. Mitochondria were energized by succinate with rotenone (complex I inhibitor). The effects of glucose, glucose 6-phosphate, glucose+ATP+Mg, fructose 6-phosphate and glucose 1-phosphate (all at 5 mmol/l and 30 mmol/l), glyceraldehyde and methylglyoxal (1 mmol/l and 6 mmol/l) on calcium-induced MPT were investigated. Mitochondrial release of hydrogen peroxide with Amplex Red was also measured in selected conditions.
Results: From all the compounds tested only 30 mmol/l glucose, 30 mmol/l glucose 1-phosphate and 6 mmol/l methylglyoxal significantly ($p<0.05$) slowed down the calcium-induced mitochondrial swelling, methylglyoxal appeared to be the strongest MPT inhibitor. On the other hand, 30 mmol/l fructose 6-phosphate significantly ($p<0.01$) accelerated the swelling. Glucose 6-phosphate or glucose+ATP+Mg did not influence the MPT, which suggests absence of glucokinase (known to affect the pore) in our isolated mitochondria. Hydrogen peroxide production by our mitochondria respiring on succinate + rotenone was found potential independent and approximately doubled during calcium-induced MPT. Cyclosporine A not only completely prevented the MPT, but also slowed down ROS production in response to Ca. In contrast, methylglyoxal simultaneously inhibited MPT and increased ROS production in response to Ca.
Conclusion: We confirm that methylglyoxal is a potent MPT inhibitor. In addition, we describe for the first time that high glucose, fructose 6-phosphate and glucose 1-phosphate concentrations affect the MPT, too. ROS production generally raised during MPT, however, inhibition of MPT by methylglyoxal was associated with increased ROS production. This mechanism can be important in development of long-term diabetic complications.
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1293

High dose metformin therapy reduces glycation and oxidative damage to apolipoprotein B100 and may decelerate atherosclerosis in patients with type 2 diabetes
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Background and aims: Formation of advanced glycation endproducts (AGEs) and oxidative damage of apolipoprotein B100 - the major protein of low density lipoprotein (LDL) - have been implicated in increased atherogenic risk of LDL in type 2 diabetes. Metformin therapy is associated with a cardiovascular protective effects and increased patient survival in
UKPDS study. As a result, metformin is the first choice of therapy in obese and diabetic patients. Metformin is already shown to reduce the concentration of dicarbonyl methylglyoxal in diabetic patients. The aim of this study, therefore, was to analyse and quantify the glycation and oxidative damage to LDL particles by methylglyoxal in diabetic patients with and without high dose metformin therapy and healthy controls.
Materials and methods: Healthy subjects as controls (HC)(n = 17), subjects with type 2 diabetes on high dose metformin (metformin dose more than 1.5 gms/d) (MD)(n = 8)
and type 2 diabetic subjects not on high dose metformin (DN) (n = 25) were recruited for this study. LDL was isolated from venous plasma, stabilised with aprotinin and collected in the fasted state, by density gradient ultracentrifugation using Iodixanol. Purified LDL was washed with argon-purged water by ultrafiltration over a 100 kDa microspin filter. The content of AGEs and oxida-

tion adduct residues in apolipoprotein B100 was analysed after de-lipidation and exhaustive enzymatic hydrolysis by stable isotope dilution analysis liquid chromatography/mass spectrometry (LC-MS/MS). The following markers of protein damage were quantified: AGEs - Nε-carboxymethyl-lysine (CML), Nε-carboxyethyl-lysine (CEL), glyoxal-derived hydroimidazolone (G-H1), methylglyoxalderived hydroimidazolone (MG-H1), 3-deoxyglucosone-derived hydroimidazolone (3DG-H) and pentosidine; oxidation markers - dityrosine and N-formylkynurenine (NFK).
Results: The AGE products, MG-H1 and 3DG-H of apolipoprotein B100 and CEL were increased in type 2 diabetes (MD and DN) compared to control subjects (HC); MG-H1(pmol/mg protein): HC (Mean ± SD) 71.6 ± 54.7, DN(Mean ± SD): 229.6 ± 48.7, MD (Mean ± SD) 55.5 ± 33.5 . (P<0.001); 3DG-H (pmol/mg protein): HC (Mean ± SD) 36.7 ± 42.6, DN(Mean ± SD): 82.8 ± 33, MD (Mean ± SD) 18.1 ± 13.5. (P<0.001); CEL(pmol/mg protein): HC (Mean ± SD) 13.9 ± 11.6, DN(Mean ± SD): 23.9 ± 15.8, MD (Mean ± SD) 4.42 ± 4.44. (P<0.01). The above results show that subjects on high dose metformin therapy have lower levels of AGE products. Protein oxidation marker: Dityrosine (pmol/mg protein): HC (Mean ± SD) 3.9 ± 7.4, DN(Mean ± SD): 19.0 ± 10.0, MD (Mean ± SD) 0.9 ± 0.6 (P<0.05). Similarly, subjects on high dose metformin therapy have lower levels of protein oxidation markers.
Conclusion: This study indicates that whilst apolipoprotein B100 of LDL suffers oxidation and glycation in type 2 diabetes, the damage seems to be significantly reduced by using high dose metformin. This could be one of the reasons by which high dose metformin therapy leads to cardiac protection.
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1294

Contribution of endoplasmic reticulum stress in oxidized LDL-induced beta cell dysfunction
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Background and aims: Induction of endoplasmic reticulum stress can be detrimental and diabetogenic as it mediates beta-cell dysfunction caused by chronic hyperglycaemia and fatty acids. Evidence for a role of oxidized LDL-cholesterol particles (oxLDL) in progression and development of diabetes is growing. First, relative increase in levels of oxLDL over native LDL, often accompanied by low plasma concentration of HDL, is measured in plasma of diabetic patients. This abnormality contributes to increase the risk of patients to develop cardiovascular disease. Second, several independent studies have shown the harmful effects of human modified lipoproteins on expression and secretion of insulin as well as cell survival. At the present time, the mechanisms whereby oxLDL exert their effect are still elusive. In this study, we investigated potential role of ER stress in beta-cell failure elicited by oxLDL.
Materials and methods: Native LDL fractions were freshly prepared from plasma of human healthy donors by sequential ultracentrifugation. In vitro oxidization of LDL by copper was adjusted to obtain oxidized LDL qualitatively similar to those observed in patients. The lipoprotein preparations were dialyzed against phosphate-buffered saline and culture medium to remove copper and salts. The mouse MIN6 insulin-secreting cell line was treated with native or modified LDL and 2.5 mM of the chemical chaperone 4-phenyl butyric acid (4-PBA) was co-added to the medium to assess the role of ER stress on oxLDL-induced dysfunction. Expression of genes involved in ER stress was monitored by quantitative PCR.
Results: OxLDL triggered a rise in the expression of ER stress markers C/EBP homologous protein (CHOP) and activating transcription factor 4 (ATF4), whereas the genes expression was unchanged in response to native LDL. However, no increase in the splicing of the unfolded protein response gene XBP-1 was observed. 4-PBA co-treatment prevented oxLDL-induced ATF4 and CHOP expression. OxLDL impair insulin synthesis, secretion and cell survival. Inhibition of the ER stress with the use of 4-PBA prevented oxLDL-induced diminution of insulin expression and cell apoptosis, whereas insulin secretion was still reduced.
Conclusion: These data highlight the contribution of ER stress in the defects of the insulin synthesis and β-cell survival induced by oxLDL.
Supported by: an EFSD/MSD grant

1295

Association between FINDRISC and systemic markers of oxidative stress
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Background and aims: Type 2 diabetes pathophysiology is causally related to increased systemic oxidative stress. The validated Finnish diabetes risk score (FINDRISC) has been successfully implemented as practical screening tool to assess the diabetes risk and to detect undiagnosed type 2 diabetes (T2D). We investigated the association between FINDRSIC and different systemic oxidative stress markers in subjects with different stages of glucose tolerance and diabetes mellitus.
Materials and methods: The cross-sectional survey included 588 participants aged 19-85 years. Individuals were examined with an oral glucose tolerance test. At baseline, FINDRISC was gathered and six different oxidative stress markers were assessed (lagtime of serum diene formation, total antioxidant capacity, oxLDL, antioxLDL antibodies, thiobarbituric acid reactive substances and paraoxonase activity)
Results: Among investigated subjects, 73% had NGT, 4% IFG, 17 % IGT, and 5% newly diagnosed T2D. The FINDRISC score varied between individuals with NGT (10.0), IGT (12.9, p<0.001) and T2D patients (15.8, p<0.001). Total antioxidant capacity was positively associated with FINDRISC score (p<0.01) as well as lagtime of serum diene formation (p<0.05). FINDRISC correlated negatively with antioxLDL antibodies (p<0.001), thiobarbituric acid reactive substances (p=0.001), and paraoxonase activity (p=0.001)
Conclusion: The significant associations between FINDRISC and oxidative stress markers underline the potential causal relationship between systemic oxidative burden and development of T2D.
Supported by: the Dresden University of Technology Funding Grant, Med Drive

1296

Inflammation, oxidative stress and endothelial function in subjects with normal, impaired and diabetic glucose tolerance
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Background and aims: Endothelial Dysfunction (ED) and Endothelial Progenitor Cells (EPC) reduction are key factors in atherogenesis.
Materials and methods: In subjects with normal (NGT, n.26), impaired (IFG/IGT, n.34) and diabetic glucose tolerance (T2D, n.18), we studied the relation of ED (flow-mediated, FMD; nitrate-induced dilatation, NID) and EPC (CD34+/KDR+ cells) with levels of biomarkers of inflammation (fibrinogen; C-reactive protein, hsCRP; serum amyloid A, SAA), proinflammatory cytokines (interleukin-6, IL-6; tumor necrosis factor-α, TNFα; monocyte chemoattractant protein-1, MCP-1), adhesion molecules (intercellular adhesion molecule-1, sICAM-1; vascular cell adhesion molecule-1, sVCAM-1), together with markers of oxidative stress (γ-glutamyltransferase, γGT; malonildialdehyde, MDA; lipid hydroperoxide, LOOH; ferric-redicing ability of plasma, FRAP) and the antioxidative activity of paraoxonase-1 (PON-1).
Results: The groups did not differ for gender, BMI, waist, dBP, total and LDL cholesterol, apoA1 and apoB, creatinine, cistatin C, and fasting insulin. IFG/IGT and T2D were older than NGT (55±5 and 58±8 vs 45±10 years, p<0.001), had higher sBP (p=0.001), fasting-, 2h-OGTT- and AUC-glucose (p<0.0001), and HbA1c (6.0±0.4 and 6.5±0.6 vs 5.5±0.4%, p<0.0001), marginally higher triglycerides (p=0.08), and lower HDL (p=0.07). NID was comparable in the three groups. FMD (and FMD/NID ratio) was lower in T2D (Δ% 4.4±3.3, m±sd), and in IFG/IGT (Δ% 6.0±2.8%) than in NGT (Δ% 7.9±3.6%, Kruskal-Wallis, p=0.0017). EPC pool was higher in NGT (533±69 cells/ml, m±se), than in IFG/IGT (440±53) and even lower in T2D (254±51 cells/ml, Kruskal-Wallis, p=0.014). The three groups did not differ for fibrinogen and SAA, but differ for hsCRP (1.68±2.22, 3.34±2.39 and 3.62±2.10 mg/L, p=0.009); not for TNF-α, but did for IL-6 (8.7±6.1, 13.8±12.5 and 15.1±15.9 pg/ml, p=0.05) and MCP-1 (201±56, 236±57 and 236±59 pg/ml, p<0.05); not for sVCAM-1, but did for sICAM-1 (244±122, 338±147 and 424±118 mg/ml, p<0.001); not for γGT, LOOH and FRAP, but did for MDA (2.33±0.57, 2.66±1.04 and 3.27±0.84 μmol/L, p=0.003) and PON-1 activity (93±46, 77±55 and 56±39 U/L, p=0.04). FMD inversely correlated with sICAM-1 (r=-0.24, p=0.04), MDA (r=-0.30, p=0.01), and FRAP (r=-0.25, p=0.03). EPC inversely correlated with

sICAM-1 (r=-0.30, p=0.008), IL-6 (r=-0.26, p=0.02), and directly with PON-1 activity (r=0.30, p=0.008). A significant association was observed between FMD and EPC levels both stratified by median value (χ2=8.67, p=0.003). Stratification by median values of FMD and EPC allows to identifying four different phenotypes among which that with both FMD and EPC below the median had the "worst" (n. 26), and that with both FMD and EPC above the median, the "best" pro-inflammatory and pro-oxidative profile; n. 26).
Conclusion: In subjects with different degree of glucose tolerance, endothelium dependent vasodilatation and EPC reduction are closely related. Pro-inflammatory, oxidative and anti-oxidative activities are already modified in the early stages of altered glucose tolerance. Mechanisms involved in different aspects of endothelial dysfunction are multifactorial at the prediabetic and diabetic states.

1297

No relationship between glucose variability and oxidative stress in type 2 diabetes

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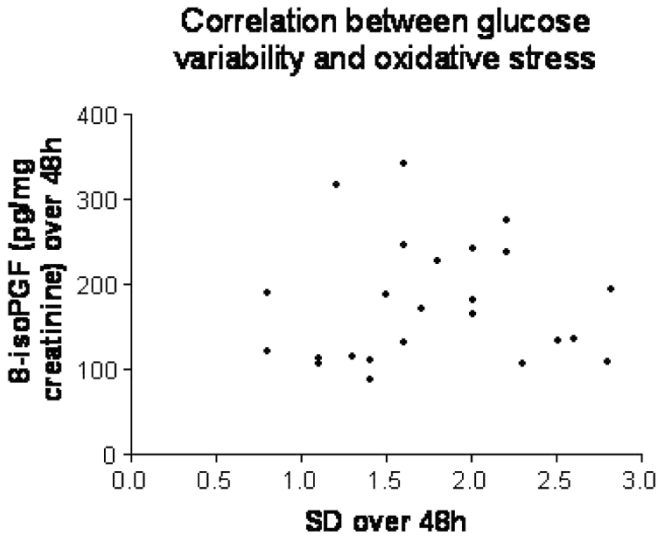
Background and aims: A strong relationship between glycaemic variability and oxidative stress has been reported. However, this relationship could not be confirmed in type 1 diabetes. The purpose of the present study is to examine whether this discrepancy relates to the difference between type 1 and type 2 diabetes or to different methods used for the quantification of oxidative stress.
Materials and methods: 24 patients with type 2 diabetes on oral antidiabetic treatment were included. The study consisted of 72 hours of continuous glucose monitoring (CGMS® system gold; Medtronic MiniMed) while patients simultaneously collected two consecutive 24 hours urine samples for determination of 15(*S*)-8-*iso*-prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) using HPLC tandem mass spectrometry. Standard deviation (SD) and mean amplitude of glycaemic excursions (MAGE) were calculated as markers of glycaemic variability over the 48 hours of continuous glucose monitoring. Correlation between markers of glycaemic variability and 15(*S*)-8-*iso*-$PGF_{2\alpha}$ excretion was calculated using Spearman's correlation. Also multivariate analysis adjusted for sex, age, HbA1c and exercise was performed. Exercise was graded into sedentary, moderately active, active and fit according to the patients' history.
Results: The patient group studied consisted of 66.7% males with a mean (range) age of 59 (36-76) years and a mean (SD) HbA1c of 6.9 (0.7) %. Median [interquartile range (IQR)] urinary 15(*S*)-8-*iso*-$PGF_{2\alpha}$ excretion was 168 (114-236) pg/mg creatinine. Median (IQR) SD was 1.65 (1.33-2.20) mmol/l and MAGE 4.68 (3.07-5.88) mmol/l. Univariate regression analysis did not show a significant correlation for SD (r^2=0.001) or MAGE (r^2=0.015) with 15(*S*)-8-*iso*-$PGF_{2\alpha}$ excretion. Multivariate regression analysis adjusted for age, sex, Hba1c and exercise did not alter this observation. Spearman correlation coefficients (ρ) between 15-(*S*)-8-*iso*-$PGF_{2\alpha}$ excretion and SD and MAGE were 0.14 (p=0.53) and 0.21 (p=0.34) respectively. A significant relationship was found between 15(*S*)-8-*iso*-$PGF_{2\alpha}$ excretion and degree of exercise (r= -0.56, p=0.009) and age (r= -0.50, p=0.012).
Conclusion: In this study we did not find a relationship between glucose variability and 15(*S*)-8-*iso*-$PGF_{2\alpha}$ excretion in type 2 diabetes. The discrepancy between these and earlier data might be attributed to the superior quantification of 15(*S*)-8-*iso*-$PGF_{2\alpha}$ by HPLC MS/MS versus enzyme immunoassay that is much more open to interfering substances or to a difference in the population studied, e.g. the much better glycaemic control in our patients than in the Monnier population (mean HbA1c 6.9% and 9.6% respectively). Finally we report a negative correlation between oxidative stress formation and age and degree of exercise.



1298

Is 1,5 AnhydroGlucitol associated with malondialdehyde a marker for oxidative stress?

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Background and aims: Hyperglycaemia, and glucose excursions especially, trigger oxidative stress. Since 1,5 AnhydroGlucitol (1,5AG) levels inversely correlate with glucose fluctuations, 1,5AG is potentially also a marker of hyperglycaemia-induced oxidative stress. The objective of this study was to investigate the interrelationship of 1,5AG, the oxidative stress marker malondialdehyde (MDA) and measures of glucose variability (GV), at various levels of glycaemia in diabetes patients.
Materials and methods: Data from 83 subjects (45 T1DM and 38 T2DM) from the A1c Derived Average Glucose (ADAG) study population were included. Glucose levels were monitored with continuous glucose monitoring (CGM- four 48-h periods during 3 months) and self-monitored blood glucose (SMBG). From CGM, we calculated mean blood glucose (MBG) and Standard Deviation (SD), Magnitude of the Amplitude of the Glycemic Excursions (MAGE) and area-under-the-curve > 180mg/dl (AUC>180) as measures of GV. Post-prandial glucose (PPG) levels were obtained from SMBG results. HbA1c, 1,5AG and MDA in plasma were measured twice at an interval of 8 weeks. Pearson correlations were calculated for 1,5AG, MDA and measures of glycaemia, at different levels of HbA1c.
Results: Significant correlations were found between 1,5AG and MDA, and between these variables and the various measures of GV, HbA1c and MBG. (Table) For MDA, the strongest correlation was found with PPG and AUC>180. After adjustment for HbA1c the association between 1,5AG and MDA was lost, leaving only a significant correlation between MDA and PPG and AUC>180. Also at HbA1c < 8 % (n = 55) a significant correlation of -0.268 (p = 0.048) between MDA and 1,5AG was lost when adjusted for HbA1c. At HbA1c > 8% (n = 17) no significant correlations were found, when adjusted for HbA1c.
Conclusion: The oxidative stress marker MDA is associated with 1,5AG and variables of GV, in particular postprandial hyperglycemia and glucose levels > 180 mg/dl. However this association becomes considerably weaker when adjusted for HbA1c. As 1,5AG levels reflect glucose excursion during the previous 2 to 3 weeks, the use of this measure of glycaemia obtained during the previous 3-month period may be regarded as a limitation of this analysis. The relationship between 1,5AG and other oxidative stress markers needs to be determined.

Table: Correlation coefficients of 1,5AG and MDA with measures of glucose control and variability over a 3-month period, for patients with T1DM and T2DM, before and after adjustment for HbA1c and for different HbA1c subgroups.

	Total population				HbA1c < 8%			
			adjusted for HbA1c				**adjusted for HbA1c**	
	1,5AG	MDA	**1,5AG**	**MDA**	1,5AG	MDA	**1,5AG**	**MDA**
N =	**75**	**75**	**72**	**72**	**55**	**55**	**52**	**52**
MDA	**-0.416****		-0.006		-0.268*		0.037	
HbA1c #	**-0.723****	**0.522****			**-0.703****	**0.416****		
SD	**-0.662****	**0.409****	**-0.404****	0.127	**-0.708****	**0.348****	**-0.467****	0.114
MAGE	**-0.587****	**0.411****	**-0.346****	0.200	**-0.638****	**0.379****	**-0.392****	0.183
AUC>180	**-0.684****	**0.536****	-0.206	**0.238***	**-0.634****	**0.473****	**-0.348****	**0.300***
All PPG values	**-0.634****	**0.608****	-0.212	**0.337****	**-0.455****	**0.542****	-0.062	**0.403***

at end of 3 months, * correlation is significant at the 0.05 level, ** correlation is significant at the 0.01 level

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1299

Whole-genome transcriptomic profile in lympho-monocytes of healthy volunteers reveals over-expression of oxidative phosphorylation and cell cycle genes in presence of increased IMT

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Background and aims: Preliminary studies have been investigating gene expression profile (GEP) of circulating peripheral mononuclear cells (PBMCs) in presence of several cardiovascular (CV) risk factors in search of transcriptomic signatures of CV risk. However, no study investigated whether PBMC's GEP correlates with markers of vascular damage, such as intima-media thickness (IMT).
Materials and methods: To address this aim, IMT and GEP were evaluated in 164 apparently healthy young-adults [91M/73F, age 37±8 y], volunteering for CV risk assessment. IMT was measured by ultrasound examination with an automated edge-detection system (Carotid Analyzer, MIA-LLC, US), and transcriptomic profile investigated using whole-genome dual-color oligo-RNA-microarrays (Agilent Technologies, CA, USA). Enrolled subjects were free of diabetes, chronic inflammatory and CV diseases and not taking pharmacological medications.
Results: Subjects were stratified by IMT quartiles and clinical characteristics compared by ANOVA. Higher IMT values were significantly correlated with several established CV risk factors, including older age ($p<0.001$), male gender (0.004), greater BMI (0.011) and waist (0.006), higher fasting plasma glucose (0.008) and LDL-cholesterol concentrations (0.013). In addition to these findings, IMT values were significantly correlated with a PBMC transcriptomic profile characterized by increased expression of groups of genes involved in cell cycle and mitosis (GO terms: "Cell division" p=1.1E-9; "Cell cycle phase" p=6.7E-9; "Cell cycle process" p=1.5E-8;"Mitosis" p=3.3E-7; etc.) and mithocondrial oxidative phosphorylation ("electron transport" p=5.5E-6; "mitochondrial electron transport" p=5.4E-5). The "oxidative phosphorylation" pathway was significantly up-regulated in high IMT (p=1.8E-8), followed by the cell cycle pathway (p=3.6E-5). Since age was independently correlated with IMT by multivariate linear regression analysis, we repeated transcriptomic analysis after adjusting IMT by age. After age-adjustment, IMT values resulted significantly correlated with the same gene groups and pathways with a higher level of significance. An increase in transcription of oxidative phosphorylation genes resulted the bio-signal most strongly correlated with age-adjusted IMT values (p=6.4E-12).
Conclusion: In conclusion, the presence of vascular damage (as assessed by increased IMT) is associated with a significantly different transcriptomic profile in PBMCs of healthy volunteers. The over-expression of several genes related to the mitochondrial oxidative and cell cycling processes appears to be the main bio-signature of high IMT. An activated phenotype is therefore already visible in inflammatory cells of apparently healthy young adults in presence of high IMT values.
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1300

A novel role for p53 in the regulation of oxidative stress in vascular cells in response to glucose fluctuation

