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ABSTRACTS

57th EASD Annual Meeting of the European Association for the Study of Diabetes

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OP 01 Macrovascular disease: large cohorts and large trials

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Association of increased intima media thickness and arteriosclerosis with elevated fasting insulin levels in middle-aged persons

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Background and aims: Recently published genetic studies could show for the first time a causal link between elevated insulin levels and cardiovascular disease (CVD) risk. We, therefore, hypothesized that increased fasting insulin levels are also associated with precursors of CVD such as increased intima media thickness (IMT > 1 mm) or arteriosclerosis (AS, defined by the occurrence of plaques).
Materials and methods: In an ongoing study, middle-aged (≥40 years) employees of Boehringer Ingelheim were regularly followed up as part of an occupational health care program. Clinical and laboratory parameters were examined. Multivariable logistic regression analyses were performed to determine odds ratios (OR) for the incidence of increased IMT or AS in relation to fasting insulin levels at baseline and its change until follow-up in comparison to basal BMI and its change in comprehensive models (Model 1: baseline laboratory and clinical parameters; Model 2: Model 1 + BMI change; Model 3: Model 1 + insulin change). Adjusted relative risk for increased IMT and AS was calculated by using Mantel-Haenszel analysis. For this purpose, fasting insulin and BMI were recoded into dichotomous variables defined as hyperinsulinemia (fasting insulin: >15 µU/ml) or overweight (BMI: ≥25 kg/m²).
Results: From n=6825 persons who entered the healthcare program, n=3332 had their first follow-up after 5.0±1.0 years. After excluding all participants with incomplete data (n=1327) or already existent cardiovascular impairments (n=366), data sets of 1639 participants were included in the analysis. Increased IMT during follow-up was diagnosed in 238 participants (15 %) and 328 (20 %) developed AS. Logistic regression analysis identified fasting insulin, BMI and smoking as risk factors for both cardiovascular endpoints (all $p<0.05$), whereas age and diastolic blood pressure were risk factors for increased IMT only, and male sex was associated with incident arteriosclerosis only (all $p<0.01$). Additional adjustment for BMI change during follow-up did not modify these associations (including fasting insulin), but adjustment for fasting insulin change during follow-up removed BMI as risk factor for both cardiovascular endpoints. Fasting insulin change during follow-up but not BMI change was found to be associated with increased IMT (OR [95% CI]: 1.055 [1.030; 1.082]) and AS (OR [95% CI]: 1.057 [1.030; 1.085]) (both $p<0.001$). Subgroup analyses showed that high baseline values of fasting insulin or BMI in combination with greater change during follow-up, respectively, yielded highest risks for both cardiovascular endpoints. The analysis of adjusted relative risks indicated that both, fasting insulin and BMI added to age and sex as risk factors. Interestingly, including the parameter smoking did not lead to a higher risk for increased IMT or arteriosclerosis than conferred by high fasting insulin levels.
Conclusion: Higher basal fasting insulin levels and increases in fasting insulin over time are associated with atherogenic progression and supersede BMI as risk factor.
Supported by: KK and SM received a research grant from Boehringer Ingelheim (grant no. 43034856)
Disclosure: **M. Röhling:** None.

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Kidney function measures and cardiovascular outcomes in people with diabetes: the Hoorn Diabetes Care System cohort