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Background and aims: Epidemiological data support the concept that postprandial oscillating and continuous high glucose levels represent independent risk factors for both cardiovascular and diabetic microvascular complications. Our group has recently shown that prolonged high glucose oscillation levels increases production of markers of oxidative stress in both *in vivo* and *in vitro*. However our previous data has also noted that key antioxidant enzymes (Zn-Cu SOD, MnSOD), were over expressed during exposure to chronic high glucose, whilst unaffected during chronic oscillating high glucose exposure even if we found, in this condition, higher level of nitrotyrosine, a marker of oxidative stress. Parallel to this hyperglycaemia induced stress shown also to activate the pro-apoptotic transcriptional factor p53, which has been reported to counteract the Nrf2-induced transcription of the ARE-containing promoters of key antioxidant genes. In order to explain the reported increased damage in response to oscillating as compared with continuous high glucose we investigated: (1) the activation of p53, (2) Nrf2 antioxidant response, (3) and their cross-talks in endothelial cells exposed to chronic continuous and chronic oscillating glucose levels, also by using cyclic pifitherin alpha, a p53 transcriptional inhibitor.
Materials and methods: Human endothelial cells (HUVECs) were exposed to high glucose media (30 mmol/l) with or without Cyclic Pifitherin alpha (100nM) for 12 hours (hr); for constant vs chronic oscillating hyperglycaemia experiments. HUVECs were grown for 3 weeks either in 5 mmol/l glucose, with 25 mmol/l mannitol for osmolarity normalisation (control), or in 30 mmol/l glucose or alternating 12 hr in normal glucose with 12 hr in high glucose (30 mmol/l). Protein expression analysis was conducted by Western blotting, gene expression analysis by Real Time PCR.
Results: HUVEC cells exposed to high glucose for 12 hr, with or without p53 inhibitor, significantly up-regulated p53 protein levels in a time dependent manner compared with control (Control: 1.0±0.0; p53 Protein, 6hr: 1.4±0.1ODU*; p53 Protein, 12hr: 2.0±0.2ODU*; *$P<0.05$). Similarly, findings were noted with TIGAR, a p53 induced gene product, protein expression, in absence of inhibitor; whilst TIGAR expression was unaffected with p53 inhibitor (p53 InHBr) compared to control. Whilst, in contrast, to TIGAR the antioxidant HOX-1 protein, had a pronounced up-regulation in the presence of the inhibitor at 12 hr (HOX-1/p53InHBr, 12hr: 1.5±0.2ODU↑*; Vs HOX-1, 12hr: 1.1±0.1ODU). mdm2 a p53 feedback inhibitor, mRNA levels, in the presence of high glucose, were significantly up-regulated at 9 and 12hr compared with controls (control Vs mdm2 mRNA 9hr: 2*fold increase; control Vs mdm2 mRNA, 12 hr: 3*fold increase;). Finally, TIGAR was significantly up-regulated after 3 weeks of oscillating glucose compared with constantly high glucose or control (TIGAR OSC 2.5±0.3* OD units vs TIGAR HG 1.4 ±0.2* OD units).
Conclusion: In summary, high glucose induces a p53 mediated intracellular response that limits the induction of Nrf2 antioxidant genes such as HOX-1. The delay of p53 feedback inhibiton may have a crucial role in determining the pattern of p53 regulation. As such this delay may account for p53 overactivation in oscillating glucose that may compromise Nrf2 antioxidant activity and therefore may lead to a more detrimental condition compared with constant high glucose.
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1301

Impact of glyoxalase 1 knock down on the heat shock protein expression

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Background and aims: Hyperglycaemia plays an important role in the pathogenesis of diabetic complications including accumulation of methylglyoxal, a highly reactive α-dicarbonyl metabolite of glucose degradation pathways and increased generation of advanced glycation endproducts (AGEs). Both AGEs and methylglyoxal contribute to the development of different pathological processes such as kidney dysfunction, retinopathy or cardiovascular disease. Methylglyoxal reacts in a non-enzymatic reaction with arginine residues of proteins to form the AGEs argpyrimidine and hydroimidazolone. In different cell lines but also in human myocardial tissue heat shock protein 27 (HSP27) was found to be the major argpyrimidine-containing protein. Furthermore, methylglyoxal is involved in regulation of different genes by modification of distinct transcription factors like mSin3A and Yap1.
Materials and methods: The rat cardiomyoblast cell line H9c2 was transfected with glyoxalase 1 (GLO1)-specific siRNA. The impact of the GLO1 knock down on the expression of HSP27 and HSP70 was analysed by immunoblotting and quantitative real-time PCR. Moreover, we investigated the effects of the knock down on the actin cytoskeleton by fluorescence microscopy and on apoptosis and oxidative stress by flow cytometry.
Results: To exclude an unspecific reaction of the siRNA we used scrambled siRNA as control and two GLO1 siRNAs, which were completely different in sequence. The GLO1 knock down using both specific siRNAs resulted in decreased expression of HSP27 on protein and mRNA level. There was no effect on the HSP70 expression. Furthermore, downregulation of GLO1 resulted in disruption of the actin cytoskeleton and increased apoptosis. It could be excluded that these observations are induced by oxidative stress, because the control siRNA also induced oxidative stress without any effects on cytoskeleton, apoptosis, and HSP27 expression.
Conclusion: It is already known that increased levels of methylglyoxal result in formation of reactive oxygen species. Results of the present study demonstrate that besides the oxidative stress, methylglyoxal induces a downregula-

tion of the anti-apoptotic and anti-oxidative protein HSP27. Further experiments are needed to explain the mechanism by which methylglyoxal affects the HSP27 expression.
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PS 123 Cardiovascular disease - epidemiology and detection

1302

Cardiovascular mortality in subjects with type 2 diabetes and in nondiabetic subjects with a first episode of myocardial infarction in southern Europe

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Background and aims: Cardiovascular risk in type 2 diabetes (DM2) patients is still controversial. Several authors suggest that DM2 is a coronary heart disease (CHD) equivalent while others disagree. No reports on this subject have been published in southern Europe. Aims: Primary objectives: To compare mortality rates from all causes, coronary , cardiovascular and stroke, and the incidence of myocardial infarction (MI) and unstable angina between groups under study. Secondary objectives: to evaluate cardiovascular risk in diabetic subjects stratified by diabetes duration (> or < 8 years), diabetes treatment (diet, oral drugs, insulin) and baseline glycemic control (HbA1c > or <7%).
Patients and methods: A cohort study was designed with a 10-year follow-up in Catalonia, north-eastern Spain. All patients included were aged between 30 and 74. DM2 patient selection: 2260 patients were randomly recruited between 1993 and 1995 in 53 primary health care centers, serving a population of 982.567 adults. Patients with prior CHD events were excluded. Previous MI patient selection: 2150 patients were recruited between 1990 and 2003 in the population-based REGICOR study. Only non-diabetic subjects with a first episode of MI were included.
Results:

Table 1. Adjusted *Hazard ratio* (HR) at 10-year for different cardiovascular endpoints

	Type 2 Diabetes N = 2260	**Myocardial infarction N = 2154**	**HR (95% CI)**
All-cause death	289 (12.8%)	482 (22.4%)*	0.42 (0.35 - 0.50)
Coronary death	41 (1.8%)	206 (9.6%)*	0.12 (0.08 - 0.18)
Stroke death	24 (1.1%)	27 (1.3%)	0.64 (0.32 - 1.25)
Cardiovascular death	99 (4.4%)	280 (13.0%)*	0.21 (0.16 - 0.28)
Unstable angina	184 (8.1%)	145 (6.7%)	0.89 (0.68 -1.17)
Non-fatal MI	126 (5.6%)	175 (8.1%)*	0.67 (0.50 - 0.89)
Fatal or non-fatal MI	161 (7.1%)	349 (16.2%)*	0.35 (0.28 - 0.44)
Coronary Heart Disease †	296 (13.1%)	475 (22.1%)*	0.44 (0.37 - 0.53)

* $p \leq 0.001$; † Unstable angina, fatal or non-fatal MI; All models are adjusted for sex, age, and baseline dyslipidaemia, hypertension and smoking status. In the different DM2 stratified groups, risk of CV death was increased with longer diabetes duration (>8 years HR: 0,29 <8years:HR:0,19, reference: 1: MI subjects) and in insulin/oral drugs treated patients (insulin-oral drugs: HR:0,34, diet:0,13, reference: 1: MI subjects). There were no statistical differences when comparing HbA1c groups (> o < 7%).

Conclusion: Patients with DM2 showed a lower incidence of cardiovascular events than patients with prior CHD. Type 2 diabetes duration and drug therapy, but not glycemic control, proved being important prognostic factors, however, the risk of cardiovascular events remains lower than in patients with first MI.
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1303

Left-sided cardiac deterioration occurs especially in women with impaired glucose metabolism - an 8-year follow-up of The Hoorn Study

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Background and aims: Patients with type 2 diabetes (T2DM) have an increased risk of left-sided heart failure, but the underlying mechanisms remain controversial. Cross-sectional data of The Hoorn Study already showed signs of left-sided cardiac deterioration in T2DM, like increasing left ventricular (LV) mass and LV systolic and diastolic dysfunction. We investigated whether people with impaired glucose metabolism (IGM) or T2DM had more left-sided cardiac deterioration than people with normal glucose metabolism (NGM) over an 8-year period.
Materials and methods: In the framework of the Hoorn Study, a population-based cohort study of diabetes, 746 people, selected for glucose status, underwent a 2D echocardiogram in 2000. At follow-up in 2008, 169 individuals with NGM (62% of the baseline participants), 91 with IGM (52%), and 121 with T2DM (41%), mean baseline age 66 years had a second 2D echocardiogram. Differences in LV mass (grams), left atrial volume (ml, diastolic function) and LV ejection fraction (%, systolic function) were calculated. T-tests were performed to analyse differences compared to baseline. Linear regression analyses were performed to investigate differences in left-sided cardiac deterioration between groups of baseline glucose status, for men and women separately. Glucose status was determined by OGTT; WHO 2006 criteria were used.
Results: Left atrial volume and LV mass increased significantly in women, but not in men, with IGM (Table 1). No significant changes were found in left atrial volume and LV mass in men and women with NGM or T2DM. The LV mass increase in women with IGM remained significantly higher in IGM compared to NGM after adjustment for age, BMI, blood pressure, fasting glucose, smoking, follow-up duration, use of antihypertensive and lipid lowering medication, prior CVD, wall motion abnormalities, and baseline value of the investigated cardiac outcome, but the increase in left atrial volume lost statistical significance after adjustment for these variables. Ejection fraction decreased significantly in all groups, but no differences in change were observed between groups of glucose status.
Conclusion: Left-sided cardiac deterioration occurs especially in women with impaired glucose metabolism. The absence of more left-sided cardiac deterioration in T2DM compared to IGM and NGM, in both men and women, may be the result of selective loss to follow-up in the T2DM group, and of aggressive risk factor treatment in T2DM patients.
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1304

Association between bone mineral density and coronary atherosclerosis in patients with type 2 diabetes

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Background and aims: The association between low bone mass and angiographically determined coronary atherosclerosis in Patients with Type 2 Diabetes is unclear.
Materials and methods: We enrolled 254 consecutive Patients with Type 2 Diabetes undergoing coronary angiography for the evaluation of established or suspected stable coronary artery disease (CAD). Type 2 Diabetes was diagnosed according to WHO guidelines. BMD was assessed by dual X-ray absorptiometry. CAD was diagnosed in the presence of any coronary artery lumen narrowing at angiography, and coronary stenoses with lumen narrowing ≥50% were considered significant.
Results: Of the total study cohort (mean age 67 ± 9 years) 36.2% (n=92) had osteopenia and 11.4% (n=29) had osteoporosis. Significant stenoses of coronary arteries were found in 65.4% (n=166). The prevalence of significant stenoses did not differ between patients with normal bone mass, osteopenia or osteoporosis (70.7%, 59.8% and 58.6%; p_{trend} = 0.173). This result did not change after multivariate adjustment for LDL cholesterol, HDL cholesterol, systolic and diastolic blood pressure, smoking, BMI, age and gender. Neither osteopenia (OR = 2.03 [95% CI 0.83-4.95], p = 0.118) nor osteoporosis (OR = 1.23 [0.50-3.00], p = 0.653) were associated with the presence of significant stenoses.
Conclusion: Low bone mass is not associated with the presence of significant coronary stenoses in patients with Type 2 Diabetes.

1305

The relationship of vascular calcification and bone mineral density with degree of albuminuria in type 2 diabetes

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Introduction: Cardiovascular disease is the most common cause of mortality in patients with diabetes. Vascular calcification is a major cause of arterial stiffness and may contribute to the progression of vasculopathy in diabetes. Vascular calcification has been studied extensively in diabetes patients with chronic kidney disease; however there is not enough information on vascular calcification in patients with normal renal function.
Aim: To investigate the relationship of vascular calcification and bone mineral density with varying degrees of albuminuria in patients with type 2 diabetes.
Research design and methods: 65 patients with type 2 diabetes with normal creatinine levels (serum creatinine < 125 μmol/l) were studied. 25 had no

Table 1: Baseline values and mean changes (sd) in LV mass, left atrial volume and ejection fraction

		Women			Men		
		NGM	IGM	T2DM	NGM	IGM	T2DM
LV mass (grams)	Baseline	142.0 (29.3)	153.3 (40.7)	166.9 (36.0)	185.7 (47.4)	179.2 (53.8)	188.0 (63.3)
	Mean change	**-0.3** (28.7)	**17.8**#** (43.6)	**1.2** (40.6)	**4.5** (39.7)	**8.3** (53.0)	**8.0** (59.9)
Left atrial volume (ml)	Baseline	41.5 (9.8)	41.0 (11.3)	47.0 (10.1)	47.1 (16.8)	47.3 (15.3)	52.2 (13.1)
	Mean change	**-2.1** (14.6)	**7.2**#* (19.0)	**-0.5** (14.1)	**2.1** (20.7)	**-0.8** (16.2)	**0.5** (19.9)
Ejection fraction (%)	Baseline	64.5 (6.7)	64.7 (5.8)	61.0 (10.1)	62.0 (7.0)	61.9 (7.6)	58.5 (8.0)
	Mean change	**-9.3**# (12.6)	**-9.0**# (11.6)	**-8.6**# (9.5)	**-9.2**# (9.5)	**-11.1**# (9.0)	**-7.9**# (10.2)

#Significant change from baseline to follow-up,
*Significantly more increase in IGM than NGM, only in crude analyses,
**Significantly more increase in IGM than NGM, in crude and adjusted analyses

albuminuria (NA), 20 had microalbuminuria (MA) and 20 had dipstick positive proteinuria (P). An x ray of the foot was carried out along with a CT scan of the femoral, posterior tibial and dorsalis pedis arteries to assess the levels of calcification along with a DEXA scan to assess the bone mineral density (BMD) of the lumbar spine, hip and left heel. In addition, routine haematology and biochemistry was carried out.
Results: The median Agatson calcification score in the left femoral artery in the NA, MA and P groups was 20, 134.5 and 260.4 respectively. The overall median score was 97.3. The proportion of patients with scores higher than the median was 32%, 55% and 65% (p = NS) in the NA, MA and P groups respectively. There was a significant correlation between calcification severity and degree of proteinuria (r = 0.251, p = 0.044). The foot x ray showed presence of calcification (based on scores of 0-4) in 36%, 30% and 45% (p=NS) in the three groups. There was a significant correlation between left femoral arterial calcification and lumbar spine BMD (r = 0.272, p = 0.034) and T score (r = 0.263, p = 0.040). The P group had longer duration of diabetes (13.4 years ±9.7: p=0.014) as compared to NA (6.6±4.9). There was no difference in calcification severity in the smoker and non-smoker groups. The groups did not differ in age, estimated creatinine clearance, serum creatinine, urea, HbA1c, Hemoglobin, serum calcium, parathyroid hormone and phosphate.
Conclusion: Vascular Calcification along with low bone mineral density is highly prevalent in Type 2 DM with normal creatinine levels. The prevalence of severe calcification increases with the degree of albuminuria.
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1306

Pre-existing macrovascular disease and glycaemic control in patients with type 2 diabetes in Europe: a matched cohort study
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Background and aims: It is widely recognized that achieving specific glycemic goals in patients with diabetes can substantially reduce morbidity. Given that pre-existing macrovascular conditions (MVC) increase the risk of diabetes-related complications, adequate glycemic control is especially important for these high risk patients. The purpose of this study was to examine the degree of glycemic control among T2DM patients in EU.
Materials and methods: This is a matched cohort study based on a multicenter, observational study with retrospective medical chart reviews of T2DM patients in Spain, France, UK, Norway, Finland, Germany, and Poland. Included patients were aged ≥30 years at time of diagnosis of T2DM, had added a SU or a TZD to failing metformin monotherapy (index date), and had pre-existing (i.e., with onset date prior to index date) MVC. A control cohort with T2DM but without pre-existing MVC was identified using 1:1 propensity score matching. Logistic and linear regression analyses were applied to identify differences in glycemic control (HbA1c<6.5% yes/no and HbA1c level) by MVC during the follow up period, after controlling for baseline demographics, clinical information, and concurrent medication use. Robust variance estimator was used to correct for multiple observations per patient.
Results: Of the 453 patients with pre-existing MVC (cases) and eligible for inclusion, 64% were male, mean (SD) age and time from T2DM diagnosis was 64.5 (9.1) and 6.2 (5.3) years, respectively. The proportion of patients with pre-existing MVC with adequate glycemic control relative to controls during the 1st, 2nd, 3rd, and 4th years after index date was 20.7 vs. 28.2, 19.3 vs. 26.5, 18.6 vs. 26.7, and 17.7 vs. 27.8 respectively. Patients with pre-existing MVC were significantly less likely to have adequate glycemic control (Odds Ratio: 0.65; 95%CI 0.49-0.86), and had higher HbA1c levels by 0.12% (P=0.049) than controls after controlling for other potential confounding covariates. This study found that T2DM patients with pre-existing MVC tended to have poorer glycemic control than those without MVC.
Conclusion: We found that T2DM patients with pre-existing MVC tended to have poorer glycemic control than those without MVC. It may suggest that management of MVC might help reach the goal of adequate glycemic control for T2DM.
Supported by: Merck and Company, Whitehouse Station, USA

1307

Long-term cardiovascular and non-cardiovascular mortality in women and men with type 1 and type 2 diabetes mellitus: a 30-year follow-up in Switzerland
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Background and aims: While several studies in Europe, the USA and Asia have documented an increased mortality in diabetic patients when compared with non-diabetic individuals, comparably little is known on the long-term mortality risk of diabetic patients in Switzerland. The present study assessed the 30-year mortality in a well defined cohort of type 1 and type 2 diabetic patients. A special focus was put on gender differences as well as on the time course of overall and cardiovascular mortality in diabetic patients compared with the general Swiss population.
Materials and methods: The present analysis is based on a 30-year follow-up of the 533 patients (225 with type 1, 308 with type 2 diabetes) included into the Swiss Cohort of the WHO Multinational Study of Vascular Disease in Diabetes. Standardised mortality ratios (SMRs) for the entire follow-up were calculated by dividing the observed number of deaths by the expected number of deaths based on estimates from the general population. SMRs were separately computed for all-cause and cardiovascular mortality. The time course of SMRs between 1974 and 2005 was assessed by using a poisson regression model to compute annual SMRs.
Results: The study comprised 10,349 patient-years of follow-up with a low drop-out rate (6.6%). During the entire follow-up there were 352 deaths (138 and 214 deaths in type 1 and type 2 diabetes, respectively). All-cause mortality was increased in diabetic patients compared with the general population (SMR [95% CI] 3.8 [3.5-4.3]), the standardised mortality ratio (SMR) being higher for type 1 compared with type 2 diabetic patients (4.5 [3.8-5.3] vs. 3.5 [3.1-4.0], p=0.032). For cardiovascular and non-cardiovascular deaths SMRs were 5.6 (95% CI 4.8-6.6) and 2.7 (2.3-3.1) and did not differ according to type of diabetes. SMRs for all-cause and cardiovascular mortality were significantly higher in women compared with men in type 1 ($p<0.05$ and $p<0.01$) and type 2 diabetes ($p<0.001$ and $p<0.01$). In both types of diabetes, SMRs significantly decreased during the last two decades (p=0.004 and p=0.002).
Conclusion: Patients with type 1 and type 2 diabetes had an increased long-term mortality compared with the general Swiss population. Excess mortality was higher in type 1 compared with type 2 diabetes and in women compared with men for both types of diabetes, but steadily decreased over the last two decades.
Supported by: Swiss National Science Foundation

1308

Global gene expression profiling displays a network of dysregulated genes in non-atherosclerotic arterial tissue from patients with type 2 diabetes
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Background and aims: Generalized arterial alterations, such as endothelial dysfunction, medial matrix accumulations, and calcifications are associated with type 2 diabetes. These changes may render the vessel wall more susceptible to injury; however, the molecular characteristics of such diffuse pre-atherosclerotic changes in diabetes are only superficially known.
Materials and methods: To identify the molecular alterations of the generalized arterial disease in type 2 diabetes, Affymetrix high-density oligonucleotide arrays were applied to examine gene expression changes in normal-appearing, non-atherosclerotic arterial tissue (arteria mammaria interna) from 10 diabetic and 11 age-matched non-diabetic men scheduled for a coronary by-pass operation.
Results: Single gene analysis demonstrated a series of differentially regulated genes in diabetes related to endothelial and smooth muscle cell functions. In addition, global pathway analysis revealed differential expression of gene

sets representing matrix metabolism, triglyceride synthesis, inflammation, as well as hormonal and cytokine effects. Gene expression data were integrated with biological interaction networks and the results showed a significant cluster of dysregulated genes coding for both intra- and extra-cellular proteins associated with several vascular cell functions. Quantitative real-time PCR validated key findings and demonstrated differential regulation of genes associated with diabetic arteriopathy.
Conclusion: Our results identify, at the mRNA level, the diffuse arteriopathy present in non-atherosclerotic arterial tissue in patients with type 2 diabetes. These abnormalities may play a role for the arterial response to injury and putatively for the accelerated atherogenesis among patients with diabetes.
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1309

Genetic variation in cyclin dependent kinase inhibitor 2A/B is associated with the risk of coronary heart disease in type 2 diabetes in Chinese population

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Background and aims: Cyclin dependent kinase inhibitor 2A/B (CDKN2A/B) encode cyclin-dependent kinase inhibitors, as a cell growth regulator, playing an antiatherogenic role. This study investigated whether genetic variations in the CDKN2A/B genes mediating these effects affect the risk of coronary artery disease (CAD) in type 2 diabetes.
Materials and methods: We selected total 7 haplotype-tagged single nucleotide polymorphisms (SNPs) of CDKN2A/2B genes and genotyped 220 CAD-positive and 89 CAD-negative subjects with type 2 diabetes by the polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) assay or directly DNA sequencing to analyze the association of all the SNPs with CAD in the given population.
Results: The major allele G of SNP rs2811708 at CDKN2A locus was protective from CAD, with the carriers of genotype GG at lower risk of CAD compared to non-carriers (OR = 0.476,*p* = 0.006,95%CI 0.281-0.808;OR* = 0.486, *p* *= 0.012, adjusted for sex, age and BMI).
Conclusion: Our findings indicated that rs2811708 at CDKN2A locus may be associated with decreased risk of CAD in type 2 diabetes in Chinese population.

Association of SNPs at CDKN2A/2B loci with CAD in type 2 diabetics

SNP	Genotype	CAD (220)	Non-CAD (89)	OR	p	CI	OR*	*p**	CI
CDKN2A-rs2811708	**GG TX**	**117 104**	**62 26**	**0.476**	**0.006**	**0.281-0.808**	**0.486**	**0.012**	**0.277-0.854**
-rs3088440	GG AX	177 43	66 21	1.310	0.373	0.724-2.371	1.284	0.433	0.688-2.398
-rs3731237	TT CX	167 52	62 22	1.140	0.657	0.640-2.030	1.338	0.341	0.735-2.438
CDKN2B									
-rs2285327	AA GX	112 82	53 24	0.618	0.093	0.353-1.083	0.647	0.148	0.358-1.167
-rs1063192	TT CX	142 70	57 32	1.139	0.623	0.733-2.202	1.290	0.361	0.747-2.228
-rs3217992	GG AX	55 159	17 72	1.465	0.221	0.795-2.699	1.430	0.268	0.760-2.690
-rs3217986	AA CX	187 31	75 13	1.046	0.901	0.519-2.107	0.967	0.931	0.459-2.039

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1310

Subclinical functional impairment in the community-dwelling older adults with type 2 diabetes

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Background and aims: Type 2 diabetes is associated with a two to three fold increased risk of disability, loss of independency leading to frequent nursing home admissions in older adults. Interventions such as resistance exercise and amino acid supplementation may need to be implemented in early stages of disablement process to delay, stop or even reverse functional limitations. The aim of the study was to investigate whether community-dwelling non-disabled older adults with type 2 diabetes have subclinical functional impairments.
Materials and methods: We have examined 623 adults (men 43.3%, age; 70.6 ± 8.7 yrs) living in Chuncheon, Korea from January to May in 2007. All subjects without previous diagnosed diabetes were taken 75g-oral glucose tolerance test (OGTT). Diabetes was defined by previous diagnosis or fasting plasma glucose ≥ 7.0 mmol/l or 2-h plasma glucose ≥ 11.0 mmol/l during an OGTT. Physical function was assessed by the "Health Aging and Body Composition Study Physical Performance Battery (H-ABC PPB)" which included semi-tandem stance, tandem stance, singe leg stance, 6m usual walk, 6m narrow walk within 20cm width, and repeated chair stand tests. Each test was scored from 0 to 3 by quartiles of results and then summed as an overall physical performance score which ranged from 0 to 12 with high score represents higher function.
Results: We have identified 159 subjects (25.6%) as having type 2 diabetes including 77 (12.4%) subjects with previously undiagnosed cases. Older adults with type 2 diabetes showed lower balance score than those without diabetes (1.75 ± 0.89 vs. 2.00 ± 0.87, p=0.002) mainly due to poor performance in single leg standing test. In fact, there were no differences in the performance on the semi-tandem stance and tandem stance in regard to diabetes. Although 6m general walking speed was not slowed in older adults with diabetes (0.89 ± 0.28 vs. 0.91 ± 0.26 m/sec, p=0.290), 6m narrow walking speed was decreased in those with diabetes compared with those without diabetes (0.87 ± 0.28 vs. 0.93 ± 0.26 m/sec, p= 0.028). Performance on the repeated chair stand test was not different between older adults with and without type 2 diabetes. The overall H-ABC PPB score was decreased in older adults with type 2 diabetes (5.98 vs. 6.63, p=0.039). We have confirmed the results in gender stratified analyses as well (data not shown).
Conclusion: In community-dwelling apparently healthy nondisabled older adults, type 2 diabetes is associated with subclinical impairment in physical function. To identify those with early stage of disabling process, physical function test should include more demanding tasks such as single leg standing and narrow walking tests.
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1311

Glucose tolerance: association with high blood pressure and metabolic syndrome in Spanish population

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Aim: to investigate the prevalence of high blood pressure (HBP) and its relationship to categories of glucose tolerance and the Metabolic Syndrome (MS).
Design, methods: A Cross-sectional survey: 3827 participants,.54.3% females (F), 35 - 74 yrs. Anthropometric parameters: Body mass Index (BMI), Waist Circumference (WC), Blood Pressure (BP). Laboratory parameters: glucose tolerance (75g), Lipid Profile. Additionally: plasma insulin, Insulin resistance (IR) by HOMAIR MS by IDF Criteria. HBP was classified by JNC (Joint National Committee) VII criteria: Normal Systolic Blood Pressure (SBP) <120 and Diastolic Blood Pressure (DBP) < 80 mmHg, Prehypertension SBP: 120-139 or DBP 80-89 mmHg; Stage 1 Hypertension: 140-159 or DBP 90-99 mmHg; Stage 2 hypertension ≥ 160 or DBP ≥ 100 mmHg.
Results: BP (median±SD) in the general population was systolic: 127±20 mmHg, diastolic 79±11 mmHg. Overall prevalence of HBP was 30.3%; in males: 30.2% and in females: 30.5%. Prevalence of HBP in normo glycaemic subjects: 24.4%, impaired fasting glucose (IFG): 36%, impaired glucose

tolerance (IGT) 44.4%, diabetes mellitus (DM) 50.7%. The prevalence of IR was similar in both sexes except for individuals with IFG (in men, 35.4%; in women, 43.5%; p= 0.026). Overall prevalence of obesity was in NG, IFG, IGT, IFG/IGT and DM 22.8%, 33.3%, 38.1%, 32.5% and 46.5% respectively. Obese individuals were more insulin resistant than non-obese counterparts (23.7% vs. 8.5%, p< 0.001) across all categories of glucose tolerance: IFG (56.6% vs. 30.7% (p< 0.001), IGT (37.5% vs. 19.8%, p= 0.010), and DM (72.1% vs. 60.9%, p= 0.037). In subjects with HBP the prevalence of metabolic syndrome was 54.4% and IR (HOMAIR) 34 %. The prevalence of metabolic syndrome was higher in hypertensive women than in men for all categories of glucose tolerance, adjusted by age and obesity (BMI ≥ 30 Kg/m2). In a logistical regression model metabolic syndrome was associated with HBP in all categories of glucose tolerance but the biggest impact was Stage 2 hypertension in Diabetic patients, OR: 33.35 adjusted by obesity, sex and age (p<0.001).
Conclusion: a) HBP was associated with features of metabolic syndrome in general Spanish population, the association had a higher prevalence in women in all glucose tolerance categories (BMI and age adjusted) b) worsening of glucose tolerance was associated with increased prevalence of HBP c) level of glucose tolerance, obesity, sex were significantly correlated with HBP and metabolic syndrome.
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PS 124 Clinical management of vascular disease

1312

Elevated plasma homocysteine level predicts progression of atherosclerotic vascular disease in type 2 diabetic patients
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Background and aims: Diabetes is known as the major risk factor for atherosclerotic vascular disease and it directly affects prognosis of diabetic patients. Homocysteine (Hcy) is considered to take part in the development of atherosclerosis. To examine the relationship between the severity of atherosclerotic vascular disease in type 2 diabetes and Hcy, we evaluated plasma Hcy level, and compared them with three parameters of atherosclerosis (IMT:mean intima-media thickness of common carotid artery, FMD:brachial endothelium dependent flow-mediated dilatation, BaPWV:brachial-ankle pulse wave velocity).
Materials and methods: We recruited a total of 205 type 2 diabetic patients (96 men, 109 women) were included in the study. The mean age of subjects was 63.8±10.3 years (mean±SD), BMI was 23.4±3.8 kg/m^2. Of the study population, 109 patients had hypertension (53.2%), 52 patients had atherosclerotic vascular disease (25.4%). Patients with reduced renal function (estimated GFR < 60mL/min/1.73m^2) were excluded from the study. Plasma total Hcy levels were measured by HPLC, and both IMT and FMD were assessed using B-mode ultrasonography. BaPWV was measured by a volume plethysmograph.
Results: Hcy levels were significantly elevated in patients with atherosclerotic vascular disease when compared with patients without vascular disease (10.52 vs 8.94 μmol/L, p<0.01). Hcy levels were associated with IMT (p<0.01), FMD(p<0.05), whereas not associated with BaPWV. Multiple regression analysis showed that Hcy levels were independently predicted by IMT. We also followed IMT for two years in 66 patients individually. It revealed that alteration of IMT in patients with high Hcy levels were progressively increased during two years compared with patients with normal Hcy levels (0.09 vs 0.04mm, p<0.01) respectively.
Conclusion: Our results indicate that the elevated Hcy level in Japanese patients with type 2 diabetes is characterized by vascular endothelial dysfunction and increased vascular thickness. Furthemore, IMT predicts Hcy independently on multiple regression analysis. Our results also indicate that high Hcy level could accelerate vascular thickness. These results imply that homocysteine is a reliable biomarker for atherosclerotic vascular disease of type 2 diabetic patients.

1313

Screening the asymptomatic diabetic patients for coronary artery disease. Cardiac echography and N-Terminal Pro-B-Type natriuretic peptide measurement may independently help in cardiac risk assessment
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Background and aims: Plasma N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels are associated with cardiac function and structure, myocardial ischemia. BNP is expressed in human coronary plaques. High levels of NT-proBNP are also predictive of cardiac events (CE) and we hypothesized that resting echocardiography and plasma NT-proBNP levels could predict silent coronary artery disease (CAD) and CE in the diabetic population.
Materials and methods: Between 1998 and 2008, 517 asymptomatic patients with ≥1 additional cardiovascular risk factor but without heart failure were prospectively screened for silent myocardial ischemia (SMI), defined as an abnormal stress myocardial scintigraphy, and subsequently for significant (>70%) CAD on angiography. The 323 patients, with type 2 (n=306) or type 1 diabetes for 13±7 years, with a feasible echocardiography and for whom NT-proBNP could be measured were selected; 233 of them were followed up for CE (cardiac death, acute coronary syndrome, cardiac failure, secondary revascularization procedure).
Results: The univariate correlates of CAD as compared with no SMI or SMI without CAD were NT-proBNP (166±507 versus 47±110 pg/ml, p<0.0001),

male gender (OR 2.4 [CI 95%: 1.1-5.1]; p<0.05), retinopathy (OR 2.0 [CI 95%: 1.02-4.0]; p<0.05), peripheral occlusive arterial disease (OR 2.9 [CI 95%: 1.3-6.6]; p<0.01), left ventricular (LV) systolic dysfunction (OR 12.6 [CI 95%: 2.0-78.4]; p<0.001), dilatation (OR 3.7 [CI 95%: 1.2-11.3]; p<0.05) and hypertrophy (OR 2.8 [CI 95%: 1.3-6.0]; p<0.05), with a trend for hypokinesia (OR 2.5 [CI 95%: 0.9-7.3]; p=0.084), for lower body mass index (28.7±4.2 versus 30.6±6.2 kg/m², p=0.06) and for nephropathy (OR 1.8 [CI 95%: 0.9-3.5]; p=0.095). The following parameters were entered into a multiple logistic regression analysis including silent CAD as a dependent variable: NT-proBNP ≥38 pg/ml (third tertile), age, body mass index, gender, retinopathy, nephropathy and peripheral occlusive arterial disease, LV systolic dysfunction, dilatation and hypertrophy, and hypokinesia. NT-proBNP ≥38 pg/ml (OR 3.9 [95% CI 1.4-10.6]; p=0.008) and LV hypertrophy (OR 3.0 [95% CI 1.1-8.0]; p=0.031) were independent predictors of silent CAD. The percentage of patients with NT-proBNP ≥38 pg/ml was significantly higher in the patients with CAD than in the ones without CAD irrespective of the presence of LV hypertrophy and type 1 transmitral flow. Nine cardiac events occurred within a follow-up duration of 3.0±1.6 years. CAD (Kaplan-Meier, adjusted Log rank 77.9, p<0.0001), NT-proBNP ≥38 pg/ml (Log rank 10, p<0.01) and their combination (no, one or both criteria: Log rank 46, p<0.0001) were predictive of CE.

Conclusion: NT-proBNP and LV hypertrophy independently predict CAD in asymptomatic diabetic patients without heart failure. CAD and NT-proBNP levels are the main predictors of CE.

1314

Association of lower limb artery intima-media thickness with three-vessel or left main coronary artery disease

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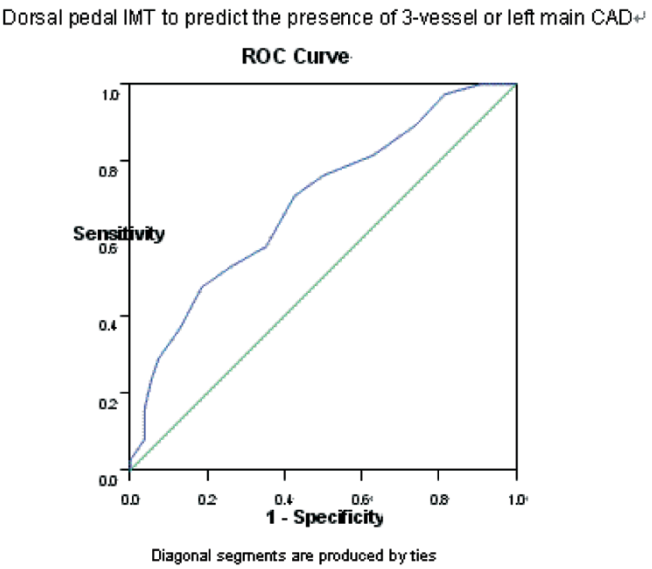
Background and aims: To examine the association between carotid and lower limb artery intima-media thickness (IMT) and occurrence of three-vessel or left main coronary artery disease (CAD) in type 2 diabetic patients.

Materials and methods: 100 type 2 diabetic patients who underwent coronary angiography for suspected CAD received B-mode ultrasound scanning of the common carotid, internal carotid, external carotid, carotid bifurcation, common femoral, superficial femoral, deep femoral, popliteal, anterior tibial and dorsal pedal artery for measurement of IMT. IMT values of carotid and lower limb system were expressed as mean values of both sides. CAD extent was evaluated by the number of diseased vessels and by Gensini score, a computerized scoring system of CAD severity.

Results: Mean IMT of the superficial femoral, deep femoral, popliteal, anterior tibial, and dorsal pedal artery were significantly higher in subjects with 3-vessel or left main CAD compare control subjects (P<0.05). Also, 3-vessel CAD group has lower high density cholesterol (HDL) level (p=0.09) and more smokers (p=0.02) in univariate analysis. Mean levels of biomarkers such as C reactive protein (CRP), intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1(VCAM-1), plasminogen activator inhibitor-1(PAI-1), and E-selectin were higher in 3-vessel CAD group, but did not gain statistical significance. Using multiple logistic regression analysis, two parameters were found to be independent predictors of 3-vessel or left main CAD: dorsal pedal artery IMT (OR 26.0; 95% CI 1.2 to 55.5; P=0.04), and smoking (OR 3.2; 95% CI 1.1 to 9.4; p=0.04). Dorsal pedal artery IMT predicted 3-vessel or left main CAD with cutoff value 0.93mm (sensitivity 71.1%, specifity 57.4%). In univariate analysis, dorsal pedal artery IMT (r=0.29; p=0.01) and course of diabetes (r=0.19; p=0.08) were correlated with Gensini score. The carotid bifurcation IMT had a weak correlation with Gensini score only in female patients(r=0.32; p=0.07). Dorsal pedal artery IMT was the only independent parameter for predicting Gensini score (p=0.01).

Conclusion: Dorsal pedal artery IMT, course of diabetes were correlated with severity and extent of CAD. Dorsal pedal artery IMT and smoking were independent predictors of three-vessel or left main CAD in type 2 diabetic patients.



1315

Glibenclamide-related excess in total and cardiovascular mortality risks: data from observational cohort study

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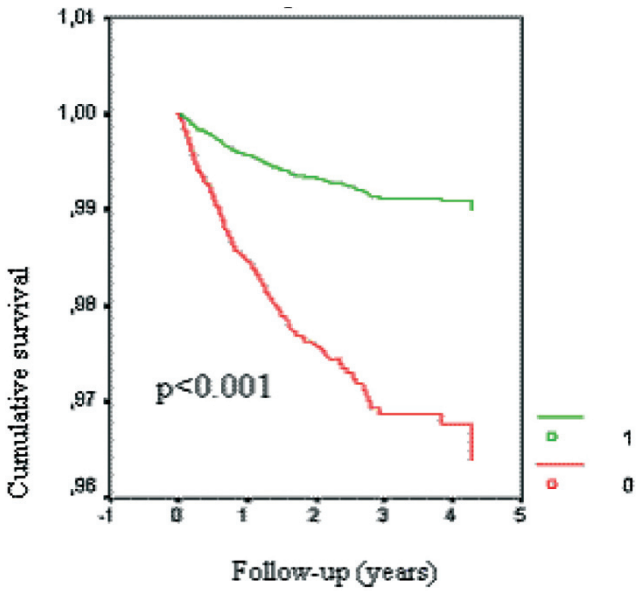
Background and aims: Negative cardiovascular effects of glibenclamide have been proven on experimental models however the CVD mortality risk had not been studied sufficiently.The aim of this study was to compare mortality risks among type 2 diabetes patients (T2D) being treated with either glibenclamide or gliclazide.

Materials and methods: Cross-sectional and cohort studies of national diabetes register were carried out. Oral antidiabetic drug (OAD) structure for 2005 and 2007 was evaluated (31 569 and 164 388 T2D patients respectively). Age at the time of death among T2D in 2002 and 2007 was compared.Risk of total and cardio-vascular (CVD) mortality was evaluated in retrospective cohort of T2D that were treated with either glibenclamide (n=21 731), or gliclazide (n=3 630). Cox regression was used for multifactor evaluation. Hazard Ratios (HRs) and 95% Confidence Intervals (95%CIs) were calculated.

Results: Age at the time of death for insulin-treated T2D had increased (p<0.001) by 1.54 (95% CI 0.81-2.26), and for OAD-treated by 6.27 (95% CI 3.67-8.87) years, p<0.001. OAD structure for 2005 and 2007 had changed: the number of glibenclamide prescriptions had decreased (p<0.001):from 64.0% (95% CI 63.5-64.5) to 59.5% (95% CI 9.7-10.4), whereas the amount of gliclazide prescriptions increased (p<0.001): from 10.1% (95% CI 9.7-10.4) to 13.4% (95% CI 13.3-13.6) respectively. Age, diabetes duration and gender-adjusted total and CVD death risks were higher (p<0.001) in the glibenclamide group: HR's 2.57 (95% CI 1.73-3.82) and 2.93 (95% CI 1.83-4.71) respectively. Further adjusting (blood glucose, body mass index, and systolic blood pressure) did not influence these results.

Conclusion: Glibenclamide treatment of type 2 diabetes is associated with much greater risk of all-cause and CVD mortality, comparing to gliclazide treatment. These results comply with recent trends of OAD structure and life span of T2D.



Cardiovascular mortality among gliclazide –treated (1) and glibenclamide – treated (0) type 2 patients

Supported by: Ukrainian National Diabetes Programme Grant

1316

Characteristics and management of diabetic patients hospitalized for myocardial infarction in France

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Background and aims: To compare characteristics and management of diabetic and non-diabetic patients six months before, during, and six months after their hospitalization for a diagnosis of myocardial infarction (MI).
Materials and methods: All hospital admissions for MI in France from January to June 2006 were selected from the national hospital discharge database. Data on medications six months before and after hospitalisation for patients covered by the general health insurance scheme (70% of French population) were merged with the reimbursement information system. Diabetic patients were identified by at least two refunds for antidiabetic medications 6 months before or 6 months after hospitalisation for MI.
Results: Of the 14 007 patients included, 2 936 were diabetics (21.0%) and 86.7% of them had antidiabetic medications before MI. After adjustment for sex and age, diabetics patients more frequently had cardiovascular medications (beta blockers, antiplatelet agents, statins, angiotensin-converting-enzyme inhibitors or angiotensin-receptor-blockers) before admission (11.2% vs. 4.2%, p<0.0001) and prior hospitalisation for stent implantation (3.9% vs. 2.2%, p<0.0001) than non-diabetics, suggesting higher prevalence of cardiovascular disease history. During hospitalization and the following month, coronary angiography was used in a similar proportion of patients (32.9% vs. 32.0%), but angioplasty (58.1% vs. 61.5%, p=0.0001) and stent implantation (55.6% vs. 59.0%, p<.0001) were less frequently used, while coronary bypass surgery was more frequent (2.9% vs. 1.7%, p=0.0003) . During the six months after MI hospitalization diabetic patients had more admission for cardiovascular diagnosis (50.8% vs.43.9% p<.0001) and received more secondary prevention medications (66.4% vs. 61.2%, p<.0001). Six months after hospitalization, 93.8% of diabetic patients had anti-diabetic medications. They were more frequently treated by insulin only (16.5% 6 months before vs. 26.0% 6 months after) or associated with oral antidiabetic drugs (OAD) (12.2% vs. 17.7%). Frequency of diabetic patients treated with OAD only decreased (55.3% vs. 50.1%).
Conclusion: In keeping with current guidelines, diabetic patients sustaining an AMI get more frequent surgical revascularisation procedures, and fewer angioplasty procedures. After the acute stage, secondary prevention medications are used more often, and the use of antidiabetic medications increases with a marked rise in the use of insulin.

1317

Primary prevention medication use in patients with type 2 diabetes who present with an acute myocardial infarction in East London

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Background and aims: NICE guidance for the treatment of type 2 diabetes states that patients are considered at high risk of CVD unless all of the following criteria are meet: not overweight (with risk stratified according to ethnic group), normotensive (<140/80 mmHg in the absence of antihypertensive therapy), no microalbuminuria, non smoker, does not have a high-risk lipid profile, has no history of cardiovascular disease and has no family history of cardiovascular disease. Those at high risk of CVD should be considered for statin and aspirin therapy depending upon a patients age and additional risk factors. The aim of this study is to assess the use of cardiovascular disease (CVD) primary prevention medication in patients with established type 2 diabetes diagnosed with an acute myocardial infarction (AMI) in accordance with national guidelines.
Materials and methods: Data was collected prospectively for 8 months (1st February to 3rd October 2008). Use of statins and aspirin for primary prevention in patients with type 2 diabetes admitted to a regional angioplasty centre with a final diagnosis of AMI were assessed on admission. During the 8 month data collection period, 573 patients were admitted with a final diagnosis of AMI, 103 patients had type 2 diabetes of whom 78 had no previous history of MI. The pre-admission medication of these patients were assessed and compared to national guidance.
Results: In patients with type 2 diabetes admitted with an AMI, primary prevention with statins and aspirin therapy was used in 48/78 (61%) and 31/78 (40%) respectively. At least 1 additional risk factor for CVD was present in 74/78 (95%) of patients. Those not taking a statin had a mean of 1.6 (±1.1) additional CVD risk factors and those not taking aspirin a mean of 2.0 (±1.1) additional risk factors.
Conclusion: These data suggest under use of statin and aspirin therapy for primary CVD prevention in patients with type 2 diabetes admitted with an AMI when compared to NICE guidelines for primary prevention. Further work is required to identify barriers to prescribing and implement solutions to improve use of primary prevention medication in patients with type 2 diabetes.

1318

Specialist multidisciplinary clinics for diabetes patients with suspected and known coronary disease are effective at reducing cardiovascular risk

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Background and aims: Diabetic patients have cardiovascular mortality rates comparable to those of non-diabetic patients with prior myocardial infarction. We have established specialist diabetic coronary disease clinics providing rapid investigation and treatment of patients with known and suspected coronary artery disease (CAD). Our specialist nurse provides lifestyle advice and risk factor modification in a model similar to that established in cardiac rehabilitation. The presentation of CAD is frequently atypical, complicating risk stratification on the basis of history alone and we therefore have a low threshold for functional imaging and invasive investigation of patients.
Methods: We conducted a prospective study of patients referred to the DCD clinic with known and suspected CAD between October 2006 until October 2008. Our DCD specialist nurse provided lifestyle and risk factor modification advice under medical supervision. Follow up was provided up to a total of 3 appointments or for a maximum of one year. Patients were assessed by cardiologists and followed established pathways of investigation. The 10-year coronary heart disease risk was calculated using the United Kingdom Prospective Diabetes Study (UKPDS) Risk Engine was used as the surrogate outcome measure. The scores were recorded at the first consultation and on discharge from nurse led follow up.
Results: A total of 233 new patients attended the clinic during the study period and were followed up for up to one year. Significant (p<0.001) reductions were recorded in systolic blood pressure 137.7(±22.8) mmHg to 129.4(±19.6) mmHg, total cholesterol 4.45(±1.14) mmol/l to 4.09(±1.08) mmol/l, LDL 2.42(±0.98) mmol/l to 2.11(±0.90) mmol/l and HbA1C 8.30(±1.72)% to

7.87(±1.43)%. Smoking prevalence was reduced from 10.7% to 6.34%. Overall, 10-year predicted risk of CHD was reduced from 20.4% to 16.0% (p<0.001).
127 of the patients seen had no past cardiac history of CAD and 74% of patients presented with atypical symptoms of angina. Exercise tolerance testing was only possible in 45% (57/127) of patients and resulted in a definitive diagnosis in only 12% (15/127). Despite this a total of 41% (53/127) of patients in the study group underwent coronary angiography. 42 patients had demonstrable CAD and 24 of these had obstructive disease for which revascularisation procedures were performed in 13 patients.
Conclusion: The study demonstrates that specialist multidisciplinary clinics with nurse led risk factor and lifestyle modification are highly effective at reducing cardiovascular risk in diabetic patients with known and suspected coronary artery disease. There is a strong case for the widespread adoption of this model as part of a strategy to improve outcomes. In addition, our data highlight the limited value of exercise ECG testing and highlight the importance of having a low threshold for functional imaging or angiography.