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Background and aims: Kidney function measures - estimated glomerular filtration rate (eGFR) and albuminuria - have been consistently associated to cardiovascular disease (CVD) risk in people with T2D. Since multiple mechanisms underlie the link between kidney dysfunction and diabetes macrovascular complications, each of the manifestations of kidney disease in T2D could contribute to increase the risk of specific CVD subtypes. Accordingly, we aimed to assess the prospective association between longitudinally repeated measures of eGFR and albuminuria and the occurrence of different cardiovascular events - including myocardial infarction (MI), coronary heart disease (CHD), stroke and heart failure (HF) - as well as cardiovascular mortality in people with diabetes.
Materials and methods: 13,507 people with T2D from The Hoorn Diabetes Care System cohort, a cohort study consisting of nearly all people with diabetes in primary care from the West-Friesland region in the Netherlands, were followed-up annually since 1998. Multivariate time-dependent Cox regression models adjusted for common cardiovascular risk factors as well as cardiovascular drug use, were used to assess the association of repeated measures of eGFR and urinary albumin/creatinine ratio (UACR) as clinical categories with CVD outcomes (time to first MI, CHD, HF and stroke events) and cardiovascular mortality. We tested for effect modification by sex, that was observed only for the association between UACR categories and HF (interaction dummies for UACR category 3.0-30.0 and >30.0 mg/mmol: both $P<0.001$).
Results: Mean age at baseline was 62.4±11.8 years and 53.7% were males**.** During a median follow-up time of 7.0 years (interquartile range 3-12) event rates per 1000 person-years were 3.08 for MI, 3.72 for CHD, 1.12 for HF, 0.84 for stroke and 6.25 for cardiovascular mortality. Categories of moderately (60-90) and severely reduced eGFR (<60 mL/min/1.73m^2) were prospectively and independently associated with a higher risk of MI, CHD and stroke but not of HF nor cardiovascular mortality, compared to eGFR>90 mL/min/1.73m^2. Conversely, categories of moderately (3-30 mg/mmol) and severely increased (>30.0 mg/mmol) UACR were prospectively associated with a higher risk of cardiovascular mortality in men and women and with a higher risk of HF in women only, compared to normal or mildly increased UACR (<3.0 mg/mmol). No significant association were observed between increased UACR and the risk of MI, CHD or stroke.
Conclusion: This study indicated differential prospective associations between each manifestation of kidney disease in T2D and cardiovascular events and mortality. In particular a longitudinal decline of eGFR associated to a higher risk of atherosclerotic CVD, while increased albuminuria associated to increased risk of HF and cardiovascular mortality. These findings suggest a regular monitoring of kidney function over time could be of potential utility to identify diabetes patients at higher cardiovascular risk.
Disclosure: **E. Dal Canto:** None.

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Comparative evaluation of GLP-1 receptor agonists and SGLT-2 inhibitors neuroprotective properties in transient brain ischaemia

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Background and aims: Nowadays guidelines for type 2 diabetes mellitus (DM) treatment focus not only at glucose-lowering properties but also at cardiovascular effects of the drugs. Recently, according to LEADER ang EMPA-REG OUTCOME studies, GLP-1 receptor agonist

liraglutide (LIRA) and SGLT-2 inhibitor empagliflozin (EMPA) have demonstrated outstanding cardioprotective potential. Importantly, ischemic stroke has high incidence in type 2 diabetes mellitus (DM) comprising the second leading cause of death in these patients. Nevertheless, there are lack of data concerning potential neuroprotective effect of abovementioned drugs. The aim of this study was to investigate neuroprotective actions of LIRA and EMPA, in comparison with metformin (MET) in acute rat brain ischemia. We have chosen MET as it is the first-line therapy for type 2 DM.

Materials and methods: male Wistar rats 200-255 g were treated with LIRA 1 mg/kg s.c. once daily for 7 days (group "LIRA", n=12), EMPA 2 mg/kg per os once daily for 7 days (group "EMPA", n=9), MET 200 mg/kg kg per os once daily for 7 days (group "MET", n=8) or 0.9% NaCl s.c. once daily for 7 days ("Control" group, n=12). Five hours after last drug administration all the animals were subjected to 30-min filament middle cerebral artery occlusion (MCAO). 48 hours after MCAO neurological deficit was evaluated by means of Garcia score - healthy animals have 18 points while maximal neurological deficit is characterized by 3 points. After that rats were euthanized and brain slices were prepared and incubated with 1% 2,3,5-triphenyltetrazolium chloride for necrosis measurement. Blood glucose level (BGL) was studied 3 times every second day during the treatment.