1319

The predictive value of diabetes mellitus for the presence and severity of angiographically established coronary artery disease and correlation with specific angiographic findings

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Background and aims: Diabetes mellitus is a well established cardiovascular risk factor of major prognostic significance for patients with coronary artery disease (CAD). However, its exact relation to the angiographically assessed anatomic extent of coronary atherosclerosis has not been conclusively delineated. In the present study, we aimed to evaluate the association between diabetes and specific coronary angiographic findings in a large unselected population of catheterized patients.
Materials and methods: The study included 1228 subjects (290 female, 938 male, mean age 64±11 years) who consecutively underwent diagnostic coronary angiography in our Catheter Laboratory for a variety of clinical indications. We compared the prevalence of diabetes between the CAD negative group and the groups with angiographically significant (>50%) narrowing of one, two and three coronary arteries and explored the relationship between diabetes and specific angiographic characteristics of the participants, such as the number of vessels affected and the selective atherosclerotic involvement of every single major coronary artery.
Results: In our cohort, diabetes was prevalent in 348 subjects (28.3%) and increased the likelihood of positive angiographic findings by approximately two-fold (Odds Ratio=2.17, p<0.001), establishing an intermediate predictive utility between dyslipidemia (Odds Ratio=3.34) and hypertension (Odds Ratio=1.65). Comparing the prevalence of diabetes among groups with 0, 1, 2 and 3 stenosed vessels, diabetes was found to correlate well with CAD severity (p<0.001), but not as strongly as smoking and dyslipidemia. The observed differences were statistically significant, when comparing the no stenosis group with double- and triple-vessel disease groups. Diabetes was also positively associated with significant obstructive lesions in left anterior descending artery (p<0.001), left circumflex (p=0.001), right coronary artery (p=0.02) and left main coronary artery (p=0.011). Concerning left main stem, diabetes was recognised as the only conventional risk factor that could reliably and independently predict significant left main stem angiographic alterations.
Conclusion: In the considerably large cohort of our study, diabetes proved to be a less important predictor of angiographic ischemic heart disease and determinant of CAD severity, compared with other traditional risk factors such as smoking and lipid disorders. However, the superiority of diabetes in terms of predicting angiographically significant left main stem disease is strongly emphasized.

1320

Patients with systemic atherosclerosis and type 2 diabetes show elevated levels of adipocyte fatty acid binding protein compared to those with normal glucose metabolism

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Background and aims: Peripheral arterial occlusive disease (PAOD) caused by lower-extremity atherosclerosis is found more frequently among people with type 2 diabetes mellitus (T2DM). The co-occurrence of PAOD and T2DM leads to higher complication and mortality rates. Recently, animal models revealed an involvement of adipocyte fatty acid binding protein (AFABP) in atherosclerosis. Furthermore, AFABP levels seem to predict T2DM development.
Materials and methods: In this study we investigated AFABP serum levels in 253 patients consisting of 85 female and 168 male patients with PAOD Fontaine stage I or II. An oral glucose tolerance test (oGTT) was performed to assess a possible influence of the patients' glucose metabolism on AFABP levels. AFAB was obtained by a commercially available ELISA (BioVendor Laboratory Medicine, Modrice, Czech Republic). Inter-assay coefficient of variation (CV) was 6.5%, and intra-assay CV was 2.9%. Statistical analyses were done with students' unpaired t-test, correlation analysis and multivariate regression modeling as appropriate. Left-skewed data were lg10 transformed before analysis in order to render the distribution normal for parametric tests. Normal data are given in mean±STD, non-parametric data as median (25; 75 percentile).
Results: After oGTT testing, 72 patients were classified as normal glucose tolerant (NGT), 45 patients had impaired fasting glucose, 32 patients showed impaired glucose tolerance, 103 patients had overt T2DM. AFABP levels of PAOD patients were significantly higher in the T2DM group (31 (23; 43) ng/ml) compared to the NGT group (26 (17; 37) ng/ml, p=0.006). Furthermore, female patients had significantly higher AFABP levels (36 (27; 44) ng/ml) compared to men (26 (19; 37) ng/ml, p<0.001). In univariate correlation analysis AFABP was positively associated with homeostatic model assessment - insulin resistance (beta=0.196; p=0.006), post-oGTT glucose (beta=0.150; p=0.018), body mass index (BMI) (beta=0.250; p<0.001), serum creatinine (beta=0.380; p<0.001), triglyceride (beta=0.158; p=0.012), alkaline phosphatase (beta=0.192; p=0.002), c-reactive protein (CRP) (beta=0.148; p=0.019), fasting insulin (beta=0.218; p=0.001) and post-oGTT insulin (beta=0.174; p=0.007). Multivariate regression modeling revealed a significant independent positive association of BMI (beta=0.245; p<0.001) and serum creatinine (beta=0.377; p<0.001) with AFABP levels. In addition, c-peptide was measured in a subgroup of 53 male PAOD patients. In this subgroup AFABP levels correlated with post-oGTT glucose (beta=0.280; p=0.042), diastolic blood pressure (beta=-0.314; p=0.022), weight (beta=0.359; p=0.008), BMI (beta=0.445; p=0.001), serum creatinine (beta=0.673; p<0.001), alkaline phosphatase (beta=0.288; p=0.037), CRP (beta=0.342; p=0.012), fasting insulin (beta=0.342; p=0.012), post-oGTT insulin (beta=0.491; p<0.001), fasting c-peptide (beta=0.278; p=0.044) and post-oGTT c-peptide (beta=0.493; p<0.001).
Conclusion: Higher AFABP levels were found in patients with diabetes compared to non-diabetic patients in severe systemic atherosclerosis. Furthermore, multivariate regression modeling revealed an independent association of AFABP with BMI and serum creatinine in PAOD patients.

PS 125 Diagnosis and management of vascular disease

1321

The impact of different types of glucose metabolism disorders on long-term outcome after an acute coronary syndrome

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Background and aims: Acute Coronary Syndrome (ACS) patients' glycemic profile is a crucial outcome factor while admission glucose and in-hospital hyperglycemia during the first days after admission or during the entire ACS hospitalization affect patient outcome after ACS. The aim of this study is to evaluate the impact of glucose metabolism disorders and glucose values (on admission, fasting, postprandial and mean values of glucose during hospitalization) on first year outcome.
Materials and methods: 520 patients who were admitted to the coronary care unit and discharged from February 2006 through October 2007 were included in this longitudinal, prospective, observational, unicenter study. First-year end points were: death (of cardiovascular cause), myocardial infarction, cardiac failure (clinical and echocardiographic determination) and unstable angina after hospitalization. Non-diabetic patients went through an Oral Glucose Tolerance Test (OGTT) one month after discharge and IGTs were categorized. To evaluate the impact of different types of glucose metabolism and glucose measurements on first-year complications after the ACS incidence, separate logistic regression models were performed for each measurement.
Results: Diabetes was previously diagnosed (Group A) in 152 (29.2%) patients, newly diagnosed (Group B) in 57 (10.9%) patients, an IGT (Group C) was observed in 110 (21.1%) patients while 201 (38.8%) were patients with normal glucose regulation (Group D). The incidence of one-year complications was 24.3%, 21.1%, 13.6% and 11.9% in groups A, B, C and D respectively (p=0.014). Concerning patient outcome during the first 12 months after ACS, group A showed the worst outcome (HR:2.66, 95%CI:1.234-5.135,p=0.001) followed by groups B(HR:1.84, 95%CI:1.129-4.328,p=0.022) and C(HR:1.24, 95%CI: 1.115-3.289,p=0.046), using group D as reference group after adjustment for age, gender, smoking, waist circumference, HDL, triglycerides, total cholesterol, metabolic syndrome (NCEP-ATP III criteria) and hypertension. The HR for the one-year outcome for patients with admission glucose in the top quartile was 2.32 (95%CI: 1.358-4.678, p<0.001), 2.11 (95%CI:1.277-5.023, p=0.001), 1.69 (95%CI:1.376-4.329, p=0.033) and 1.39 (95%CI:1.261-3.679, p=0.046) for Groups A, B, C and D respectively compared to those in the bottom quartile. Among other glucose measurements, a statistically significant difference in outcome between upper and lower quartile was in fasting glucose for Group A [HR=1.88 (95%CI:1.341-5.237, p=0.026)] and Group B [(HR=1.67 (95%CI: 1.461-5.673, p=0.039)], in postprandial glucose just for Group A [(HR=1.79 (95%CI:1.337-4.905, p=0.031)], and in the mean glucose values for Groups A [(HR=1.61 (95%CI: 1.218-5.892, p=0.036)] and B [(HR=1.47 (95%CI: 1.288-4.926, p=0.046)].
Conclusion: Patients with known diabetes mellitus have a worse long-term outcome after Acute Coronary Syndome compared with newly diagnosed and prodiabetes patients. Among different glucose measurements only admission glucose in ACS shows a more significant correlation with long-term outcome after an ACS even for normoglycemic patients.

1322

Impact of different glucose values during hospitalization on one-year outcome after an acute coronary syndrome

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Background and aims: Hyperglycemia on admission is common and associated with markedly increased in-hospital complication rates in patients hospitalized with acute coronary syndrome (ACS) with and without diabetes mellitus. Admission glucose represents only a single measurement in time while in-hospital hyperglycemia during the first days after admission or during the entire ACS hospitalization period is obtained by the use of multiple glucose values. The aim of this study is to evaluate the impact of different glucose values (admission, fasting, postprandial and mean hospitalization glucose) for these patients' first-year outcome.
Materials and methods: 520 patients were admitted to the coronary care unit and discharged February 2006 - October 2007 were included in this longitudinal, prospective, observational, study. First-year end points were: death (of cardiovascular cause), myocardial infarction, cardiac failure (clinical and echocardiographic determination) and unstable angina after hospitalization. Non-diabetic patients went through an Oral Glucose Tolerance Test one month after discharge and IGTs were categorized. To evaluate the impact of different glucose measurements on first-year complications after the ACS incidence, separate logistic regression models were performed for each measurement. The accuracy of these logistic regression models in predicting complications for the predetermined time period was assessed using the Receiver- Operating Characteristic (ROC) curves, and their respective areas under the curve (AUC).
Results: Diabetes was previously diagnosed (Group A) in 152 (29.2%) patients, newly diagnosed (Group B) in 57 (10.9%) patients, an IGT (Group C) was observed in 110 (21.1%) patients while 201 (38.8%) were patients with normal glucose regulation (Group D). The incidence of one-year complications was 24.3%, 21.1%, 13.6% and 11.9% in groups A, B, C and D respectively (p=0.014). AUC of ROC curves for the probabilities of the logistic regression models (adjusted for age, gender, smoking, waist circumference, HDL, triglycerides, total cholesterol, metabolic syndrome (NCEP-ATP III) and hypertension for the admission glucose was 0.697 (95%CI:0.565-0712,p=0.001), 0.627 (95%CI:0.536-0.682,p=0.010), 0.601 (95%CI:0.524-0.679,p=0.022), and 0.578 (95%CI:0.502-0.657,p=0.049) for A, B, C and D Group respectively. AUC for the fasting glucose was 0.632 (95%CI:0.527-0.764,p=0.021), 0.581 (95%CI:0.523-0.699,p=0.033), 0.522 (95%CI:0.484-0.640,p=0.124), 0.494 (95%CI:0.414-0.574,p=0.580) for groups A, B, C and D respectively. Statistically significant AUC for the postpandial glucose was just for Group A [0.611 (95%CI:0.588-0.722,p=0.033)], while significant AUC for the mean value of the glucose during hospitalization was 0.597 (95%CI:0.532-0.691,p=0.026) and 0.533 (95%CI:0.508-0.634,p=0.097) for Groups A and B respectively.
Conclusion: Admission glucose in ACS incidents shows a more significant correlation with first-year end-points even in normoglycemic patients. Other values do not have the same impact while prolonged hyperglycemia affects newly diagnosed as well as known diabetic patients.

1323

Susceptibility to ischaemia-reperfusion injury in isolated human diabetic myocardium *versus* non diabetic

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Background and aims: Results from a number of experimental studies using animal models of diabetes are inconsistent in sensitivity of the diabetic heart to ischemia-reperfusion injury, demonstrating no change, increased or decreased sensitivity to ischemia. These controversies may be explained by important differences in experimental models of diabetes mellitus, species differences. However, important limitations of these experiments must be highlighted: it must be emphasized that glucose concentrations reported in these experimental studies are far greater (two- to three-fold higher) than those observed in diabetic patients. Consequently, the clinical relevance of experimental studies remains limited. Thus, studies on isolated human myocardium obtained from diabetic patients may have importance. The aim of the present study was, *in vitro*, to examine the sensitivity of human diabetic myocardium to simulated ischemia reperfusion in comparison to non diabetic.
Materials and methods: After the approval of local medical ethics committee, right atrial appendages were obtained during cannulation for cardiopulmonary bypass from patients (non diabetic (ND, n=9), with diabetes mellitus type 1 (insulin-dependent diabetes mellitus (IDDM), n=9) and type 2 (non-insulin-dependent diabetes mellitus (NIDDM), n=9) scheduled for routine coronary artery bypass surgery and aortic valve replacement. The force of contraction (34°C, stimulation frequency 1 Hz) of human right atrial trabeculae was recorded during 30-min hypoxia followed by 60-min reoxygenation. The results are expressed in % of baseline. The force of contraction (FoC) was compared (mean ± SD) between the groups by a variance analysis and post

hoc test. In these three groups, patient's glycemia was measured in a blood sample taken at the time of obtaining of right atrial, and their glycemic control as measured by HbA_{1C}.
Results: Hypoxia decrease the FoC in three groups; after 30 min of exposure to hypoxia the FoC is not significantly different in 3 groups: FoC was: 20 ± 13% of baseline in ND group; 31 ± 13% in IDDM group (P=0.21 vs ND), and 25 ± 14% in NIDDM group (P=0.16 vs ND; P=0.34 vs IDDM).
Reoxygenating resulted in a partial recovery of FoC, at the end of reoxygenation period, the FoC is not significantly different in 3 groups: 53 ± 5% in ND group; 54 ± 6% in group IDDM (P= 0.29 vs ND), and 52 ± 10% in the group NIDDM (P=0.78 vs ND; P=0.18 vs IDDM). The glycemia and HbA_{1C} of the patients were respectively: 5.2 ± 0.5 mM and 5.6 ± 0.5% in ND group; 8.2 ± 2.2 mM and 7.2 ± 0.7% in IDDM group; 5.7 ± 0.6 mM and 7.0 ± 1.2% in NIDDM group.
Conclusion: The present results showed that, *in vitro,* human diabetic myocardium has the same sensitivity to hypoxia-reoxygenation injury than non diabetic myocardium. However, it should be highlighted that HbA_{1C} was comparable between groups, suggesting that glycemic control was good in the diabetic patients included in the study, contrary to the experimental studies using animal models of diabetes. We hypothesized that these results could be extended to patients with diabetes mellitus.
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1324

Newly diagnosed diabetes patients show worse short-term outcome compared to know diabetic and prodiabetic patients after an acute coronary syndrome
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Background and aims: Patients' glycemic profile after an Acute Coronary Syndrome (ACS) is a crucial outcome factor determining complications during hospitalization as well as after discharge, with known diabetic patients showing more in-hospital complications. The impact of different categories of glucose metabolism on patient outcome after discharge varies according to elapsed time after ACS. The aim of this study is to determine the prevalence of several categories of glucose metabolism in ACS patients, as well as the correlation of these categories with the incidence of short-term complications.
Materials and methods: 520 patients mean aged 66.14±11.94 years who were admitted to the coronary care unit (CCU) and discharged from February 2006 through October 2007 were included in this longitudinal, prospective, observational, study. First 30 days end- points were: death (of cardiovascular cause), myocardial infarction, cardiac failure (clinical and echocardiographic determination) and unstable angina after hospitalization. Non-diabetic patients went through an Oral Glucose Tolerance Test (OGTT) one month after discharge and IGTs were categorized. Adjusted and unadjusted logistic regression analyses were carried out in order to find the correlation between the glycemic status of the patients and the incidence of complications during the first 30 days after their discharge.
Results: Out of the study's 520 patients, diabetes was previously diagnosed (Group A) in 152 (29.2%) patients, newly diagnosed (Group B) in 57 (10.9%) patients, an IGT (Group C) was observed in 110 (21.1%) patients while 201 (38.8%) were patients with normal glucose regulation (Group D). The incidence of the first 30-days complications was 10.5%, 12.8%, 7.3% and 5% in groups A, B, C and D respectively (p=0.031). Regarding the patient outcome after the first 30 days following ACS, group B had the worst outcome (HR:2.63, 95%CI: 1.122-4.476, p=0.001) followed by groups A (HR=2.41, 95%CI:1.354-5.783, p=0.006) and C (HR=1.41, 95%CI:1.116-3.231 p=0.026), using group D as a reference group. The correlation of glycemic status after adjustment to age, gender, smoking, waist circumference, HDL, triglycerides, total cholesterol, metabolic syndrome (NCEP-ATP III) and hypertension was similar to that of the unadjusted model, with group B still showing the worst prognosis, (HR:2.15, 95%CI: 1.109-4.156, p=0.001), followed by groups A (HR:1.87, 95%CI: 1.228-5.231, p=0.003) and C (HR:1.22, 95%CI: 0.976-2.985, p=0.112), again using group D as a reference group.
Conclusion: Disturbed glucose metabolism is common among patients with ACS. Newly diagnosed diabetic patients with ACS show a worse short-term outcome compared to known diabetic patients due to the fact that those patients have diabetes that was neither appropriately recognized nor treated before hospitalization. Insulin therapy and the achievement of nornoglycemia improve outcome but hyperglycemic patients without known diabetes are much less likely to be treated with insulin even when glucose levels are markedly elevated.

1325

Aortic stiffness as a risk factor for recurrent cardiovascular events in diabetic men with recent-onset ischaemic heart disease
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Background and aims: Aortic stiffness (AS) has been shown to correlate with the possibility of recurrence of coronary events in non-diabetic patients. However, its predictive value for cardiovascular morbidity in diabetic patients with ischaemic heard disease is not yet established. The aim of this prospective cross-sectional study was to test the hypothesis that estimating AS could predict cardiovascular morbidity in diabetic patients.
Materials and methods: One hundred consecutive diabetic men with no history of cardiovascular disease, admitted to the coronary care unit between February 2006 and June 2007, were evaluated at discharge for arterial stiffness by calculating carotid-femoral pulse wave velocity (PWV). Routine biochemistry was also obtained. Follow-up telephone interviews at 12 months after discharge assessed the primary endpoints: re-hospitalisation for any cardiovascular event (unstable angina, revascularization, myocardial infarction, stroke) or death. The independent sample t-test and chi-square test were used to compare parametric and non-parametric values of risk factors for cardiovascular disease between groups of those who developed a cardiovascular event in 12 months and those who did not. Cox proportional hazard models were used to estimate cause-specific hazards of the end-points. To facilitate analysis, PWV values were divided into two halves, upper and lower. Statistical analysis was performed using SPSS 15.0.
Results: Mean age was 63.86±10.09 years, diabetes duration 11.93±9.64 years, body mass index (BMI) 28.42±4.65 Kgr/m2, waist circumference 105.81±11.77 cm, A1c glycaeted hemoglobin (HbA1c) 7.50±2.31 %, glucose at admission 166.95±52.04 mg/dl, total cholesterol (TC) 184.45±55.85 mg/dl, triglycerides 147.77±87.69 mg/dl, HDL-cholesterol 36.27±11.74 mg/dl, LDL-cholesterol 83.99±62.99 mg/dl. Frequency of smoking was 36.7%, hypertension 58.8%, dyslipidemia 44%. Overall 12 cardiovascular events (unstable angina: 6, myocardial infarction: 3, revascularization: 2, stroke: 1) and 2 deaths were recorded in the population during the 12 month period. Those in the upper compared to those in the lower half of PWV were older (68.22±7.18 vs. 58.96±10.36 years, p<0.001), had a longer diabetes duration (13.6±10.43 vs. 6.71±5.95 years, p=0.003), and had more increased TC (178.26±52.50 vs. 216.66±56.7 mg/dl, p=0.025). No statistical significant differences were detected for waist, BMI, glucose levels, troponine I, HbA1c, triglycerids, HDL-C, proportion of those receiving a lipid lowering agent, frequency of hypertension and smoking. After adjustment for age, diabetes duration, TC, and smoking, PWV was independently associated with the risk for recurrent cardiovascular morbidity (hazard ratio=2.408, 95%CI=1.313-6.88, p=0.032).
Conclusion: Increased AS was associated with increased one-year risk for recurrent cardiovascular event in diabetic men, thus representing a useful and practical measure of cardiovascular morbidity in such patients. We propose that AS could be routinely used for risk stratification in the management of diabetic men after the first coronary event.

1326

The association between the -374T/A polymorphism of the RAGE gene and blood pressure and arterial stiffness is modified by glucose metabolism status. The Hoorn and CoDAM studies
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Background and aims: Receptor for advanced glycation endproducts (RAGE)-ligand interaction triggers signal transduction pathways, which may lead to vascular complications. Genetic variation in RAGE has been shown

to alter expression and/or activity of RAGE. In the present study, we investigated whether RAGE single nucleotide polymorphisms (SNPs) were associated with markers of micro- and macrovascular disease among individuals without and with type 2 diabetes (DM2).
Materials and methods: Nine tag SNPs that cover the common RAGE gene variation were genotyped in 1291 individuals from 2 large population-based cohort studies in the Netherlands, aged 64.5±8.6 years, with normal glucose metabolism (NGM; 44%), impaired glucose metabolism (IGM; 23%) or DM2 (33%). We used linear regression analyses to compare levels of blood pressure, markers of atherosclerosis (i.e. carotid IMT and ankle-arm index), arterial stiffness and renal function across genotypes, and examine effect modification by glucose metabolism status.
Results: No consistent associations between RAGE SNPs and markers of micro- and macrovascular disease were found. However, the AA genotype of SNP -374 T/A (rs1800624) was consistently associated with lower systolic blood pressure (SBP) [-4.7 mmHg (95%-CI: -10.1; 0.7)], diastolic blood pressure (DBP) [-4.0 mmHg (-7.0; -1.1)] and pulse pressure (PP) [-2.2 mmHg (-6.3; 1.9)], as well as with less arterial stiffness [-0.54 SD (95%-CI: -1.02; -0.06)] in individuals with *NGM*, but with greater SBP [6.2 mmHg (0.9; 11.4)], DBP [2.0 mmHg (-0.9; 4.8)], PP [4.1 mmHg (0.5; 7.8)] and arterial stiffness [0.10 SD (-0.26; 0.47)] in individuals with *IGM* or *DM2* (p for interaction ≤0.05 in all analyses). Similar results were found for the haplotype that includes the -374A allele.
Conclusion: In individuals with NGM, the -374A allele of the RAGE gene is protectively, whereas in individuals with IGM or DM2 it is adversely associated with levels of blood pressure and arterial stiffness. These differences are currently unexplained, but may be related to differences in cellular expression of RAGE in response to hyperglycaemia.
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1327

Endothelial dysfunction and arterial stiffness in subjects with type 2 diabetes mellitus
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Background and aims: Arterial stiffness, recently proposed as an independent predictor of cardiovascular mortality, is regulated by a number of factors, including vascular tone. Diabetic subjects have both impaired endothelial function and increased stiffness of the large arteries. However, the relationship between endothelial dysfunction and arterial stiffness has been poorly explored in type 2 diabetic (T2DM) patients.
Materials and methods: Therefore we have tested the hypothesis that endothelial function independently affects aortic pulse wave velocity (PWV) in 39 subjects (18 men, 21 women) with T2DM (58±8 years old, diabetes duration: 12±8 years, BMI: 32.7±5.6 kg/m2, fasting C-peptide: 4.0±2.4 ng/ml, HbA1c: 8.3±0.7%).
Results: Endothelial function was measured as flow-mediated dilation (FMD) and sublingual nitroglycerin response (NTG) of the brachial artery. Carotid-femoral pulse wave velocity (PWV), and central augmentation index (Aix/HR75) were measured at rest by applanation tonometry (SphygmoCor). Time to reflection and central blood pressures were also obtained from pulse wave analysis. Central PWV correlated with age (r=0.33, p=0.04), BMI (0.53, p=0.001), waist circumference (r=0.45, p=0.05) and fasting blood glucose (r=0.41, p=0.01), but not with blood pressure parameters as estimated by 24-hour ambulatory blood pressure monitoring (ABPM). PWV was closely correlated with central BP parameters, but not with Aix/HR75 or NTG. FMD was inversely correlated with age (r=-0.46, p=0.004) and with 24-hour systolic and mean BP (r=-0.37/-0.42, p<0.05/=0.025, respectively), but not with central BP parameters, Aix/HR75 or NTG. More interestingly, FMD was significantly and inversely correlated with central PWV (r=-0.50; P<0.001). After adjusting for potential confounders, endothelial function remained independently and inversely associated with aortic PWV. On stepwise regression analysis, BMI (step 1, R2=30%), FMD (step 2, R2 change=24%), and fasting glucose (step 3, R2 change=10%), but not ABPM parameters, were independent predictors of PWV. AixHR75 and time to reflection were related only to age (R2=24% and 37%, respectively).
Conclusion: In subjects with type 2 diabetes, a decline in endothelial function seems to be independently associated with increased large artery stiffness.