Results: Brain infarct volume was significantly smaller in "LIRA" group in comparison with "Control" (5.50 (3.97; 5.50) and 16.56 (13.33; 24.65) % of total brain volume, respectively). Brain infarct volume in "EMPA" group (4.91 (2.67; 14.49) % was also significantly smaller than in "Control" group. Importantly, there was no difference between "LIRA" and "EMPA" groups. Treatment with MET also led to brain damage volume decrease (8.67 (5.39; 30.07) %, comparing with control, but still it was significantly larger than both in "LIRA" and "EMPA" groups. Rats in group "LIRA" had less prominent neurological deficit and more points according to Garcia score (14.0 (11.5; 15.5) comparing with "Control" group (12.0 (9.0; 14.0). Neither EMPA, nor MET diminished neurological deficit, comparing with "Control" group (12.0 (9.5; 14.0) and 12.0 (6.5; 12.5) points vs 12.0 (9.0;14.0)). BGL was normal in all groups, with no hypoglycemic episodes during treatment.

Conclusion: LIRA, EMPA and MET are neuroprotective in rat transient brain ischemia and this effect is not connected with influence on glucose metabolism. Infart-limiting effect of LIRA and EMPA is similar and is more prominent than that of MET. Only LIRA administration prior to ischemia modelling causes neurological deficit diminishing.

Disclosure: **A. Simanenkova:** None.

4

Insulin resistance and risk of first stroke in type 2 diabetes: a nationwide cohort study

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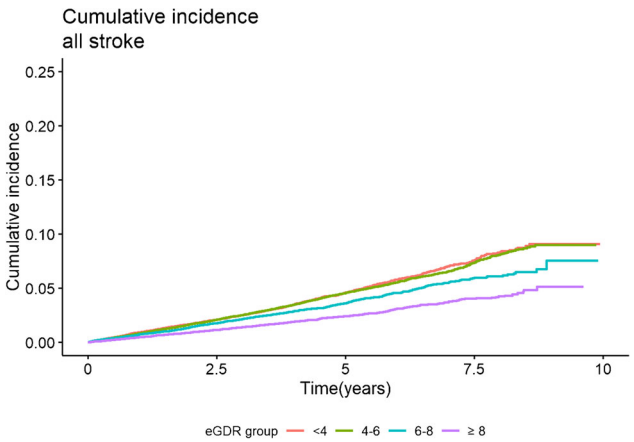
Background and aims: Insulin resistance contributes to the development of type 2 diabetes (T2D) and is a cardiovascular risk factor. The aim of this study was to investigate the potential association between insulin resistance measured by estimated glucose disposal rate (eGDR) and risk of first stroke and death in people with T2D.

Materials and methods: This was a nationwide population based observational cohort study that included all T2D patients from the Swedish national diabetes registry between 2005 to 2016 with full data on eGDR which was calculated by the formula (based upon the euglycemic hyperinsulinemic clamp technique): eGDR (mg/kg/min) = 21.158 - (0.09 * WC) - (3.407 * HT) - (0.551 * HbA1c) [WC = waist circumference (cm), HT = hypertension (yes=1/no=0), and HbA1c = HbA1c (DCCT %)]. eGDR was categorized as following: <4 (highest grade of insulin resistance), 4-5.99, 6-7.99, and ≥8 mg/kg/min). We calculated the crude incidence rates and 95% confidence intervals (CIs) and used multiple Cox regression to estimate hazard ratios (HRs) to assess the association between the risk of stroke and death and the eGDR categories in which the lowest category served as a reference. The relative importance of each factor in the eGDR formula was measured by the R^2 (±SE) values by calculating the explainable log-likelyhood that was attributable to each risk factor.

Results: A total of 104 697 T2D patients (woman 44.5%) with a mean age of 63 years was included in this study. During a median follow uptime of 5.6 years, 4201 strokes occurred (4.0%). Crude survival curves for freedom of stroke are shown in the figure. After multivariate adjustment the HR (95% CI) for stroke in patients with eGDR categories between 4 to 5.99, 6 to 7.99 and >8 were: 0.77 (0.69-0.87), 0.68 (0.58-0.80) and 0.60 (0.48-0.76), compared to the reference, i.e. eGDR <4). The corresponding numbers for risk of death were: 0.83 (0.76-0.89), 0.77 (0.69-0.77) and 0.72 (0.59-0.88). The estimated explained relative risk (R2) for each factor in the eGDR formula was for the risk of stroke: hypertension (0.045±0.0024), HbA1c (0.013±0.0014), and waist (0.006±0.0009), respectively.