1328

Increased arterial wall stiffness caused by hyperglycaemia is partially reversible in early diabetic patients
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Background and aims: Increased arterial wall stiffness (AWS) has been reported to be complicated by metabolic syndrome, impaired glucose tolerance and diabetes. The detailed relation between hyperglycemia and AWS has not been fully clarified. We investigated the influence of hyperglycemia on AWS using cardio-ankle vascular index (CAVI) which is a new method for estimating AWS.
Materials, methods and results: Three investigations were carried out in order to achieve the aim.
FIRST INVESTIGATION: We measured CAVI in 42 diabetic patients (DM) with short duration (less than 5 years) without microangipathy and 43 age-matched non-diabetic subjects (NDM), and compared the CAVI values. The CAVI of DM was significantly higher than that of NDM (7.12 vs 6.70, p<0.04). Multiple regression analysis revealed that significant risk factors for the elevation of CAVI were the presence of diabetes and age but not triglyceride (TG), HDL, LDL, systolic blood pressure (SBP) and body mass index (BMI).
SECOND INVESTIGATION: We measured CAVI in 37 DM who were hospitalized shortly with poor glycemic control. After 8 weeks, the CAVI were re-examined. In well improved DM (HbA1c improvement>1.0%, n=21), CAVI were significantly improved (8.00 to 7.44 p=0.0016). In the less improved DM (HbA1c improvement<1.0%, n=16), however, CAVI did not significantly improve (8.72 to 8.57 p=0.4793). The TG, HDL levels and SBP did not change significantly.
THIRD INVESTIGATION: We measured CAVI in 135 established DM (average duration 14.3 years) twice at interval of 18 weeks, and the change in CAVI (delta CAVI) was calculated. The clinical risk factors associated to high CAVI or CAVI elevation were evaluated by multiple regression analyses. With the multiple regression analysis using baseline CAVI as a dependent variable, age, high SBP and low BMI were significantly associated to high CAVI. On the other hand, HbA1c, lipids levels, diabetic duation and microangiopathy were not related to baseline CAVI. With the multiple regression analysis using delta CAVI as a dependent variable, no significant risk factor of CAVI elevation was detected.
Conclusion: These findings suggest that progression of AWS can be observed in the early stage of diabetes and that is partially reversible by glycemic control. The effect of glycemic control on AWS elevation may decrease in the established diabetic patients.

1329

Ambulatory arterial stiffness index and insulin sensitivity in type 1 diabetes mellitus
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Background and aims: Increased arterial stiffness may predict cardiovascular mortality in patient with hypertension or diabetes. Ambulatory arterial stiffness index (AASI) has been proposed as an indirect measure of arterial stiffness. Insulin resistance is a risk factor of cardiovascular diseases. The aim of this study was to estimate the relationship between AASI and insulin sensitivity in type 1 diabetic children and adolescents without hypertension.
Materials and methods: 88 patients (36 girls and 52 boys) aged 11-19 years (mean SD: 16±2.4), with diabetes duration 0.5-17.3 years (mean SD: 4.6±3.9) were included into the study. Ambulatory arterial stiffness index was defined as 1 minus the regression slope of diastolic over systolic BP readings obtained from 24-hour recordings. Euglycemic-hyperinsulinemic clamp by de Fronzo was performed to estimate insulin resistance. Glucose disposal rate (M value) determined during the last 30 min of the test was calculated as a surrogate of insulin resistance. The height, weight and waist circumference were measured and body mass index (BMI-SDS) and waist-SDS were calculated. HbA1c was measured (HPLC).
Results: In present group AASI was 0.24±0.19 (mean±SD). There was no difference between boys and girls (p=0.97). AASI correlated with patients age (0.23; p=0.03) and BMI-SDS (0.26; p=0.014).There was no relationship with

diabetes duration, waist-SDS and actual HbA1c. The M value ranged from 2.5 to 19 mg/kg/min (6.7±2.7). The correlation between AASI and M value was also found (r=-0.27; p=0.011). In multiple regression analysis after adjustment to patient age M value still correlated with AASI (beta=-0.23; p=0.036). But the model better describing AASI included patient age and BMI-SDS (adjusted R^2:0.1; p=0.008).
Conclusion: Among type 1 diabetic children and adolescents a relationship between arterial stiffness and insulin resistance and BMI has been found.
Supported by: Medical University of Lodz

PS 126 Biomarkers and assessment of cardiovascular disease

1330

Brachial-ankle pulse wave velocity is more correlated with visceral fat thickness measured by ultrasonography than waist circumference in type 2 diabetic patients
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Background and aims: Measurement of pulse wave velocity (PWV) is a useful non-invasive index of arterial distensibility, and predicts cardiovascular morbidity and mortality. Several studies demonstrated positive relationships between obesity and PWV in non-diabetic and type 2 diabetic patients. Although waist circumference (WC) is a fairly good indicator of the amount of total abdominal fat, it cannot distinguish the amount of visceral fat from the amount of subcutaneous abdominal fat. Ultrasound (US) measurements have been recently shown to correlate better with cardiometabolic risk factors than anthropometric measurements. We investigated the associations of PWV with obesity-related parameters including body mass index, WC, and US-measured visceral fat thickness (VFT) in type 2 diabetic subjects.
Materials and methods: 245 type 2 diabetic patients participated in the study: 131 men (mean age 58.8±12.4 years) and 114 women (mean age 60.0±13.0 years). Anthropometric, clinical, and laboratory data were measured. US procedures were performed by the same examiner using a 3.5-MHz probe. US-determined VFT was defined as the distance between the internal face of the rectus abdominis muscle and the anterior wall of the aorta. The PWV was measured between the brachial and ankle regions (baPWV), and the baPWV was measured in all patients using a waveform analyser.
Results: Strong positive correlations existed between VFT and BMI, VFT and WC, and BMI and WC ($r = 0.68$, $r = 0.79$, $r = 0.81$, respectively, $p<0.01$). The baPWV was significantly ($P<0.05$) higher in subjects with general obesity (BMI ≥ 25 kg/m^2) (1794±317 cm/s) as compared with those without general obesity (BMI < 25 kg/m^2) (1584±261 cm/s). Age ($r = 0.412$; $p< 0.01$), duration of diabetes ($r = 0.300$; $p< 0.05$), WC ($r = 0.235$; $p< 0.05$), and US-determined VFT ($r = 0.294$; $p< 0.01$) were significantly correlated with baPWV. But BMI, lipid profiles, HbA_{1C}, and systolic and diastolic BP were not correlated with baPWV. Multiple logistic regression analyses showed that the baPWV was independently associated with age ($p<0.05$) and US-measured VFT ($p<0.05$), but not WC when adjusted for various confounding factors.
Conclusion: Our data suggests that the baPWV appears to be associated with VFT measured by ultrasonography more closely than with BMI and WC in type 2 diabetic patients.

1331

Sustained improvement of glycaemic control reduces urinary leukotriene E_4 excretion in patients with type 1 diabetes
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Background and aims: The development of atherosclerosis is multifactorial. The increased risk of cardiovascular disease (CVD) among patients with either type of diabetes is only partially explained by traditional risk factors, there is substantial evidence linking chronic hyperglycemia to an increased risk. Recently, the DCCT-EDIC and 10-year UKPDS follow-up support the importance of glucose lowering in reducing the risk of coronary events; indeed CVD might be more strongly glucose mediated in type 1 diabetes and intervening on blood glucose could ameliorate CVD to a greater extent in type 1 than in type 2 diabetes. Compelling evidences suggest a role of the 5-lipoxygenase pathway (5-Lox) in the pathogenesis of CVD. This pathway generates leukotrienes that possess vasoactive, chemotactic and pro-inflammatory properties. Increased urinary leukotriene E_4 excretion (LTE_4-UE) has been reported in patients with poorly controlled diabetes. Therefore the effect of an intensive glucose strategy on LTE_4-UE was investigated prospectively.
Materials and methods: Intensive therapy (education program and revised insulin dosing or insulin initiation) was decided in 20 type 1 (T1) and 19 type 2 (T2) consecutive patients with A1c > 8.5% (age 35 and 60 y, diabetes duration 14 and 11 y, retinopathy 40 and 26% respectively, 44% of T2 previously

treated with insulin). Concomitant conditions known to be associated with an increased LTE_4 production were exclusion criteria. LTE_4 and 11 dehydro TXB_2 were measured at baseline and day 90±10 (LCMS/MS, pg/mg creatinine, data shown as Inter Quartile Ranges).
Results: A1c (7.9±1.1 vs. 9,7±1.4) and LTE4-UE (IQR: 25 - 44 - 65 vs. 39 - 63 - 99, p=0.008) decreased in T1 vs baseline. The changes in A1c and LTE_4-UE were correlated with baseline A1c (r: 0.72 and 0.69, p<0.02 and <0.005). In a subset of 11 T1, an additional sampling was possible on day 5 (LTE_4 UE not different vs. baseline: 32 - 52 - 87 vs. 36 - 56 - 98). LTE_4-UE was unchanged in T2 (IQR: 39 - 64 - 94 vs. 57 - 65 - 97) despite a similar change in A1c (7.5 vs. 10.1) vs. baseline. TXB_2-UE significantly decreased in T1 (426 - 574 - 759 vs. 577 - 766 - 1123) and T2 (151 - 380 - 750 vs. 551 - 829 -1490), whereas hs-CRP (higher in T2) and s-ICAM were unchanged vs. baseline.
Conclusion: Results suggest that hyperglycemia induces a reversible activation of the 5-Lox pathway in type 1 diabetes that is consistent with CVD appearing strongly glucose-mediated in this type of diabetes. A similar 3-month improvement of hyperglycemia did not change LTE_4-UE in T2, which suggest that the activation of the cysteinyl leukotriene pathway is multifactorial in this type of diabetes associated with complex underlying inflammation (NCT00324792)
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1332

Risk factors for vulnerable plaque rupture in patients with type 2 diabetes and acute coronary syndrome with ST elevation
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Background and aims: Type 2 diabetes is an independent risk factor for cardiovascular disease, in particular coronary artery disease. Rupture of unstable atherosclerotic plaque and thrombus formation results in the acute coronary syndrome (ACS). Risk factors for rupture of a vulnerable plaque include sudden increase in arterial blood-pressure, vasospasmus reaction and inflammatory reaction. Main destabilizers of atherosclerosis plaque are metaloproteinases - MMP-9. Vasoprotective factors include adiponectin, a cytokine with a diverse antiatherosclerotic activity. The aim of the study was to assess relationship between elevation of MMP-9 concentrations, selected inflammatory markers: visfatin, TNFα, IL6 and adiponectin in patients with type 2 diabetes and ST-elevation in acute coronary syndrome in relation to the severity of lesion in coronary arteries.
Materials and methods: 90 subjects were enrolled into the study: 55 women and 35 men with type 2 diabetes treated with sulphonylurea derivatives, diagnosed with ACS with ST-segment elevation, who underwent percutaneous coronary angioplasty. The patient were divided into two groups based on the Gensini Score (GS): the study group - 50 patients (31 women, 19 men, mean age 66±9,5 years, with severe atherosclerosis, GS>32 points [more advanced]), control group - 40 patients (24 women, 16 men, mean age 65±10 years, with less severe atherosclerosis, GS<32 points). Within 12 hours since ACS MMP-9, visfatin, TNFα, IL6, adiponectin, HbA1C were measured in the patients. Glucose and insulin levels and lipids profiles were obtained after overnight fast conditions
Results: There were no significant differences in gender, age BMI, WHR, waist, lipids profiles, HOMA-IR nor in hypertension or smoking prevalence between the two groups. The duration of type 2 diabetes was significantly longer in the study group than in the controls (6.3±2.4 vs 3.4±2.2years; p<0.05). Mean HbA1c in the study group was 7.8±1.9% vs 6,7±1,7% in the controls (p<0.05). STEMI patients with more advanced lesions presented with a significantly higher plasma MMP-9 (1112±503 vs 349±288 ng/ml; p<0.05), visfatin (187±65 vs 116±44 ng/ml; p<0,05), TNFα (62±20 vs 53±20 pg/ml; p<0.05), IL6 (17.1±14 vs 8.1±3.5 pg/ml; p<0.05) and lower adiponectin concentration (5.1±4.4 vs 8.7±3.4 mcg/ml; p<0.05) as compared to the controls.
Conclusion: Severity of inflammatory process and hypoadiponectinemia could contribute to the progression of atherosclerosis lesions in the coronary arteries in patients with type 2 diabetes.
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1333

Chemerin is associated with markers of inflammation and components of the metabolic syndrome but does not predict coronary atherosclerosis
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Background and aims: Chemerin is a recently discovered adipokine that regulates adipocyte differentiation and modulates chemotaxis and activation of dendritic cells and macrophages. Given the convergence of adipocyte and macrophage function, chemerin may provide an interesting link between obesity, inflammation and atherosclerosis in humans. We sought to examine the relationship of 1) chemerin and markers of inflammation, 2) chemerin and components of the metabolic syndrome, and 3) chemerin and coronary atherosclerotic plaque burden and morphology.
Materials and methods: Serum chemerin levels were determined in 303 patients with stable typical or atypical chest pain who underwent dual-source multi-slice CT-angiography to exclude coronary artery stenosis. Atherosclerotic plaques were classified as calcified, mixed or non-calcified.
Results: Chemerin levels were highly correlated with hsCRP (r=0.44, p<0.0001), IL-6 (r=0.18, p=0.002), TNF-α (r=0.24, p<0.0001), resistin (r=0.28, p<0.0001) and leptin (r=0.36, p<0.0001) concentrations. Furthermore, chemerin was associated with components of the metabolic syndrome including BMI (r=0.23, p=0.0002), triglycerides (r=0.29, p<0.0001), HDL-C (r=-0.18, p=0.003) and hypertension (p<0.0001). In bivariate analysis, chemerin levels weakly correlated with coronary plaque burden (r=0.16, p=0.006) and the number of non-calcified plaques (r=0.14, p=0.02). These associations, however, were lost after adjusting for established cardiovascular risk factors (OR 1.17, 95%CI 0.97 to 1.41, p=0.11 for coronary plaque burden; OR 1.06, 95%CI 0.96 to 1.17, p=0.22 for non-calcified plaques).
Conclusion: Chemerin is strongly associated with markers of inflammation and components of the metabolic syndrome. Chemerin, however, does not predict coronary atherosclerosis.

1334

Fibulin-1, a novel molecular marker for arterial disease in diabetes?
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Background and aims: Cardiovascular diseases are frequently seen in diabetes, principally because of accelerated development of atherosclerotic plaques in the arterial wall. We have recently identified a cluster of dysregulated genes at the mRNA level in non-atherosclerotic tissue from patients with type 2 diabetes using global gene expression profiling. The dysregulated genes may represent markers for pre-atherosclerotic arterial changes in diabetes and the most upregulated in diabetes was fibulin-1 (FBLN1). FBLN1 is a secreted glycoprotein of the extracellular matrix, localized in the neighbourhood of elastic fibres, but it is also a plasma protein. In this study we investigated the distribution of FBLN1 in arterial wall and the concentration in plasma from patients with diabetes *in vivo*, and in addition studied the influence of relevant factors on the production of FBLN1 *in vitro*.
Materials and methods: Patients undergoing artery by-pass graft surgery at Aarhus and Odense University Hospitals were recruited. Participants included ten men with more than 2 years known duration of type 2 diabetes, defined as known diabetes diagnosed after adolescence, and eleven non-diabetic individuals matched according to sex and age. From each subject, the waste part of the repair artery i.e. *arteria mammaria interna* was obtained and quickly dissected into intima-media and surrounding tissue. For immunohistochemistry, in total nine of the tissues from diabetic patients and eleven from non-diabetic were used. The day before operation, HbA1c, fasting glucose and lipids were measured and plasma were gathered for FBLN1 measurement (immunoassay). For *in vitro* studies, primary human vascular smooth muscle cells (HVSMCs) cultures were used as *in vitro* model of arterial physiology. HVSMCs were established from explants of healthy aortic tis-

sue obtained from excess donors' vasculature at kidney transplant operations. HVSMCs were stimulated using various substances of relevance and FBLN1 mRNA and the protein expression levels were measured *via* RT-qPCR and *via* Western blotting and ELISA respectively.
Results: Immunohistochemical studies showed a high concentration of FBLN1 in the arterial wall throughout all the tunicae with a distinct distribution in close relation with elastic fibres. Semi-quantitative analysis revealed a significantly higher amount of FBLN1 at the media/adventitia boundary, in relation to the external elastic membranes. ELISA immunoassay of serum samples showed a significant higher concentration of circulating FBLN1 in diabetic patients. *In vitro* studies, with HVSMCs, pointed at a possible up-regulation of FBLN1 at the protein level following stimulation with BMP-4 and at a possible regulatory role of progesterone on FBLN1. However we did not find influences of insulin and glucose.
Conclusion: FBLN1 occurs in higher amounts both in normal appearing arterial tissue and in plasma from patients with diabetes and therefore appears as a candidate biomarker for early changes of diabetic artery. The pathophysiological role of FBLN1 in diabetes demands further investigations.
Supported by: NOVO Nordisk Foundation, Danish Medical Research Council, Danish Diabetes Association

1335

Functional roles of transcriptional factor mafB *in vivo* assessed by siRNA technique and microarray analysis
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Background and aims: Maf is a family of transcription factor proteins characterized by a typical bZip structure, and its members have been reported to regulate developmental processes, cell differentiation, and the establishment of endocrine and non-endocrine cell function in the pancreas. It is well known that mafA is a strong transactivator of insulin, whereas mafB activates glucagon gene expression. However, mafs have been speculated to have multiple functions, especially in the networking of glucose, lipid, and energy balance. Previously, we reported that mafA suppression alternated gene expression profile not only in the pancreas but also in adipose tissue and central nervous system. Since less information is available regarding mafB than mafA, the aim of this study was to elucidate the role of mafB in tissues and organs, including the pancreas.
Materials and methods: We have established an mRNA interference technique that makes it possible to modify the level of targeted mRNAs in the mouse *in vivo*. An expression vector carrying synthetic maf-siRNA was rapidly injected into the tail vein of 8-week-old-mice (n=5), and the resulting alterations of the gene profile were analyzed with a microarray system. The role of mafB and cross-talk between the mafs in the pancreas and other tissues was explored by performing a microarray analysis (Afymetrix) to characterize the gene expression profile, and the level of each transcript was confirmed by real-time PCR.
Results: The level of mafB expression in the pancreas, and in the kidney of the siRNA-treated mice was approximately 50% less than in the stop-siRNA-treated animals. Interference with the mafB mRNA level in the pancreas by siRNA resulted in changes in the level of expression of several genes, including down-regulation of the genes coding glucagon and lipocalin 2 and up-regulation of the genes coding Ddit3 and Nurpr1. MafB mRNA suppression in the kidney of the siRNA-treated mice revealed that genes related to the immune response and lipid or iron metabolism were down-regulated, that genes coding complement component C3, lipocalin2 and fibrinogen Bβ/Aα polypeptide were markedly down regulated (to~1/10), that the genes coding clusterin, serine protease inhibitor 1-1 were also down-regulated, and that the genes coding serumglucocorticoid regulated kinase (Sgk) and thioether S-methyltransferase (Temt) were up-regulated. MafB may regulate not only endocrine hormones in the pancreas, but genes coding inflammation and immune-response-related functions and lipid metabolism in the pancreas and kidney as well. Lipocalin 2, which is abundantly expressed in the kidney, Ngal, has been reported to promote insulin resistance, whose expression was likely to be regulated by mafB. The effects of mafB suppression were pancreas- and kidney-specific or unknown, so other organs, especially adipose tissue are under examination, but the overall actions of mafB in regard to glucose and lipid metabolism are in the opposite direction to those of mafA. Preliminary evidence shows that c-maf, another maf that regulates glucagon expression, tends to have effects on gene profiling similar but with some of them are not overlapping, to those observed by mafB suppression.
Conclusion: Taken together, the findings in this study suggested potential roles of mafB in glucose/lipid metabolism or inflammatory reaction in the kidney as well as in the pancreas.
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1336

Long time culture of human aortic smooth muscle cells in high glucose concentration up-regulates ERK1/2 activation and PTP1B protein expression
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Background and aims: Recent studies have provided important insights into hyperglycemia-induced signaling dysregulation in vascular smooth muscle cells (SMCs), shedding light on activated phosphorylation cascades, and on their regulation by dephosphorylation reactions exerted by phosphatases. This study aimed to investigate the effect of long time (7 days) exposure to high glucose concentration (22.5 mM and 30mM D-glucose) on multilayered human aortic SMCs with the intent to mimic situation in type 2 diabetes in which vascular wall is exposed for long term to circulating hyperglycemia; cells grown for 12 days in 5.5mM D-glucose were used as control (low glucose condition).
Methods: The level of PI3-kinase, Akt and ERK1/2 phosphorylation, as well as of PTP1B, an enzyme regulating tyrosine phosphorylation-linked signaling events were examined comparatively in oxidant (1mM H2O2, for 10 min) and insulin (1μM, for 10 min) stimulated cells. Immunoblotting and immunofluorescence techniques have been used.
Results: The results show that compared to low glucose condition, long time exposure of multilayered SMCs to high glucose induced: (i) down-regulation of PI3-kinase and Akt pathways, and (ii) up-regulation of ERK1/2 phosphorylation and of PTP1B protein expression. Cells in high glucose exposed to oxidative stress conditions displayed (vs. non-stimulated cells): (i) decreased viability, proliferation, and p-nitrophenyl phosphatase enzymatic activity that was partly recovered in the presence of reducing DTT (20 mM), and (ii) activation of PI3-kinase, Akt, and ERK1/2. Stimulated with insulin in high glucose conditions, SMCs showed (vs. non-stimulated cells): activation of PI3-kinase, Akt, and ERK1/2 and down-regulation of PTP1B protein level, while augmenting the enzymatic activity of p-nitrophenyl phosphatase in cell lysates.
Conclusion: In conclusion, increased ERK1/2 phosphorylation and PTP1B protein expression are the common events produced at long term exposure of multilayered SMCs to high glucose concentration, as well as after stimulation by oxidative stress and insulin; the latter induced activation of PI3-kinase, Akt, and ERK1/2, while down-regulating PTP1B protein level.
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1337

Vascular effects of sphingomyelinase
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Background and aims: Sphingolipids, derived from sphingomyelin metabolism, have been implicated as important mediators of inflammatory processes in diabetes. The first step of sphingolipid biosynthesis is catalyzed by sphingomyelinase (SMase) enzymes which are reportedly upregulated in diabetes. In the present study we aimed to analyze the vascular effects of SMase and sphingosine-1-phosphate (S1P).
Materials and methods: The experiments have been carried out in thoracic aorta segments isolated from adult male C57Bl/6 mice. Vascular reactivity has been analyzed in vitro by isometric tension recording. After a 60-min equilibration period a reference-contraction has been induced by 124 mM K^+. Thereafter functional integrity of the endothelium has been tested by NO-mediated relaxations induced by acetylcholine after precontraction of the vessels with 10 μM phenylephrine (PE). Finally, the vascular effects of neutral SMase and S1P have been determined without and at two levels of precontraction induced by 0.1 or 10 μM PE. SMase- and S1P-induced con-

tractile and relaxant responses are presented as mean±SEM percentage of the K^+-induced reference-contraction and the PE-induced precontraction, respectively.
Results: The vascular effects of SMase and S1P were highly dependent on the level of precontraction: they induced contraction and relaxation in the absence and presence of 10 μM PE, respectively. After moderate precontraction induced by 0.1 μM PE, both SMase and S1P evoked a biphasic vascular reaction with an initial contraction and subsequent relaxation. These effects were dose-dependent and the maximal responses were induced by 0.2 U/ml SMase and 10 μM S1P. Inhibition of the final step of S1P synthesis by 10 μM sphingosine kinase inhibitor (SKI) decreased the contractile effect of 0.2 U/ml SMase from 32±7 to 14±3 % ($p<0.05$) while increased SMase-induced relaxation from 63±9 to 87±7 % ($p<0.05$). In contrast, inhibition of NO synthesis by 100 μM N^G-nitro-L-arginine methyl ester (L-NAME) increased the contractile effect of 0.2 U/ml SMase from 20±4 to 64±7 % ($p<0.001$) while decreased SMase-induced relaxation from 45±6 to 0±0 % ($p<0.01$).
Conclusion: Both SMase and S1P induce biphasic changes of the vascular tension with an initial contraction and subsequent relaxation. The contractile effect of SMase appears to be mediated by S1P. However, SMase-induced vasorelaxation is induced by a yet unidentified sphingolipid and mediated by NO. In case of diminished bioavailability of NO, like in type 2 diabetes, the vasoconstrictor effect of SMase may become dominant and contribute to the development of vascular dysfunction. SKI appears to be able to prevent the contractile effect of SMase without negatively influencing its vasorelaxant action.
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1338

Serum levels of endothelial monocyte-activating polypeptide-II in type 2 diabetes
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Background and aims: Endothelial dysfunction is implicated in the pathogenesis of diabetes and atherosclerosis. Endothelial monocyte-activating polypeptide-II (EMAP-II) is a multifunctional polypeptide with proinflammatory and antiangiogenic activity. EMAP-II induces procoagulant activity on the surface of endothelial cells, increases expression of E- and P-selectins and tumor necrosis factor-1, directs migration of monocytes and neutrophils, induces apoptosis in endothelial cells. However, the role of this cytokines in diabetes is not understood. The aim of this study was to investigate serum level of EMAP-II in obese and non-obese patients with type 2 diabetes.
Materials and methods: We studied 34 obese diabetic patients (age: 50,36±1,62; BMI: 36,5±1,24); 34 non-obese diabetic patients(age: 52,9±1,69; BMI: 24,3±0,42); 22 obese non-diabetic subjects (age: 50,28±1,24; BMI: 37,95±1,8) and 22 control subjects (age: 49,4±0,4; BMI: 24,03±0,61). Serum levels of EMAP-II were determined by immunoenzyme assay. Statistical analysis was performed by use Student's test and Person's. The data were presented as means±SD.
Results: We found an increase of serum level of EMAP-II in obese diabetic patients compared to obese subjects without diabetes (4,44±0,58 and 2,41±0,47ng/ml , respectively, p=0,006), also, in non-obese diabetic patients compared to control subjects (4,23±0,94 and 1,26±0,31 ng/ml respectively, p=0,005). Moreover, it was significant elevation of serum EMAP-II in obese patients without diabetes compared to control subjects (2,41±0,47 and 1,26±0,31ng/ml respectively, p<0,02). In obese and non-obese patients with diabetes it was significant correlation between HbAc1, blood glucose and EMAP-II levels (r=0,58, r=0,69 and 0,56, r=0,67 respectively, p<0,05), also between total cholesterol, triglycerides and EMAP-II (r=0,57, 0,44 and r=0,68, r=0,29, respectively p<0,05). In obese non-diabetic patients it was significant correlation between BMI, triglycerides, total cholesterol and EMAP-II (r=0,7, r=0,39, r=0,44, respectively, p<0,05).
Conclusion: The revealed change of EMAP-II serum level reflects an endothelial dysfunction in patients with type 2 diabetes. Hyperglycemia, dyslipidemia and obesity appear to be significant factor to contributing elevation of EMAP-II.

1339

N-terminal fragment of pro-brain natriuretic peptide (NTproBNP) has a high negative predictive value to rule-out silent myocardial ischaemia (SMI) in type 2 diabetes mellitus
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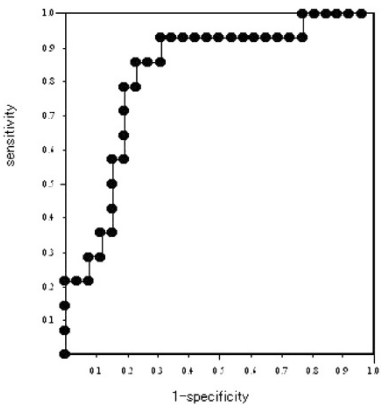
Background and aims: Early identification of cardiovascular risk is of vital importance for comprehensive diabetes management, since it allows early, targeted intervention. Screening for kidney and retinal complications has already been established, but there is no comparable screening for cardiac complications. Data about the significance of NT-proBNP in type 2 diabetes are limited. It is reported that NT-proBNP is elevated in type 2 diabetic patients reflecting asymptomatic left ventricular dysfunction and is also a strong predictor of the cardiovascular mortality in diabetes independent of conventional cardiovascular risk factors. With consideration to causality, we hypothesized that NT-proBNP might be a marker for SMI in diabetes.
Materials and methods: Forty type 2 diabetic subjects without evident coronary artery disease history or symptoms were consecutively recruited. There were essentially no singns of ischemia in their resting ECG. Serum NT-proBNP (ECLusys pro BNP) were measured and multislice CT (MSCT) of the coronary arteries were performed. Additional coronary angiographies were performed on subjects with positive MSCT findings.
Results: The patients' mean age, HbA1c and serum creatinine were 64.7, 8.33% and 0.78mg/dl respectively. Fourteen patients (35%) had SMI (defined as >50% significant stenosis of coronary arteries demonstrated by coronary angiographies). NT-proBNP levels were significantly higher in SMI(167.2±44.0 vs. 62.4 ±11.8 pg/ml,mean±SE, p<0.005) but HbA1c, lipid profiles and creatinine were similar in two groups. SMI subjects were older(70.4±2.0vs. 61.9±1.9,p<0.01), however, by after adjustment for age, HbA1c, creatinine, log NT-proBNP was identified as an independent predictor of SMI(p=0.025).
ROC analysis with a cut-off value of 52.06pg/ml showed 92.8% sensitivity and 69% specificity of predicting SMI (positive predictive value 61.9%, negative predictive value 94.7%).
Conclusion: As a biochemical screening tool for investigating SMI, NT-proBNP is useful because of its non-invasiveness and low cost. Its outstanding high negative predictive value in ruling out SMI enables the clinician to focus on high coronary risk patients within a large diabetic population.



PS 127 Cardiovascular disease - early detection and prevention

1340

Role of physical activity on endothelial function of healthy subjects

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Background and aims: A moderate physical activity is considered a protective factor against cardiovascular disease and a sedentary life style is associated to endothelial dysfunction, the first step to atherosclerosis. Nevertheless, it is still unclear to which extent a decreased active energy expenditure (EE) relates to the presence of subclinical cardiovascular disease, also because of the lack of reliable systems to continuously measure spontaneous EE .
The aim of our study was to evaluate the relationship between average EE, assessed by continuous monitoring with a validated wearable device (Armband, SenseWear), and endothelial function (EF), the earliest vascular abnormality related to cardiovascular disease, in a cohort of healthy young adults.
Materials and methods: To quantify endothelial function we performed a brachial artery Flow Mediated Dilatation (FMD) test, a non invasive, ultrasound-based technique, that records the endothelial vasodilatation of conduit arteries. As EE measurement we evaluated average Metabolic Equivalents (aMETS), spent over a one week period with the support of Armband SenseWear device. To evaluate the relationship between energy expenditure and endothelial function, we enrolled a population of 157 healthy volunteers (87 M; 70F, age =37 ± 8 years), after having excluded diagnosis of diabetes, previous cardiovascular events and other relevant diseases.
Results: Stratifying the study population by FMD quartiles, we observed that a worse endothelial function (lowest FMD quartiles) was significantly associated with a greater prevalence of male gender, smoking habit, and lower both average hours of sleep in the week in which armband was dressed and the number os hours of sleep the night before FMD test. Besides, a reduced endothelial vasodilatation was associated to higher values of BMI, waist circumference, and systolic blood pressure ($p< 0.05$ for all), as well as a bigger baseline Brachial Diameter ($p<0.01$) and significantly lower aMETS and average steps ($p<0.05$ for both). A multivariate linear regression analysis confirmed the presence of an independent relationship between aMETS and FMD, and a prediction model including average METS, the hours of sleeping the night before the test and brachial artery diameter was able to account for almost 20% of the variability in FMD values.
Conclusion: The spontaneous average physical activity, together with the hours of sleep and the brachial artery diameter (strictly correlated with gender) independently predicts the presence of endothelial dysfunction, a marker of subclinical atherosclerosis, in a population of apparently healthy young adults.

1341

Effect of exercise training on cardiopulmonary functions in type 2 diabetic rats during endotoxaemia

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Background and aims: Diabetic patients are susceptible to E. coli lipopolysaccharide (LPS) infection, resulting in endotoxemia with cardiopulmonary failure and high mortality rate. However, heat shock protein 72 (HSP72) overexpression could reduce the mortality rate and organ damage in septic shock and attenuate hemodynamic alterations due to LPS. We have demonstrated that HSP72 can be detected in various organs from normal and type I diabetic rats with exercise training. Therefore, the purpose of the current study was to characterize effects of exercise preconditioning on endotoxemia-induced cardiopulmonary dysfunction in the rat model of type 2 diabetes.
Materials and methods: Male Wistar rat fed 60% fructose-rich chow for 6-8 weeks. When loss of plasma glucose lowering response to tolbutamide were considered as type 2 diabetes with insulin resistance and randomly assigned to sedentary or exercise groups. The trained rats run gradually on a treadmill 5 days/week, 30-60 min/day with intensity 20-30m/min for 4 weeks. Twenty-four hours after the last exercise session, we compared the temporal profiles of mean arterial pressure, heart rate, cardiac output, and stroke volume of urethane-anesthetized rats after intravenous injection of LPS. At the 4th hour after LPS injection, we determined arterial blood gas, tumor necrosis factor-alpha level in serum and lavage, and lung edema. In addition, the HSP72 expression in multiple organs were determined in different groups.
Results: After exercise training, we found that HSP72 expression in multiple organs were significantly greater in rats with exercise training than sedentary rats. The survival time was significantly longer in exercised-diabetic rat than that in sedentary diabetic rats after administration of LPS (260.0 ± 23.8 min versus 712.8 ± 69.9 min, $P < 0.05$). Tumor necrosis factor-alpha level in serum were significantly lower in diabetic rats with exercise preconditioning than those in diabetic rats without exercise training (22.08 ± 14.09 pg/ml versus 0.09 ± 0.03 pg/ml, $P < 0.05$). In addition, prior exercise training could significantly diminish cardiovascular dysfunction in diabetic rats during endotoxemia.
Conclusion: Exercise training could overexpress HSP72 in multiple organs, prolong the survival time, ameliorate the survival rate and cardiopulmonary functions of type 2 diabetic rats.
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1342

Circulating endothelial progenitor cells (EPC) and endothelial function in subjects with normal, impaired and diabetic glucose tolerance

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Background and aims: Endothelium is the key regulator of vascular homeostasis. Endothelial dysfunction occurs early in diabetes and reduced number and impaired function of EPC has been described even in nondiabetic hyperglycemia. Our aims were: 1. to investigate the relationship between endothelial function and circulating EPC in subjects with normal (NGT), impaired and diabetic glucose tolerance; 2. to explore the impact of the G801A gene polymorphism of the mobilizing factor SDF-1α (stromal cell-derived factor-1α) on circulating SDF-1α, EPC levels and endothelial function.
Materials and methods: Brachial artery flow-mediated dilatation (FMD) and nitrate-induced dilatation (NID) were measured in 26 subjects with NGT (13 with, NGT/Fam+, and 13 without, NGT/Fam-, first degree family history of diabetes), 34 IFG and/or IGT, and 18 with screening-diagnosed diabetes (T2D). CD34+/KDR+ cells from peripheral blood were measured by flow cytometry as phenotype for circulating EPC. SDF-1α concentration was measured by ELISA assay and the G801A SDF-1-3'A polymorphism screened by PCR-RFLP and confirmed on PCR-DHPLC. Glucose and insulin levels in response to a 75-g oral glucose load were also obtained.
Results: The groups did not differ for gender, BMI, waist, dBP, total and LDL cholesterol, apoA1 and apoB, creatinine, cistatin C, γGT, fibrinogen, hs-CRP, SAA, and serum insulin. IFG/IGT and T2D were older than NGT (55±5 and 58±8 vs 45±10 years, $p<0.001$), had higher sBP ($p=0.001$), fasting-, 2h-OGTT- and AUC-glucose ($p<0.0001$), and HbA1c (6.0±0.4 and 6.5±0.6 vs 5.5±0.4%, $p<0.0001$), marginally higher triglycerides ($p=0.08$), and lower HDL cholesterol ($p=0.07$). NID was comparable in the four groups. FMD (and FMD/NID ratio, expression of the selective alteration of the endothelium-mediated dilatation) was lower in T2D (Δ% 4.4±3.3, m±sd), and in IFG/IGT (Δ% 6.0±2.8%) than in NGT (Δ% 7.9±3.6%, Kruskal-Wallis, $p=0.0017$) with no difference between NGT/Fam+ and NGT/Fam-. EPC pool was higher in NGT/Fam- (608±87 cells/ml, m±se), similarly reduced in NGT/Fam+ (457±68) and in IFG/IGT (440±53) and even lower in T2D (254±51 cells/ml, Kruskal-Wallis, $p=0.013$). Serum SDF-1α levels were modulated by the SDF-1α-3'A G801A polymorphism (GG, n. 45: 2522±430 pg/ml; A carriers, n. 33: 2220±572 pg/ml, $p<0.01$). Both FMD and EPC showed a negative correlation with fasting glucose ($r=-0.39$, $p=0.006$; $r=-0.20$, $p<0.05$), 2h-OGTT glucose ($r=-0.31$, $p=0.009$; $r=-0.25$, $p=0.03$), AUC glucose, and HbA1c ($r=-0.40$, $p=0.001$; $r=-0.24$, $p=0.03$). Logistic regression analysis revealed the SDF-1α-3' G/A variant to be a marginally significant ($p=0.09$) predictor of EPC level independent of HbA1c ($p=0.017$) or AUC-glucose ($p=0.022$), a role that was significant in IFG/IGT ($p<0.03$).
Conclusion: Our data suggest that: 1. both FMD as well EPC count are inversely related to the degree of hyperglycemia; 2. endothelial dysfunction and impairment in endothelial repair capacity, both early markers of macrovascular disease, are present in subjects with prediabetes; 3. EPC pool seems

to be impoverished even in NGT subjects with a positive family history for diabetes; 4. 3'G/A variant of SDF-1α, a recognized mobilizing factor for EPCs, may play an independent role in regulating EPC pool.

1343

Leukocyte telomere length correlates with subclinical atherosclerosis and is independently correlated with endothelial progenitor cell number in young adult healthy subjects

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Background and aims: Leukocyte telomere length (LTL) and endothelial progenitor cells (EPCs) are putative new cardiovascular (CV) markers. Telomeres, repeats of the DNA sequence necessary for chromosomal integrity, lose repeats with self-replication and therefore LTL is considered a marker of cellular senescence. EPCs are considered to play a key role in vessel repair following endothelial damage. LTL and EPC number are reduced in conditions characterized by increased CV risk such as diabetes. No studies are available to date investigating LTL in a population with low CV risk. Aim of the study was to evaluate LTL as novel CV risk factor and the possible correlation between LTL and EPC number in a population of young healthy adults.
Materials and methods: LTL was determined in 82 healthy subjects (49M/33F; age 37±9yrs), normotensive and not taking any medication. Fasting blood samples were drawn in all subjects for the determination of lipid profile, white blood count, high sensitive C-reactive protein (hsCRP), uric acid, PAI-1 and EPC number. EPCs were identified as cells positive for CD34, CD133 and Kinase insert Domain Receptor (KDR) cell-surface antigens by flow cytometry analysis. Mean and maximum Intima Media Thickness (IMT) (B-mode ultrasound) and Framingham Risk score (FRS) were evaluated in all subjects. LTL was assessed with a specific real-time PCR reaction in DNA samples isolated from Peripheral blood mononuclear cells (PBMCs). Data were analysed with Pearson correlation test. A multivariate analysis was performed to identify variables independently correlated to LTL.
Results: LTL resulted significantly inversely correlated with age (r-0.236; p<0.05), waist circumference (r=-0.28; p<0.001), triglycerides (r=-0.25; p<0.05), PAI-1 (r=-0.28; p<0.05), hsCRP (r=-0.23; p<0.05), IMT mean (r=-0.24; p<0.05), FRS (r=-0.28; p<0.05) and directly correlated with HDL-cholesterol (r=0.35; p<0.001) and EPC number (r=0.35; p<0.001). EPC number resulted also inversely correlated with the main CV risk factors as insulinaemia (r=-0.2; p<0.05), IMT (r=-0.318;p=0.010); PAI-1 (r-=0.197; p=0.030); FRS(r=-0.273;p=0.022). At a multivariate analysis, EPC number was associated with LTL independently of the other variables considered. Subjects were divided into two CV risk groups (higher LTL/EPC number) and (lower LTL/EPC number), the latter showed lower HDL-cholesterol (p<0.05), higher IMT (p<0.05), FRS (p<0.05) and PAI-1 (p<0.05) values compared to the lower risk group.
Conclusion: These data suggest that LTL and EPC number can be considered putative markers of CV risk and subclinical atherosclerosis also in a young adult healthy population with low CV risk. The independent correlation between LTL and EPC number suggests a shared mechanism of impairment and possible pathophysiological implications. The use in combination of the two markers might be a better indicator of subclinical atherosclerosis and of cumulative CV risk.
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1344

Polymorphism for gene of connexin 37 is associated with subclinical atherosclerosis in obese women from general population and in women with diabetes type 1

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Background and aims: Connexin 37 (Cx37) is a key protein in gap junctions, and thus important for inter-cell communication. Potential association of genetic polymorphism of Cx37 with atherosclerotic changes is matter of controversy and the proposed association of this gene with cardiovascular disease has not been proved so far. The aim of this study was to evaluate association of Cx37 gene with preclinical atherosclerosis in population of obese women from general population and in women with diabetes type 1.
Materials and methods: Women aged 45-55 representing 5% of population sample (n=892) and 102 women with diabetes type 1 aged 22-55 were genotyped for Cx37 C1019T polymorphism. Traditional cardiovascular risk factors were analyzed. Ankle/brachial blood pressure index (ABI) was measured and calculated in both groups by standardized method. The differences between carriers of different genotypes were evaluated by non-paired t-test and by test for trend (STATA). Traditional cardiovascular risk factors including smoking were also included into analyses.
Results: The prevalence of TT, CT and CC genotype in obese women from general population (body mass index more than 30 kg/m^2; n=157) was 8.3, 48.7, and 42.9 %. Similar prevalence was found in women with diabetes type 1 (11.8, 44.1, and 44.1 %). In obese women, significantly lower ABI was found in CC than in CT/TT carriers (1.024 ± 0.07 vs. 1.056± 0.09, p=0.018, p for trend 0.03). In women with diabetes type 1 and without impaired nutrition (body mass index more than 18.5 kg/m^2; n=98) similar results were obtained (0.979 ± 0.09 vs. 1.043 ± 0.2, p=0.043, p for trend= 0.065). In non-obese women from the population sample no such differences were found.
Conclusion: The gene for Cx37 was associated with preclinical atherosclerosis expressed as ankle/brachial blood pressure index in obese women from general population. Similar but weaker association was found in women with diabetes type 1.
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1345

Early atherosclerosis and dehydroepiandrosterone sulphate in patients with type 2 diabetes mellitus

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Background and aims: DHEA-S, the sulphated ester of dehydroepiandrosterone (DHEA), decreases with age in both sexes. It has been shown that this prohormone can increase the levels of nitric oxide (NO), improve insulin sensitivity and inhibit accumulation of cholesteryl ester in macrophages. Moreover, DHEA exerts an antioxidant effect on LDL. In humans, some studies showed an inverse correlation between DHEA-S and CHD, DHEA-S and atherosclerosis in men with type 2 DM, as well as with progression of atherosclerosis in healthy men and women.
To our knowledge, the association between endogenous DHEA-S and ultrasonographically measured common carotid intima-media thickness (CCIMT), a marker of preclinical atherosclerosis, in type 2 DM patients of both sexes has not been evaluated.
Materials and methods: Characteristics of participants are shown in table. DHEA-S was quantified using an electrochemiluminescent immunoassay (Coat-A DHEA-S Kit, ROCHE, Europe), on a fully automated sample selective analyzer (Elecsys 2010, Roche). High-resolution real-time ultrasonography with 7,5 MHz transducer was used to measure the left and right CCIMT. Traditional cardiovascular (CV) risk factors and alcohol use were also assessed. All participants provided written informed consent and the study was approved by the Local Ethical Committee.
Results: DHEA-S were negatively correlated with age (r=-0.278, p=0.05) and CCIMT (r= -236, p=0.002). Besides DHEA-S, CCIMT was correlated with age (r=0.325, p<0.001), systolic BP (r=0.248, p=0.001), diastolic BP (r=0.166, p=0.031) and duration of DM (r=0.169, p=0.028). In multiple regression analysis between CCIMT and several CV risk factors, DHEA-S, age, and BP were the only independent predictors of CCIMT (p values and standardized beta coefficients were for DHEA-S (log10) 0.002/-0.246, for age 0.016/0.200, and for BP 0.015/0.189).
Conclusion: In this cross-sectional study, endogenous DHEA-S levels were inversely related to CCIMT. This relationship was independent of other CVD risk factors. In our study, in agreement with other reports, CCIMT was positively associated with age and both systolic and diastolic BP. The age related decline of DHEA-S is negatively associated with CCIMT in both sexes, implicating a possible atheroprotective role of DHEA-S in patients with type 2 DM.