Conclusion: Insulin resistance, measured as eGDR, is associated with an increased risk of stroke and death in people with T2D. The relative importance of the predictors in the eGDR formula for the risk of stroke was highest for hypertension followed by glycemia.



Disclosure: **A. Zabala:** None.

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Gender differences in cardiovascular risk, treatment, and outcomes: a post-hoc analysis from the REWIND trial

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Background and aims: Cardiovascular disease (CVD) is the leading cause of death in women and more common in those with type 2 diabetes (T2D). Evidence suggests that the development of T2D adversely affects metabolic and CVD risk factor profiles more in women than men. The aim of this analysis was to investigate gender differences in risk factor management and outcomes in the REWIND trial.

Materials and methods: Analysis was performed on a subset of the REWIND participants by excluding patients with missing data at baseline (BL) or 2 years for HbA1c, systolic BP, LDL-cholesterol, and concomitant medications, or BL history of CVD either missing or unknown. Gender differences in BL characteristics, cardioprotective therapies use at BL and after two years, achievement of relevant treatment targets, and observed cardiovascular (CV) outcomes were analysed. The risk for CV outcomes including fatal/nonfatal stroke, fatal/nonfatal myocardial infarction, CV death, all-cause mortality, and heart failure hospitalisation in women versus men were analysed using Cox proportional hazards models adjusted for randomised treatment and key baseline characteristics identified using stepwise variable selection. Time-to-event analysis was performed in the subgroups with and without CVD history using Cox proportional hazards regression models, including gender, subgroup, randomised treatment, and the gender-by-subgroup interaction.

Results: Of 9901 study participants with either high CV risk or established CVD, 4589 (46.3%) were women. Significantly fewer women than men had a history of CVD (20.0% vs 41.4%; P<0.001). Although the majority of women met clinically relevant treatment targets for blood pressure (96.7%) and lipids (72.8%) at BL, fewer women than men were at target for relevant clinical targets of ACE inhibitor/ARB use, lipid control/statin use, or aspirin use (P<0.001 for all). Overall, women had a lower risk than men for all CV outcomes except fatal/nonfatal stroke, a pattern echoed among the subgroup without a history of CVD at BL. Compared to men (Figure), women with a history of CVD had a similar risk for stroke, heart failure hospitalisation, all-cause mortality, and CV death.

Conclusion: In this international trial cohort of patients with T2D and high CV risk or established CVD, women, overall, were less likely than men to reach treatment targets for CV risk management. Nonetheless, they remained at lower risk for all adverse CV outcomes except stroke. These findings warrant further investigation in women with T2D.

Cardiovascular Outcomes	Patients with events (%)	Incidence (per 100 pt-yr)	Patients with events (%)	Incidence (per 100 pt-yr)	HR (95% CI)	P-value
With history of CVD	Women (N=740)		Men (N=1771)			
MACE-3 composite outcome	93 (12.6%)	2.43	334 (18.9%)	3.76	0.64 (0.51, 0.81)	<0.001
Fatal or non-fatal stroke	32 (4.3%)	0.82	95 (5.4%)	1.03	0.80 (0.53, 1.19)	0.270
Fatal or non-fatal MI	41 (5.5%)	1.06	163 (9.2%)	1.80	0.58 (0.41, 0.82)	0.002
CV death	46 (6.2%)	1.16	134 (7.6%)	1.42	0.81 (0.58, 1.13)	0.222
All-cause mortality	75 (10.1%)	1.89	201 (11.3%)	2.13	0.88 (0.68, 1.15)	0.361
HF requiring hospitalization	40 (5.4%)	1.03	133 (7.5%)	1.45	0.71 (0.50, 1.01)	0.057
Without history of CVD	Women (N=2791)		Men (N=2406)			
MACE-3 composite outcome	191 (6.8%)	1.27	212 (8.8%)	1.66	0.77 (0.63, 0.93)	0.008
Fatal or non-fatal stroke	83 (3.0%)	0.55	65 (2.7%)	0.50	1.09 (0.79, 1.51)	0.595
Fatal or non-fatal MI	60 (2.1%)	0.40	80 (3.3%)	0.62	0.64 (0.46, 0.90)	0.009
CV death	78 (2.8%)	0.51	98 (4.1%)	0.75	0.68 (0.51, 0.92)	0.011
All-cause mortality	138 (4.9%)	0.90	194 (8.1%)	1.48	0.61 (0.49, 0.76)	<0.001
HF requiring hospitalization	72 (2.6%)	0.48	93 (3.9%)	0.72	0.66 (0.48, 0.90)	0.008

N = total number of patients in each subgroup. MACE-3, major adverse cardiovascular events; MI, myocardial infarction; CV, cardiovascular; HF, heart failure; p-yr, patient years; HR, hazard ratio.