Characteristics of participants (Values are mean (SD) unless otherwise specified)

	Men	Women	Total	p
n (%)	102 (60,4)	67 (39.6)	169	
Age, years	66.5 (10.4)	66.3 (7.8)	65.2 (8.9)	0.13
BMI, kg/m^2	30.3 (4.2)	32.9 (5.5)	31.9 (5.1)	<0.001
Systolic BP, mmHg	144.9 (18.40)	146 (16.8)	145.6 (17.4)	NS
Diastolic BP, mmHg	79.9 (9.5)	79.9 (8.4)	79.9 (8.8)	NS
Hypertension n (%)	63 (94.0)	98 (96.0)	161 (95.3)	NS
Well controlled	4 (6.4)	8 (8.2)	12 (7.5)	
Moderate	26 (41.3)	35 (35.7)	61 (37.9)	
Poor	33 (52.4)	55 (56.1)	88 (54.7)	
HbA1c (%)	8.0 (2.0)	7.7 (1.9)	7.8 (1.9)	NS
Duration of DM, years	8.8 (6.9)	10.4 (7.7)	9.8 (7.4)	NS
Total chol	199.8 (40.5)	208.7 (39.3)	205.1 (39.9)	NS
HDL-chol	52.8 (8.2)	59.7 (10.5)	56.9 (10.2)	<0.001
LDL-chol	112.2 (33.5)	117.5 (31.5)	115.4 (32.3)	NS
Triglycerides (mg/dl)	170.2 (96.4)	166.5 (101.7)	168.0 (99.4)	NS
Pack years n (%)	20 (29.9)	93 (91.2)	113 (66.9)	0.002
0-3	23 (34.3)	9 (8.8)	32 (18.9)	0.016
>3-50	24 (35.8)	0	24 (14.2)	NA
>50				
Antihypertensive drugs, no/yes (%)	6/94	3.9/96.1		NS
	65.7/34.3	55.9/44.1		NS
Hypolipidemic drugs, no/yes (%)				
DHEA-S, µg/l	1783.1 (1019.0)	1355.8 (891.1)		0.005
Mean CCIMT, cm	0.073 (0.014)	0.070 (0.013)	0.071(0.013)	NS

1346

Acute restoration of euglyacemia improves endothelial reactivity in type 2 diabetes but alters relative contribution of nitric oxide to vasodilatation

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Background and aims: Endothelial dysfunction commonly occurs in type 2 diabetes (DM). We wished to determine the effects of acute restoration of euglycemia on forearm endothelial function.
Materials and methods: To do so we studied ten subjects with DM (age 56.5±2.5 yrs, DM duration 6.5±1.2 yrs, BMI 29.5±2.3 kg/m^2, HbA1c 7.2±0.2%), all on metformin 2000 mg/day. Venous occlusion plethysmography was used to measure brachial artery reactivity to intra-arterial graded infusions of acetylcholine (Ach) (2, 4 and 8 µg/100 ml forearm volume/min) and sodium nitroprusside (NTP) (0.5, 1 and 2 µg/100 ml forearm volume/min) before and after intra-arterial L-NMMA infusion. Measurements were recorded after an overnight fast at ambient hyperglycemia (glucose 9.8±0.7 mM; insulin 67.0±12.8 pM) and after 3 hours of euglycemia (glucose 5.5±0.1mM; insulin 131.5±11.8 pM) obtained with an insulin infusion.
Results: Baseline forearm blood flow was higher ($p<0.05$) in the presence of euglycemia then hyperglycemia (3.7±0.5 vs. 2.2±0.3 ml/100 ml/min). Peak Ach (i.e. endothelial dependent) vasodilatation also was greater ($p<0.05$) in the presence of euglycemia than hyperglycemia (14.8±2.3 vs. 10.9±1.7 ml/100 ml/min). On the other hand peak NTP induced (i.e. endothelium independent) vasodilatation did not differ in the presence of euglycemia and hyperglycemia (16.3±1.8 vs. 14.7±1.0 ml/100 ml/min). Of note, while L-NMMA inhibited ($p<0.01$) peak Ach mediated vasodilatation in the presence of hyperglycemia (8.0±1.7 vs. 10.9±1.7 ml/100 ml/min; post vs. pre L-NMMA), it did not alter Ach mediated vasodilatation in the presence of euglycemia (15.1±2.1 vs. 14.8±2.3 ml/100 ml/min; post vs. pre L-NMMA) implying a nitric oxide mediated effect in the presence of hyperglycemia but not in the presence of euglycemia. On the other hand, L-NMMA did not alter NTP mediated vasodilatation in the presence of either hyperglycemia (15.3±1.6 vs. 14.7±1.0 ml/100 ml/min) or euglycemia (17.7±1.7 vs. 16.3±1.8 ml/100 ml/min).
Conclusion: Thus, in people with DM, acute restoration of euglycemia improves Ach mediated vasodilatation via a non-nitric oxide mediated mechanism but does not alter NTP mediated vasodilatation.
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1347

High plasma fetuin-A is associated with increased carotid intima-media thickness in a middle-aged population

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Background and aims: The secreted liver protein fetuin-A (α2-Heremans-Schmid-glycoprotein, ASHG) is increased in fatty liver and is strongly associated with insulin resistance, and the metabolic syndrome. In addition, fetuin-A promotes a state of subclinical inflammation. Therefore, fetuin-A represents a possible candidate linking fatty liver to type 2 diabetes and atherosclerosis. We investigated whether elevated plasma fetuin-A levels are associated with increased intima-media thickness (IMT) of the common carotid artery in a middle-aged population at risk for type 2 diabetes, and therefore, may serve as an early biomarker of vascular defects.
Materials and methods: We studied 315 (121 males and 194 females) non-diabetic subjects at increased risk for type 2 diabetes and cardiovascular disease. IMT was measured with high resolution ultrasound (13 MHz). Total body fat was measured using magnetic resonance imaging.
Results: In a forward stepwise multivariate linear regression analysis including gender, age, total body fat, plasma fetuin-A levels, systolic and diastolic blood pressure, HDL-cholesterol and triglycerides as independent variables only age ($p<0.0001$), gender ($p<0.0001$), plasma fetuin-A ($p=0.04$) and HDL-cholesterol ($p=0.1$) predicted IMT at significance level of $p<0.15$. In an additional multivariate regression model, plasma fetuin-A was an independent predictor of IMT ($p=0.04$) after adjustment for the independent variables age, gender and HDL-cholesterol.
Conclusion: The present study provides novel evidence that high fetuin-A levels are independently associated with early anatomical changes of the vessel wall already in middle-aged subjects without manifest cardiovascular disease. Thus, plasma fetuin-A represents an interesting candidate in the pathophysiology of atherosclerotic lesions in patients at risk for type 2 diabetes and/or cardiovascular disease.
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1348

Impaired diffusing capacity for carbon monoxide in children and adolescents with type 1 diabetes: is this the first sign of long-term complications?

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Background and aims: The pathogenesis of diabetic complications is still matter of debate and is thought to involve both microangiopathic process and non enzymatic glycosylation of tissue proteins. The aim of our study was to assess the presence of lung dysfunction in adolescents with type 1 diabetes, and if the alteration of the pulmonary capacity depends more on the reduction of the diffusing capacity of the alveolar-capillary membrane or of the pulmonary capillary blood volume.
Materials and methods: We evaluated 42 consecutive young patients (23 males), ages 15±3 yrs, with type 1 diabetes for 8±5 yrs (height 164±12cm, weight 59±15 kg). As controls we evaluated 30 healthy age and sex-matched peers. Both patients and controls were non smokers and didn't report any lung disease. Lung volumes and spirometric dynamic parameters were assessed by plethysmography (VMAX277 Autobox V6200), performed in accordance with European Respiratory Society and American Thoracic Society criteria. Single-breath DLCO was measured according to the recommendation above mentioned. Double measurements were accepted when estimates of DLCO and effective alveolar volume differ by 5%. The interval between measurements was 5 min and the tests were performed in the standing position. The carbon monoxide transfer coefficient (KCO) was derived. The diffusing capacity of the alveolar-capillary membrane and the pulmonary capillary blood volume were calculated using the Roughton and Foster equation
Results: Anthropometric data and pulmonary function tests of the patients and controls are shown in the Table.
Conclusion: Lung volumes, the transfer factor (TLCO), KCO and the pulmonary capillary blood volume (Vc) were significantly reduced in young patients with type 1 diabetes when compared to controls. However, when differentiat-

ing the alveolar-capillary membrane component and the pulmonary capillary blood volume component, we observed a significant impairment only about pulmonary capillary blood volume component. If this might be seen as the 'first' sign of microangiopathic involvement in patients with type 1 diabetes have to be confirmed on larger groups but is still fascinating.

Anthropometric data and respiratory function vales in diabetes patients and controls.

	Patients with type 1 diabetes n = 42	Controls n = 30	Significance
Age (yrs)	15±3	16±1	NS
Height (cm)	164±12	167±9	NS
Weight (kg)	59±15	63±6	NS
FVC (%predicted)	101±14	120±5	0.0001
FEV1 (5 predicted)	95±13	114±7	0.0001
TLCO (% predicted)	107±14	115±13	0.05
RV/TLCO (% predicted)	107±24	99±12	NS
DLCO (% predicted)	78±16	120±16	0.0001
KCO (% predicted)	86±15	115±12	0.0001
DM (ml/mim/mmHg)	23±14	26±3	NS
Vc (ml)	34±20	88±18	0.0001

PS 128 Cardiovascular disease and diabetes

1349

Insulin sensitivity, hyperglycaemia and risk of all-cause mortality and cardiovascular disease events in the general population - the AusDiab study

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Background and aims: To assess the independent associations of insulin sensitivity, glucose and HbA_{1c} with all-cause mortality and cardiovascular disease (CVD) events in individuals without diagnosed diabetes.
Materials and methods: Between 1999-2000, baseline measures of fasting insulin, plasma glucose and lipids, two-hour plasma glucose, HbA_{1c}, blood pressure, self-reported medication use, smoking and history of CVD were collected from 8533 adults aged >35 years from the Australian Diabetes, Obesity and Lifestyle (AusDiab) study. Insulin sensitivity was estimated by the homeostasis model assessment (HOMA2-%S). Five year all-cause mortality and fatal or non-fatal CVD events were ascertained through linkage to the National Death Index and adjudication of medical records.
Results: After a median 5.0 years there were 277 deaths and 225 CVD events. HOMA2-%S was not associated with all-cause mortality. However, compared to those who were most insulin sensitive, the CVD (fatal or non-fatal) HR (95% CI) for quintiles of decreasing HOMA2-%S were 1.0 (0.6-1.8), 1.4 (0.9-2.3), 1.6 (1.0-2.5), 1.9 (1.2-3.0), adjusting for age and sex (p for trend <0.01). Smoking, CVD history, hypertension, lipid-lowering medication and total cholesterol attenuated this relationship; but the association was rendered non-significant by adding HDL-C (p for trend = 0.16). Fasting plasma glucose (FPG), but neither 2hPG nor HbA_{1c}, was significantly associated with CVD independent of HOMA2-%S.
Conclusion: In individuals without diagnosed diabetes, HOMA2-%S showed no association with all-cause mortality. The significant association between HOMA2-%S and CVD disappeared after adjusting for HDL-C. FPG was a significant predictor of CVD independent of HOMA2-%S.
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1350

Five-year incidence of coronary heart disease in type 2 diabetes. Comparison with the predicted risk by Framingham and UKPDS engines

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Background and aims: To assess the incidence of Coronary Heart Disease (CHD) in patients with type 2 diabetes mellitus (T2DM) and its relation to various risk factors, as well as to assess the predictive value of Framingham and UKPDS risk engines in a Greek population of patients with T2DM.
Materials and methods: We followed 941 consecutive patients with T2DM, who were examined for the first time at the outpatient clinic. At the 5th year of follow-up complete data was available, for 886 of them (94.2%). At baseline 658 of the patients (47.6% men) did not have macroangiopathy and were included in the present analysis. The mean age was 58.3 years and median diabetes duration 5 years. Hypertension was present in 45.6%, dyslipidemia in 41.0%, while 47.9% of the patients were current or ex-smokers. CHD was diagnosed according to UKPDS criteria, based on ECG findings, myocardial infarction, coronary angioplasty or grafting or CHD mortality. HbA1c was measured at least 15 times during the 5 years of follow-up and lipid levels at least 5 times.
Results: The 5-year incidence of CHD was 6.8%, much lower than the predicted for this population by Framingham (10.01%, p<0.05) and UKPDS (9.99%, p<0.05) risk calculators. The above risk engines did not differ between them in risk prediction. At baseline, patients who later developed CHD did not

differ in sex, age, diabetes duration, BMI, blood pressure, lipid levels, HbA1c, hypertension and dyslipidemia prevalences and smoking habits from those who did not develop CHD. During follow-up, patients without CHD showed significant improvement from baseline in HbA1c (7,5vs7,1%), in lipid levels (Cholesterol 225vs214mg%, Triglycerides 161vs149mg%, LDL 149vs138 mg%) and creatinine (1,03vs0,97 mg%), p<0,01 for all. Patients who developed CHD shoed improvement only in triglyceride levels (212vs144 mg%).
Conclusion: a) The 5-year incidence of CHD in Greek patients with T2DM is significantly lower than that predicted by Framingham and UKPDS risk engines, meaning that these engines should be used with caution in the Greek and possibly other populations b) These engines provide similar prediction of CHD in T2DM c) In spite of the short duration of follow-up, metabolic control improvement (HbA1c, lipid levels) may possibly decrease CHD incidence.

1351

Glycaemic control reduces the platelet-activating factor acetylhydrolase activity (PAF-AH activity) in type 2 diabetes mellitus

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Background and aims: The platelet-activating factor acetylhydrolase activity (PAF-AH activity) is a phospholipase A2 associated to the lipoproteins that hydrolyzes the platelet-activating factor (PAF) and oxidizes the phospholipids. It is considered that plays an important role in the development of atherosclerosis and its complications. The aim of our study was to determine the relationship between the PAF-AH and the glycaemic control in type 2 diabetes mellitus.
Materials and methods: In a cross-sectional study, we evaluated 131 type 2 diabetes mellitus subjects and 89 healthy individuals as controls. We did a new evaluation in 54 patients after improving the glycaemic control. We determined anthropometric parameters, HbA_{1c}, lipid profile, apolipoproteins A and B, leptin and adiponectin. The PAAF-AH activity was determined using 2 thio-PAF as a substrate (Cayman Chemical).
Results: In diabetic subjects total PAF-AH was 20.7±6.9 µml/min/mL and it was 18.9±6.3µml/min/mL in healthy controls (p=0.058). In the diabetic subjects, PAF-AH had a correlation with HbA_{1c} (r=304, p<0.001) and LDL cholesterol (r=353; p<0.001) and apolipoprotein B (r=223, p<0.01) concentrations. The improvement in glycaemic control (HbA_{1c} 9.7±1.8% vs 7.6±1.1%; p<0.01) carried a reduction in PAF-AH (19.9±8.2 vs 17.2±5.6µml/min/mL, p<0.001).
Conclusion: Type 2 diabetic patients show a tendency to a better PAF-AH activity and it is related to the glycaemic control.

1352

Prevention of cardiovascular disease through glycaemic control in type 2 diabetes. A meta-analysis of randomized clinical trials

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Background and aims: Randomized clinical trials (RCTs) aimed at the assessment of the efficacy of lowering blood glucose in the prevention of diabetic complications have always failed to detect a significant effect on cardiovascular events. Aim of this meta-analysis is the assessment of the effects of improvement of glycemic control on the incidence of cardiovascular diseases in patients with type 2 diabetes.
Materials and methods: The RCTs were included in this meta-analysis if: a) the between-group difference in mean HbA1c during the trial was at least 0.5%, b) they had a planned duration of treatment of at least 3 years, c) if they had a cardiovascular endpoint. Data for analysis were extracted independently by two observers and potential contrasts were resolved by a senior investigator.
Results: Five studies (17,267 and 15,362 patients in the intensive and conventional therapy groups, respectively) were included. Intensive treatment, which reduced mean HbA1c by 0.9% on average, was associated with a significant reduction of incident cardiovascular events and myocardial infarction (OR 0.89[0.83-0.95] and 0.86[0.78-0.93], respectively), but not of stroke or cardiovascular mortality (OR 0.93[0.81-1.07] and 0.98[0.77-1.23], respectively). In meta-regression analysis, a higher BMI duration of diabetes, and incidence of severe hypoglycaemia were associated with greater risk for cardiovascular death in intensive treatment groups.
Conclusion: Intensified hypoglycaemic treatment in type 2 diabetic patients leads to a significant reduction of the incidence of myocardial infarction, while it does not affect the incidence of stroke and cardiovascular mortality. Hypoglycemia induced by intensified treatment could be associated with increased cardiovascular mortality.

1353

Osteoprotegerin is associated with markers of atherosclerosis in type 2 diabetes patients

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Background and aims: Osteoprotegerin is a glycoprotein from the tumor necrosis factor receptor superfamily, involved in bone remodeling. In general population, osteoprotegerin levels are positively correlated with the presence and severity of coronary artery disease and progression of atherosclerosis. However it is little known about the relation of osteoprotegerin and cardiovascular disease in type 2 diabetes. The aim of this study was to determine the relationship between serum osteoprotegerin concentration and some markers of atherosclerosis and heart failure assessed by measurements of pulse wave velocity and NT-pro-BNP concentrations in a group of patients with type 2 diabetes.
Materials and methods: Material included 98 consecutive patients with type 2 diabetes from diabetes outpatient clinic, aged 40-70 years. In each subject osteoprotegerin, NT-pro-BNP, lipid profile (total, high density lipoprotein [HDL], low density lipoprotein [LDL] cholesterol and triglycerides), homocysteine, fasting glucose and glycated hemoglobin were evaluated. All subjects had bone mineral density assessed by dual-energy X-ray absorptiometry (DEXA). Total and percentage of visceral fat were assessed by DEXA. Serum lipids were measured by enzymatic methods using Roche reagents and homocysteine by enzyme-linked immunosorbent assay using R&D reagents. Osteoprotegerin was analyzed by ELISA method, NT-pro-BNP by electrochemiluminescence method (ECLIA Roche). Glycated hemoglobin was determined by high pressure liquid chromatography (HPLC). Carotid - femoral pulse wave velocity (PWV) was assessed using Complior system.
Results: Mean age of patients was 59,6±10,7 years, mean glycated hemoglobin values 7.3±1.6 %, BMI 31,2±3,8kg/m^2. Mean values of osteoprotegerin were 4.528±1.38pmol/l, in men 4.27±1.2, in women 4.83±1.36, p=ns. Mean values of serum homocysteine were 16.4+/-5.4 µmol/l, higher in men than in women (17.6+/-5.2 and 14.47+/-5.2 µmol/l, respectively [p<0.05]). Total cholesterol, LDL and HDL cholesterol and triglyceride levels were as follows: 5.4±1.74 mmol/l, 2.8±1.2 mmol/l 1.2±0.42 mmol/l and 3.4±5.4 mmol/l. Mean and median values of NT-pro-BNP were 186.6±341 and 63.2 pg/ml, higher in women than in men: 265.4±400.4, median 102.2 pg/ml vs 141.1±300, median 51.03 pg/ml, respectively (p<0.05). In the whole group of patients osteoprotegerin concentration correlated significantly positively with age (r=0.48), NT-pro-BNP (Spearman correlation coefficient R = 0.41), pulse wave velocity (r=0.43), pulse pressure (r = 0.43) and with percentage of total fat (r = 0.58). No correlation between plasma lipids, homocysteine levels, blood pressure, glycated hemoglobin or bone mineral density with osteoprotegerin levels were observed. Multiple regression analysis with osteoprotegerin as dependent variable and NT-pro-BNP, PWV and percentage of total fat as independent variables, revealed that only PWV (beta = 0.379, p = 0.02) and percentage of total fat (beta = 0.473, p = 0.005) were significantly associated with osteprotegerin level.
Conclusion: The results of this study indicate that serum osteoprotegerin is associated with arterial stiffness and percentage of fat mass in patients with type 2 diabetes.

1354

Copeptin predicts and explains the association between IGFBP-1 and cardiovascular events in patients with type 2 diabetes and myocardial infarction

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Background and aims: High levels of the IGF Binding Protein-1 (IGFBP-1) are associated with impaired prognosis in patients with type 2 diabetes (DM) and acute myocardial infarction (MI). Copeptin, a surrogate marker for vasopressin, is a novel cardiovascular prognostic predictor. Infusion of vasopressin increases IGFBP-1 in humans. The present study analyzed the relation between copeptin and IGFBP-1 and their impact on the prognosis in patients with DM and MI.
Materials and methods: Copeptin and IGFBP-1 was analysed at the time for hospitalisation in 393 patients with type 2 DM and MI (a subgroup from the DIGAMI 2 trial). No patient was lost to follow and all endpoints were independently adjudicated. Multiple Cox proportional hazard regression was used to study the relation between copeptin, IGFBP-1 and cardiovascular (CV) events (cardiovascular death, reinfarction and stroke) and to adjust for a number of possible confounders of which only age was of significant importance.
Results: There was a positive correlation between the levels of copeptin and IGFBP-1 (Spearman's rank correlation r = 0.53; p<0.001). During a median follow-up of 2.1 years 95 (24%) patients died, 77 of cardiovascular causes. Fifty-nine (15%) patients had a nonfatal reinfarction and 25 (6%) a nonfatal stroke. The age adjusted Hazard Ratio (HR) for CV mortality was 1.32 (95% CI 0.99-1.74; p = 0.05) for log IGFBP-1 and 1.33 (95% CI 1.08-1.64; p = 0.008) for log copeptin. The age adjusted HR for CV events was 1.06 (95% CI 0.87-1.299; p = 0.55) for log IGFBP-1 and 1.36 (95% CI 1.16-1.59; p < 0.001) for log copeptin.
Conclusion: This study shows that copeptin, a surrogate marker for vasopressin may, at least partially, explain the prognostic impact of IGFBP-1 in patients with type 2 DM and MI. Since copeptin is an independent predictor for fatal and non-fatal cardiovascular events it becomes of interest to study agents decreasing the action of vasopressin as a possibility to improve the serious prognosis in this category of patients.
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1355

Type 2 diabetes and the coronary angiographic state are mutually independent predictors of future vascular events among angiographied coronary patients

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Background and aims: Type 2 diabetes (T2DM) in cross-sectional studies is associated with coronary artery disease (CAD) and prospectively confers a strongly increased risk of vascular events. It is not certain to what extent the baseline CAD state accounts for the increased vascular risk of diabetic patients in prospective studies because angiography usually is not performed.
Materials and methods: We therefore enrolled 750 consecutive patients undergoing coronary angiography for the evaluation of stable CAD. At angiography, CAD was diagnosed in the presence of any irregularities of the vessel wall. Stenoses ≥50% were considered significant, and the extent of CAD was defined as the number of significant stenoses in a patient. Vascular events were recorded over 8 years.
Results: The prevalence of CAD (87.8% vs. 80.4%; p = 0.029) and of significant stenoses (69.5% vs. 58.4%; p = 0.010) as well as the extent of CAD (1.7 ± 1.5 vs. 1.4 ± 1.5; p = 0.014) were significantly higher in patients with T2DM (n = 164) than in nondiabetic subjects (n = 586). Prospectively, vascular events occurred in 257 patients (34.3% of the study population). T2DM after multivariate adjustment strongly predicted vascular events (adjusted hazard ratio (HR) = 1.55 [1.17-2.05]; p = 0.002). Also, the presence of CAD (HR = 3.59 [2.11-6.13]; p <0.001), the presence of significant stenoses (HR = 2.29 [1.70-3.09]; p <0.001) and the extent of CAD (standardized adjusted HR = 1.40 [1.25-1.56]; p <0.001) significantly predicted vascular events. These angiographic characteristics still predicted vascular events after additional adjustment for T2DM (HR = 3.46 [2.03-5.91], p <0.001; 2.24 [1.66-3.02], p <0.001; and 1.38 [1.24-1.54], p <0.001, respectively). Conversely, T2DM remained strongly and significantly predictive of future vascular events after adjustment for the presence and extent of CAD (HR = 1.41 [1.07-1.87]; p = 0.016).
Conclusion: Among angiographied coronary patients, the presence and the extent of CAD are higher in patients with T2DM than in nondiabetic individuals. Prospectively, T2DM and the baseline CAD state are mutually independent predictors of future vascular events.

1356

The metabolic syndrome is associated with maladaptive carotid remodeling - The Hoorn Study

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Background and aims: The metabolic syndrome (MetS) is associated with an increased risk of ischemic stroke. The underlying pathophysiology is poorly understood. Arterial remodeling, i.e. structural changes of the arterial wall, could play an important role as maladaptive remodeling (i.e., an increase in circumferential wall stress (CWS) despite changes of the arterial wall) is a risk factor for ischemic stroke. The purpose of this study was therefore to investigate, in a population-based cohort, whether MetS is associated with maladaptive remodeling.
Materials and methods: We studied 385 (n=195 women) non-diabetic, elderly subjects. A continuous MetS *z*-score (average of the sex-specific *z*-scores of the 5 individual MetS traits) was constructed. Intima-media thickness (IMT) and inter-adventitial diameter (IAD) of the carotid artery were assessed by ultrasonography, and lumen diameter (LD) and CWS were calculated. Multiple linear regression analyses were used to investigate the associations between MetS and carotid arterial properties.
Results: After adjustment for potential confounders (i.e. sex, age, height, LDL-cholesterol, use of statins, prior CVD and smoking), the MetS z-score was independently associated with greater IAD (regression coefficient (β) per SD increase in MetS z-score (95%CI)), 0.46 mm (0.27; 0.64)), LD (0.41 mm (0.23; 0.59)) and CWS (11.17 kPa (7.17; 15.17)). No statistically significant association was found between the MetS z-score and IMT (0.025 mm (-0.003; 0.053)). In addition, the reported associations were not mediated by inflammatory, metabolic or hemodynamic factors (expressed as high sensitivity C-reactive protein, adiponectin and the homeostatic model for the assessment of insulin resistance (HOMA-IR), and pulse and mean arterial pressure, respectively).
Conclusion: MetS is associated with greater CWS, which is the result of changes in LD, IAD and, to a lesser extent, IMT of the carotid artery. This process of arterial remodeling thus seems maladaptive and may therefore explain, at least partially, the increased risk of ischemic stroke in MetS.
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1357

Paraoxonase 192 Q/R gene polymorphism, enzyme activity and coronary heart disease in type 2 diabetes

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Background and aims: It has been known the high prevalence coronary heart disease (CHD) and dysglycaemia in Ukrainian population. Paraoxonase 1 (PON1) is a HDL-associated enzyme playing an important role in organophosphate detoxification and prevention of atherosclerosis (PON1 can protect LDL and HDL from oxidation at a certain extent). Variations in the PON1 gene (192 Q/R) which can be risk factors for CHD have been

described in different population but not in Ukrainian one. The study was undertaken to identify the PON1 Q192R polymorphism and to explore its impact on CHD in Ukrainian population including type 2 diabetes mellitus (T2D) patients.
Materials and methods: Two groups of subjects of both sexes were enrolled and compared: 123 healthy control subjects (C, M/F 86/47, age 55.3±2.5 yrs) and 96 T2D patients with CHD (T2Ds, M/F 28/68, age 56.2±1.4 yrs, diabetes duration 9.4±1.0 yrs, BMI 30.7±0.8 kg/m^2) under suboptimal or poor glycaemic control. DNA was amplified with PCR followed by restriction enzyme digestion. The products were size-separated on 2% agarose gel, stained with ethidium bromide, and pictured on an ultraviolet light box. Serum PON1 activity toward paraoxon was measured spectrophotometrically.
Results: The overall PON1 genotype distribution in C (QQ 39.0 %; QR 52.0 %; RR 9.0 %) was consistent with Hardy-Weinberg equilibrium (χ^2=2.52, p>0.05), and the allele frequencies were similar to those in other Caucasian populations. There was no gender differences in allele frequencies (F: Q/R 0.68/0.32 vs M: Q/R 0.64/0.36, p>0.05). In T2Ds allele frequencies were similar to C (F: Q/R 0.67/0.33 vs M: Q/R 0.79/0.21, p>0.05). But we observed difference in the PON1 genotype distribution between groups (χ^2=13.4, p<0.01). The genotype distributions in T2Ds were: QQ 57.0 %, QR 26.0 %, RR 17.0 %. Thus, there were more homozygous T2Ds (for both Q and R alleles) as compared to C. Relative risk (the odds ratio) of studied pathologies development in population for QQ and RR homozygote was 1.46 [95% CI 1.18 - 1.74, p<0.05] and 1.86 [95% CI 1.13 - 2.60, p=0.05], respectively, and for QR heterozygote was 0.50 [95% CI 0.12 - 0.88, p<0.05]. Serum PON1 activity was significantly decreased in T2Ds as compared to C (p<0.01), and PON1 genotype had the effect on enzyme activity level: the presence of QQ genotype was accompanied by lower (4-fold) PON1 activity than RR genotype (p<0.002).
Conclusion: The study provides first evidence for the potential role of PON1 Q192R polymorphism in protection of T2D patients from CHD in Ukrainian population and demonstrates heterosis effect at this locus, which may be due to different type of enzymatic activities of isozymes. These findings warrant further investigation to clarify their pathogenic mechanisms.