Clinical Trial Registration Number: NCT01394952
Supported by: Eli Lilly and Company
Disclosure: **G. Ferrannini:** None.

6
The importance of addressing multiple risk markers in type 2 diabetes: results from LEADER and SUSTAIN 6

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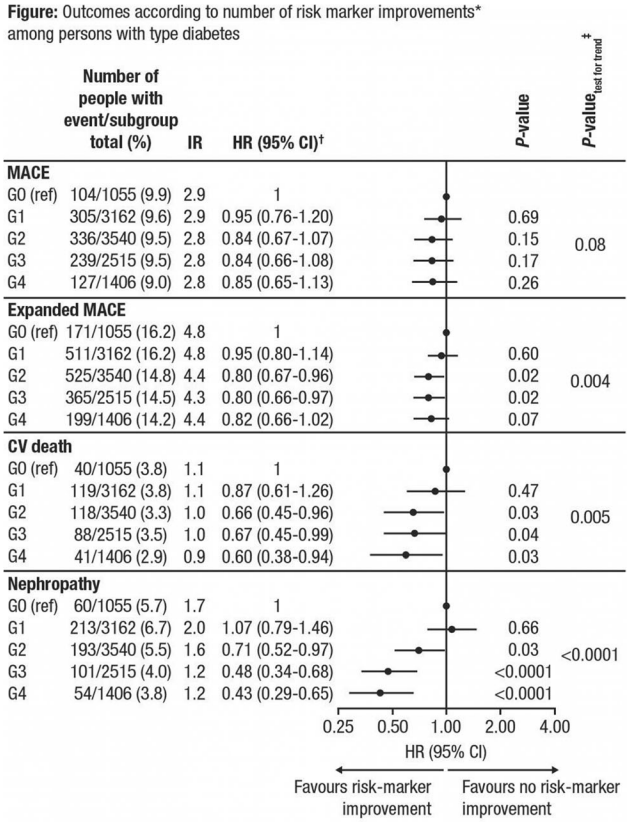
Background and aims: The extent to which improvements in multiple risk markers affect outcomes in type 2 diabetes (T2D) is unclear. Our aim was to investigate whether improvement in multiple risk markers confers lower risk of vascular disease in patients with T2D and cardiovascular disease (CVD)/high risk for CVD, independent of specific treatments.

Materials and methods: This was a post-hoc analysis of the LEADER (n=8638; median follow-up 3.8 years) and SUSTAIN 6 (n=3040; median follow-up 2.1 years) cardiovascular outcome trials in patients with T2D. Patients had baseline and year-1 assessment of at least one of the parameters of interest. We pooled the liraglutide/semaglutide- and placebo-treated groups and categorised them by number of risk markers with clinically relevant improvements after 1 year. We investigated risk of major adverse cardiovascular events (MACE: cardiovascular death, nonfatal myocardial infarction and nonfatal stroke); expanded MACE (MACE + coronary revascularisation and hospitalisation for heart failure or unstable angina pectoris); cardiovascular death; or nephropathy (new onset of macroalbuminuria or doubling of the serum creatinine level and eGFR ≤45 mL/min/1.73 m^2, or the need for continuous renal-replacement therapy, or death from renal disease). Clinically relevant change: body weight loss ≥5%, HbA_{1c} reduction ≥1%, systolic blood pressure reduction ≥5 mmHg, low-density lipoprotein cholesterol reduction ≥0.5 mmol/L, eGFR reduction ≥0 mL/min/1.73m^2 and urinary albumin-to-creatinine ratio reduction ≥30% of baseline value. Numbers of risk markers with change were classified as: none (G0) to ≥4 (G4). Cox regression was used; models were adjusted for continuous baseline levels of risk markers and treatment group (liraglutide/semaglutide and placebo) and stratified by trial.