1358

Relationship between carotid intima-media thickness with plaque and brachial-ankle pulse wave velocity in patients with type 2 diabetes

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Background and aims: Carotid intima-media thickness (IMT) and pulse wave velocity(PWV) have been shown to be good surrogate markers of clinical atherosclerosis. Recently, brachial-ankle PWV(baPWV) has been developed as a more noninvasive and convenient methods for assessment of arterial stiffness. Several studies have demonstrated a significant correlation between aortic PWV and carotid IMT. However, the interrelation between carotid IMT and baPWV in type 2 diabetes patients has not yet been fully clarified. We determined the relationship between carotid IMT with plaque and baPWV in patients with type 2 diabetes mellitus(DM)
Materials and methods: One hundred twenty two patients (56 men, 66 women, mean age 60.8±10.5 years, mean DM duration 7.0±7.3 years) were included. Subjects with type 1 diabetes, peripheral artery obstructive disease were excluded. Carotid IMT and plaque formation was assessed using B-mode ultrasonography at both common carotid artery(CCA)[10mm distal to the bifurcation], bifurcation and internal carotid artery(ICA)[10mm proximal to the bifurcation]. baPWV was measured using an automated device.
Results: Maximal baPWV was 1648±363.2 cm/sec, mean IMT of Rt. carotid artery was 0.73±0.20mm, mean IMT of Lt. carotid artery was 0.79±0.26mm, maximal IMT of carotid artery was 1.19±0.41mm, and plaque was found in 11% of subjects. In bivariate correlation analysis, maximal baPWV was significantly correlated with IMT of Rt. CCA (r=0.322, p<0.01), mean IMT of Rt. carotid artery(r=0.235, p<0.01), IMT of Lt. CCA (r=0.221, p=0.02), mean IMT of Lt. carotid artery(r=0.412, p<0.01), maximal IMT of carotid artery (r=0.409, p<0.01) and plaque (r=0.369, p<0.01). Maximal IMT of carotid artery was significantly associated with age(r=0.314, p<0.01), systolic blood pressure(SBP)(r=0.369, p<0.01) and DM duration (r=0.200, p<0.03). The existence of plaque was significantly associated with age(r=0.265, p<0.01), SBP(r=0.271, p<0.01) and DM duration (r=0.224, p=0.02). Multiple regression analysis was performed with maximal IMT of carotid artery and plaque as a dependent variable respectively. Maximal IMT of carotid artery was significantly correlated with SBP(β =0.269, p<0.01). The existence of plaque was significantly correlated with maximal baPWV (β =0.280, p=0.02).
Conclusion: baPWV was significantly correlated with carotid IMT and plaque in patients with type 2 diabetes.

1359

Impact of glycaemic control on the prognosis of diabetic patients with critical limb ischaemia

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Background and aims: Critical limb ischaemia (CLI) has a poor prognosis for both survival and limb salvage and often requires a revascularization procedure. Diabetes mellitus is one of the major risk factors for CLI and its prevalence is considerably high in patients with CLI. Few studies have, however, examined predictive indicators for mortality and lower extremity amputation in diabetic patients with CLI, especially treated with endovascular therapy. The aim of this study was to reveal the influence of a variety of possible risk factors including poor glycaemic control on their outcome.
Materials and methods: We recruited 176 consecutive patients with diabetes mellitus, who underwent percutaneous transluminal angioplasty (PTA) for CLI between 2003 and 2008. The outcome measures were mortality and major amputation, and Cox proportional hazards regression analyses were performed. The variables in the analyses included age, gender, activity of daily living (ADL) assessmemt, history of cardiovascular disease, haemoglobin A1c (HbA1c), C-reactive protein (CRP), ejection fraction, and receiving regular haemodialysis.
Results: HbA1c levels were 7.1 ± 1.4% and follow-up period was 1.5 ± 1.2 years (mean ± SD). One hundred and sixteen patients had infrapopliteal lesions revascularized. Forty seven patients (26.7%) underwent major amputations and 54 patients (30.7%) died. In the multivariable model, HbA1c, as well as CRP and low ADL, was significantly associated with major amputation; adjusted hazard ratios (HR) of HbA1c, CRP and low ADL were 1.36 per 1% increase (p = 0.029), 1.16 per 1 mg/dl increase (p = 0.028), and 2.96 (p = 0.008), respectively. On the other hand, independent predictors for mortality were low ADL (HR 3.13; p = 0.002), receiving regular haemodialysis (HR 2.98; p = 0.009) and impaired ejection fraction (HR 1.02 per 1% decrease; p = 0.016). There was no significant association between HbA1c and mortality.
Conclusion: Poor glycaemic control, indicated by an elevated value of HbA1c, predicts major amputation, but not mortality, in diabetic patients with CLI undergoing PTA. Prognostic predictors seem somewhat different between survival and limb salvage in the population.

PS 129 Non-conventional risk factors for cardiovascular disease

1360

A proinflammatory and prothrombotic environment in young individuals with type 1 diabetes: the effects of glycaemic control

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Background and aims: Raised C-reactive protein (CRP) and complement C3 plasma levels are associated with increased risk of cardiovascular events. In addition to inflammatory molecules, fibrin clot structure has been linked to cardiovascular disease, as compact clots are associated with premature and more severe atherothrombotic conditions. Although the atherosclerotic process starts at an early age, little is known about CRP and C3 plasma levels in younger individuals with type 1 diabetes (T1DM). Also, studies investigating blood clot structure in this cohort are still lacking. The present work analyses CRP and C3 plasma levels and evaluates fibrin clot structure in children with T1DM and results are compared to normal control individuals.
Materials and methods: ELISA was used to determine CRP and C3 plasma levels in 30 Type 1 DM children [14 yrs (range 11-17)] and 17 age matched controls, whereas fibrin clot structure was studied using a validated turbidimetric assay.
Results: T1DM children had higher C3 levels (mean±SEM) compared with controls (1.13±0.24 versus 0.83±0.24 mg/ml respectively; p<0.001), whereas the difference in CRP levels failed to reach statistical significance (0.85±1.36 versus 0.5±1.21 mg/l respectively; p=0.06). Clot final turbidity, an indicator of clot density, was higher in diabetes children at 0.38±0.01 au, compared with controls (0.31±0.02 au ;p<0.01). Clot final turbidity correlated positively with C3 plasma levels (r=0.39, p<0.01) but not CRP, suggesting an interaction between C3 and clot structure.
Conclusion: This is the first report describing a denser ex vivo clot structure and elevated C3 levels in children with T1DM, further confirming that the vascular inflammatory/thrombotic process starts at a young age in individuals at risk.

1361

Vitamin D levels and mortality in type 2 diabetes

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Background and aims: Low levels of vitamin D is independently associated with an increased all-cause mortality risk in the general population. This study evaluated vitamin D deficiency as a predictor of overall and cardiovascular mortality in patients with type 2 diabetes.
Materials and methods: In a prospective observational follow-up study 315 caucasian type 2 diabetic patients were followed for a median (range) of 15.5 (0.2-17.0) years. Samples available in 290 subjects, mean age 54 years, normoalbuminuria (n=173), microalbuminuria (n=73), and macroalbuminuria (n=44) at baseline. Vitamin D, 25(OH)D_3, levels were determined by high performance liquid chromatography/tandem mass spectrometry (LC-MS/MS) on stored baseline samples. Samples were available in 290 subjects, mean age 54 years. Severe vitamin D deficiency was defined as the lower 10% (<13.9 nmol/l).
Results: Median (range) vitamin D level was 35.7 (5 to 136.7) nmol/l. Vitamin D levels were not associated with age, sex, eGFR, urinary albumin excretion rate, or hemoglobinA_{1c},at baseline, but low levels were weakly associated with elevated systolic blood pressure (R=0.13, p=0.03). Of the patients, 142 died (49%), 119 of them (72%) due to cardiovascular causes. All-cause mortality was increased in patients with severe vitamin D deficiency (hazard ratio [95% CI] 2.03 [1.26 to 3.26], p=0.003). The association persisted after adjustment for urinary albumin excretion rate, glomerular filtration rate, hemoglobinA_{1c} and conventional cardiovascular risk factors (covariate adjusted hazard ratios 1.94 [1.19 to 3.16], p<0.01). Vitamin D deficiency was also associated with increased cardiovascular mortality (unadjusted hazard ratios 1.93 [1.10 to 3.39], p=0.02; covariate adjusted 1.49 [1.156 to 1.93] p=0.002).
Conclusion: In patients with type 2 diabetes, severe vitamin D deficiency is a risk factor for excess overall and cardiovascular mortality, being independent of urinary albumin excretion rate and conventional cardiovascular risk factors. Whether vitamin D substitution in type 2 diabetic patients can improve the prognosis remains to be determined.

1362

Free fatty acids are associated with Q-T interval length in men, but not women, with type 1 diabetes

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Background and aims: Free fatty acids have been shown to be associated with ischemic heart disease in the general population and in some reports with Q-T interval lengthening. As free fatty acids are chronically elevated in type 1 diabetes and account for a significant amount of the counterregulatory response to hypoglycemia, it has been hypothesized that this, via Q-T interval lengthening, might account for some of the silent (or unrecognized) coronary artery disease observed in this population.
Materials and methods: We therefore sought to determine whether free fatty acids were associated with Q-T interval lengthening and 183 participants (87 men and 96 women) from the Pittsburgh Epidemiology of Diabetes study, a cohort of childhood onset (age < 17 years) type 1 diabetes. As we have previously noted sex-specific free fatty acid associations with pulse pressure, i.e. an association in women, but not men, we also sought to determine whether any free fatty acid association with Q-T interval length also varied by sex. Mean age and diabetes duration were 44 and 33 years, respectively, at time of investigation. Free fatty acids were measured using the colorimetric method (Wako Pure Chemical Industires, Ltd). Corrected Q-T intervals were calculated using Hogdes Formula (Corrected QT=QT+ (1.75*(vent rate-60))). Free fatty acids were naturally logarithmically transformed before analyses and all analyses were controlled for serum albumin.
Results: Mean free fatty acid levels were 0.95 mmol/l and did not vary by sex (0.93 vs 0.96 mmol/l for men and women respectively, p=0.76). Overall, age- and serum albumin adjusted free fatty acids showed no association with the Q-T interval (r=0.05, p=0.51). However, upon sex-specific examination, age- and serum albumin -adjusted free fatty acids were associated with the Q-T interval in men (r=0.22, p=0.04), but not in women (r=-0.05, p=0.62). After further allowing for systolic blood pressure, HbA_{1c}, albumin excretion rate, and HDL cholesterol, free fatty acids remained associated with the Q-T interval in men (p=0.03), but continued to show no association in women (p=0.61).
Conclusion: Free fatty acids are associated with an increased Q-T interval in men, but not women, with type 1 diabetes. Free fatty acids may contribute to the increased risk of cardiovascular disease in type 1 diabetes, albeit their effects may vary by gender.
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1363

Association between leukocyte telomere shortening and CVD in patients with type 2 diabetes mellitus

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Background and aims: Many studies have measured leukocyte telomere length with the aim to identify associations with aging-related diseases in humans, including type 2 diabetes mellitus (T2DM). However, is unclear which biomarkers and/or T2DM complications could be significantly correlated to telomere shortening observed in these patients. We performed a cross-sectional study to examine the differences in telomere length between healthy subjects (CTR), T2DM and diabetic patients with myocardial infarction (T2DM +MI), with the aim to clarify if telomere length could be a reliable marker associated with MI in T2DM patients. Secondary end point is

the identification of possible correlations between leukocyte telomere length and selected biomarkers of pro-inflammatory status, of glycemic control and of lipidic profile.
Materials and methods: A total of 307 elderly subjects, 103 T2DM (mean age 70 ± 4 years, 59% males), 100 T2DM +MI (mean age 70 ± 7 years, 65% males), and 104 CTR (mean age 69 ± 7 years, 50% males) were studied. Telomere length, defined as T/S (Telomere-Single copy gene ratio), was determined in leukocytes by quantitative real time polymerase chain reaction (Real-Time PCR)-based assay. Moreover, we assessed: 1) high sensitive C reactive protein (hsCRP), fibrinogen and plasminogen-activator inibitor-1 (PAI-1) as inflammatory markers; 2) fasting glucose, insulin, glycated haemoglobin (HbA_1C) and waist to hip ratio as markers of glycemic control; 3) total-cholesterol, HDL-cholesterol and triglycerides as markers of lipidic profile, in all sample population. Use of statins and sulfonylurea, and presence of some relevant diabetes complications (nephropathy and retinopathy) were also assessed.
Results: The main result of the present study is that T2DM +MI patients have leukocyte telomere lengths shorter than those of T2DM patients and healthy CTR. Moreover, glucose, HbA_1C and waist to hip ratio, variables related to glycemic control, showed a significant inverse correlation with leukocyte telomeres length.
Conclusion: Our results suggest that leukocyte telomere length may be a possible reliable marker of MI, one of the most frequent and clinically relevant complication of diabetes, in elderly T2DM patients.
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1364

Morbidity and mortality associated with hepatobiliary disease in type 2 diabetes: the Fremantle Diabetes Study
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Background and aims: There are few studies examining morbidity and mortality associated with hepatobiliary disease in type 2 diabetes. The aim of this study was to analyse detailed relevant longitudinal data collected as part of the observational community-based Fremantle Diabetes Study (FDS).
Subjects and methods: We studied the 1294 type 2 FDS participants who were recruited between 1993 and 1996 and invited to subsequent annual assessments. Additional data were obtained from the Western Australian (WA) State Government registers which record details of all deaths and hospital admissions to public and private hospitals. These data were linked to the FDS database from April 1993 to end-June 2007. All hospital admissions with ICD-9CM and ICD-10AM codes for hepatobiliary disease were extracted, as were coded causes of death.
Results: At FDS entry, the patients had a mean ± SD age of 64.1 ± 11.3 years, 48.8% were male and they had been diagnosed a median (inter-quartile range) 4.0 (1.0-9.0) years previously. The crude cumulative incidence of hospitalisation for hepatobiliary disease was i) 24.7/1000 patient-years in FDS type 2 patients during a mean of 10.3 (range 0.04-14.2) years follow-up, and ii) 5.9/1000 person-years in the general population of WA (approximately 1.7 million people) during the same period. The age-standardised incidence rate was 68% higher in the FDS type 2 cohort. By end-June 2007, 532 (41.2%) FDS type 2 participants had died, 11 (2.1%) from liver disease including cancer, alcohol-related cirrhosis, primary biliary cirrhosis, failed liver transplant, or cirrhosis/hepatic encephalopathy of unspecified cause. No death was attributed to non-alcoholic fatty liver disease (NAFLD). A history of liver disease at baseline, including viral hepatitis (13.2% hepatitis B core antibody positive, 1.8% hepatitis C positive) and at least one C282Y allele (12.2%), was not associated with all-cause mortality in a Cox proportional hazard model after adjusting for a range of significant independent predictors.
Conclusion: The incidence of hospitalisation for hepatobiliary disease is increased in community-based patients with type 2 diabetes relative to that in the local general population. However, deaths associated with liver disease are uncommon and rarely due to NAFLD. These data question the need for directed screening for NAFLD in patients with type 2 diabetes.
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1365

YKL 40 levels are elevated in patients with peripheral arterial occlusive disease and type 2 diabetes mellitus
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Background and aims: Peripheral arterial occlusive disease (PAOD) constitutes a generalized atherosclerosis of the vessels with the highest risk of cardiovascular disease (CVD) events of all its latter manifestations forms. The co-occurrence of PAOD with type 2 diabetes mellitus (T2DM) results in an even further increased CVD risk and a high risk of death within four years. YKL-40, an inflammatory protein, involved in the development of atherosclerosis, is participating in plaque rupture. In the present study we investigated the association of YKL-40, T2DM and PAOD.
Materials and methods: Our study group consisted of 132 patients with PAOD. By oral glucose tolerance testing (OGTT) 47 were identified as normal glucose tolerant and 85 as T2DM. YKL-40 levels were measured using a commercially available ELISA (Quidel Corporation, San Diego, CA). Statistical analyses were done with students' unpaired t-test, ANOVA, correlation analysis and multivariate regression modeling as appropriate.
Results: YKL-40 levels were higher in the patients with T2DM (99±68 ng/ml) versus non-diabetic PAOD patients (68±45ng/ml; p=0.002). Within one year of follow up 9 % of non-diabetic versus 25 % of the diabetic subgroup suffered a cardiovascular event. PAOD patients who developed a cardiovascular event had higher YKL-40 levels (138±77) versus those (80±55 ng/ml p=0.004) who were spared. Interestingly, YKL-40 was neither associated with insulin resistance determined by the homeostatic model assessment nor by vascular inflammation estimated by C-reactive protein in the total study population. In contrast, YKL-40 values were strongly associated with urinary albumin excretion rate (UAER; beta=0.236, p=0.007). In the 47 patients without T2DM, UAER (beta=0.388, p=0.007) and creatinine (beta=0.460, p=0.001) were associated with YKL-40 values in univariate fashion. In the 85 patients with T2DM, low-density lipoprotein cholesterol (beta=-0.232, p=0.033) and aspartate amino transferase (beta=0.327, p=0.002) were associated with YKL-40 values in univariate fashion. YKL-40 levels were higher in symptomatic PAOD (stage IIa and IIb) with T2DM (96±65 ng/ml) versus patients without T2DM (61±42 ng/m p=0.005).
Conclusion: YKL-40 levels were higher in symptomatic and asymptomatic PAOD patients with T2DM versus patients with normal glucose metabolism. Patients who had cardiovascular events during the first year of follow up had significantly higher YKL-40 levels.

1366

Glucosepane and CML glycation modifications in human elastic arteries
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Background and aims: The reason for the increased cardio-vascular disease in both type 1 and type 2 diabetes is not known in detail. However, early stiffening of arteries is central to the pathogenesis. Advanced Glycation Endproducts (AGE) are involved in the initiation and progression of atherosclerosis in diabetes and may be involved in the stiffening of arteries. We therefore studied the presence of two AGE modifications, Carboxymethyllysine (CML), a glycoxidation product, and Glucosepane, a major newly discovered lysine-arginine crosslink, in human aorta and carotid arteries .
Materials and methods: Proteins were extracted from carotid arteries and aorta from 5 diabetics and 10 non-diabetics, having different degree of atherosclerotic lesions. The extracts were analysed by one and two-dimensional electrophoresis followed by western-blotting using four different antibodies against CML and an antibody against Glucosepane. Trypsin and endopeptidase Glu-C were used to cleave proteins into peptide fragments. A Dionex Ultimate 3000 nano-LC system connected to a linear quadrupole ion trap - Orbitrap mass spectrometer (LC-MS/MS) was used to identify proteins. UltraVision ONE Detection System was used for immunohistochemical studies of tissue slices.

Results: In western-blotting the strongest signal with antibodies against CML corresponded to a molecular weight of 230 kDa. LC-MS/MS analyses showed the presence of three different myosin heavy chains and some other proteins at that position. The extracted proteins were therefore digested with trypsin and endoproteinase Glu-C, respectively. Two-dimensional electrophoresis of the digests, followed by western-blotting using antibodies against CML and the proteins identified by LC-MS/MS, showed overlapping signals with antibodies against CML and smooth muscle myosin heavy chain (SM-MHC). SM-MHC could therefore be a main target for CML-modification in human arterial walls. Immunohistochemical (IHC) studies gave strong CML-signals in smooth muscle cells that were positive for SM-MHC, compatible with the western-blotting results. Fibulin-5, vimentin and annexin IV and V were identified for the first time, by western-blotting, as arterial proteins that were modified by Glucosepane. By immunohistochemistry, Glucosepane was mainly distributed extracellularly around long fibre-like structures that probably contained elastin. Glucosepane was found associated with the internal and external elastic lamina, in the medial layer, and close to basal membranes.
Conclusion: Our results support earlier work localizing CML to smooth muscle cells in vessel wall and point out smooth muscle myosin heavy chain as a possible main target for CML modification in vivo in human elastic arteries. Putative main targets for modification by Glucosepane were also identified. CML and Glucosepane target different proteins,and both these AGE modifications may play a role in arterial stiffening.
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1367

Antibodies to oxLDL/beta(2)-GPI in type 2 diabetic subjects with and without coronary artery disease

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Background and aims: High levels of oxLDL are present in metabolic disorders including type 2 diabetes mellitus (T2DM). β_2 glycoprotein 1 is an inhibitor of platelet aggregation and makes complexes with oxLDL. The complex oxLDL/beta(2)-GPI is an antigen leading to the production of autoantibodies, the activation of atherosclerotic process and, therefore, increasing the risk of cardiovascular disease. The aim of the present study was to compare the levels of IgG and IgM antibodies to oxLDL/beta(2)-GPI in T2DM subjects with and without coronary artery disease (CAN).
Materials and methods: A total of 35 with T2DM and CAN and equal number of T2DM subjects without CAN were studied [mean age ± SD: 65.1 ± 9.3 vs. 64.9 ± 12.1 years (P=0.95), duration of diabetes: 13.8 ± 8.5 vs. 12.4 ± 10.7 years (P=0.57), respectively]. A detailed medical history, current use of medications and smoking habits was obtained and a thorough physical examination was performed. Antibodies to oxLDL/beta(2)-GPI were measured by ELISA.
Results: Diabetic subjects with CAN higher levels of IgG antibodies to oxLDL/beta(2)-GPI compared with diabetic subjects without CAN (10.9 ± 12.7 vs. 7.0 ± 10.2, P=0.02). No difference was observed between the two study groups regarding the levels of IgM antibodies to oxLDL/beta(2)-GPI (16.9 ± 5.7 vs. 16.1 ± 1.2, P=0.87). Multivariate linear regression analysis in diabetic subjects with CAN demonstrated a positive relationship between IgG antibodies to oxLDL/beta(2)-GPI with HbA1c (P=0.01), plasma fasting glucose levels (P=0.04) and triglycerides, and a negative association with body mass index (P = 0.08). However, multivariate analysis, after adjustment for the above factors, showed no statistical significant relationships between antibodies to oxLDL/beta(2)-GPI and the study parameters.
Conclusion: High levels of IgG antibodies to oxLDL/beta(2)-GPI in T2DM subjects with CAN compared with T2DM subjects without CAN shows the significance of oxLDL/beta(2)-GPI to the initiation and progress of atherosclerosis as well as to the development of cardiovascular complications.

Author Index

B

C

D

H

L

N

Q

R

S

T

Z

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