Results: Baseline characteristics were similar in each subgroup. Compared with patients with 0 risk-marker improvements, patients with 2, 3 or ≥4 improved risk markers had reduced risk of expanded MACE (HR 0.80; 0.80; 0.82), cardiovascular death (HR 0.66; 0.67; 0.60) and nephropathy (HR 0.71; 0.48; 0.43) (**Figure**). One improved risk marker conferred no risk reduction. The trend of decreased risk with each additional risk marker improvement was observed for expanded MACE (p=0.004), cardiovascular death (p=0.005) and nephropathy (p<0.0001). We observed an increasingly higher number of patients on liraglutide/semaglutide treatment in groups with 0, 1, 2, 3 or ≥4 risk marker improvements as follows: 30.5% in G0, 38.0% in G1, 48.8% in G2, 61.6% in G3 and 75.3% in G4.

Conclusion: In patients with T2D, improvements in ≥2 risk markers confers reduced risk of CVD and nephropathy as compared with 0 or 1 improved risk marker. The results stress the importance of multifactorial intervention targeting all risk markers and the benefit of pleiotropic antidiabetic treatments.

Figure: Outcomes according to number of risk marker improvements* among persons with type diabetes



Hazard ratios show risk for outcomes according to number of risk markers with a clinically relevant improvement among patients with type 2 diabetes. Post-hoc analysis of data from the LEADER and SUSTAIN 6 trials included 11,678 persons with type 2 diabetes. Patients were categorized according to number of risk markers with an improvement at year 1 [none (group G0), 1 (G1), 2 (G2), 3 (G3) and ≥4 (G4)].
*Adjusted by baseline variables; †compared G1–G4 to G0 (the reference group); ‡test for trend was evaluated in a Cox regression model with number of risk marker improvements as a continuous variable adjusted for treatment and baseline levels of the risk markers. CI, confidence interval; CV, cardiovascular; HR, hazard ratio; IR, incidence rate per 100 patient years of observation; MACE, major adverse cardiovascular events. Reproduced with permission from Nephrol Dial Transplant (ERA-EDTA).

Clinical Trial Registration Number: NCT01179048; NCT01720446
Supported by: Novo Nordisk A/S
Disclosure: **E. Hein Zobel:** None.

OP 02 Deep and shallow looks at human beta cell gene expression

7

About time: functional and molecular effects of prolonged ex vivo human islet culture

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Background and aims: Several studies have addressed human islet features during *ex vivo* culture under different conditions. It is, however, unclear if and to which extent culture time affects human islet cell functional and molecular traits. Here we studied whether glucose-stimulated insulin secretion (GSIS), insulin content and transcriptome signatures of human islets change upon 8 days of culture after isolation.
Materials and methods: Islets were prepared by enzymatic digestion and density gradient purification from 17 non-diabetic organ donors (age: 70±4 years, mean±SEM; BMI: 24±1 Kg/m^2; sex: 14F/3M) and cultured in control M199 medium. GSIS, insulin content and RNA-sequencing were studied 2 (D2), 4 (D4) and 8 (D8) days from isolation. Transcriptomes were compared to identify gene expression changes over time.
Results: GSIS (n=17), expressed as insulin stimulation index, was similar at D2 (3.5±0.4), D4 (3.1±0.4) and D8 (3.1±0.3). Insulin content was 301.2±28.6 µU/islet at D2, 258.1±35.6 µU/islet at D4 (p=0.13 vs D2) and 205.5±27.4 µU/islet at D8 (p=0.20 vs D4, p<0.01 vs D2 after Tukey's correction). Gene expression trajectories (n=11) revealed that, at D8 vs D2, 1125 genes were differentially expressed (FDR≤0.05, absolute fold-change≥2), of which 425 and 770 were respectively up- and down-regulated. The top 5 up- and down-regulated genes were: AC004017.1, PTH2R, NKIRAS2, CTD-3199J23.6, KLHL22; GJC1, ZNF469, WNT5A, COL4A1, CAV1. Gene Set Enrichment Analysis retrieved 40 significant (FDR≤0.05) KEGG pathways. Twenty of them were positively enriched (including Fatty Acid Metabolism, Peroxisome and Glycolysis/Gluconeogenesis), and 20 were negatively regulated (including Cell Cycle, ECM receptor interaction, Apoptosis and DNA Replication).
Conclusion: In the present study prolonged *ex vivo* culture of human islets did not significantly affect beta cell insulin responsiveness to glucose stimulation, but reduced insulin content. The respective, associated transcriptomic signatures could unveil some of the molecular mechanisms regulating beta cell function and insulin reservoir, to be possibly surveyed in the study of beta cell resilience or subsidence.
Supported by: RHAPSODY, INNODIA HARVEST
Disclosure: **M. Suleiman:** None.

8

Identification of mRNA and microRNA transcripts that differ in expression across islet donor sex, age and BMI

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Background and aims: Donor age, sex and body mass index (BMI) have been recognized to influence pancreatic islet gene expression.

These traits have also been implicated with metabolic health and/or associated with diabetes risk. Since human islets are widely used as a research tool in understanding metabolic pathways in disease, it is important to identify transcriptomic features that are significantly different merely due to sex, age, or BMI of islet donors. Such data obtained from several human islets is an important resource to understand true effects of metabolic stressors on human islet gene expression profiles.
Materials and methods: We analyzed mRNA (58,190 transcripts) and microRNA (miRNA; 754 known/validated) profiles from 131 non-diabetic donor islet preparations (males n=38, female n=25 for mRNA dataset; male n=53, female n=48 for miRNA dataset; of which 33 (male n=18 and female n=15) from the 131 samples, both mRNA and miRNA data were available) generated via bulk RNA-sequencing and TaqMan real-time quantitative PCR respectively. Islet transcriptome profiles were then assessed along with islet metadata on sex, age, BMI and other donor characteristics. Univariate analyses, adjustments to other co-variates and machine-learning penalized regression with bootstrap (at 1000 iterations) analyses were applied. Correlation matrix and predictive target tools were used to identify potential miRNA-mRNA transcript interaction within islets.
Results: Analyses on entire human islet transcriptome identified 1628 significantly different gene transcripts across sex (excluding sex-chromosome linked transcripts), 2208 transcripts correlated with age (range 16 to 69 years) and 1774 transcripts correlated significantly with BMI (range 17.9 to 47.3 kg/m^2). Of these, 85 transcripts were common between the sex and age comparison; and 56 transcripts were common between the sex and BMI comparison, while three transcripts were common between each comparison. Interestingly, there were 142 (sex-), 292 (age-) and 267 (BMI-) associated transcripts which were also present in their respective regression bootstrap model. Analyses across the miRNA profiles in human islet samples identified 40 miRNAs associated with sex differences, 23 and 82 miRNAs correlated with age (range 16 to 66 years) and BMI (range 17.9 to 43.6 kg/m^2) respectively. Of these, miR-147b was common between the sex and age comparison; and 18 miRNAs were common between the sex and BMI comparison, while miR-99c-3p was common between all three comparisons. Regression bootstrap analysis identified 17, 18 and 37 miRNAs as significantly associated with sex, age and BMI respectively that are common to univariate analysis. Correlative analysis in our dataset and predictive target gene tools identified potential miRNA-mRNA interactions in human islets.
Conclusion: Our analyses reveal multiple genes and miRNAs that are associated with different islet phenotype characteristics. These findings serve as an important resource to understand the underlying mechanisms between donor variables and gene expression profiles in human islet biology.
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Disclosure: **W.K.M. Wong:** None.

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A single cell atlas of de novo beta cell regeneration in adult zebrafish identifies hybrid cell states that facilitates diabetes reversal

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Background and aims: To achieve restoration of β-cell mass after near-compete β-cell destruction, it will be imperative to stimulate endogenous β-cell formation from progenitor-differentiation or via trans-differentiation of non β-cells. However, we lack a systematic understanding of the cellular sources underlying β-cell regeneration. In our study, using the regeneration competent model zebrafish (*Danio rerio*), we focused on mapping the plasticity of different pancreatic cells during β-cell regeneration. To this end, we generated a transcriptomic profile of the adult zebrafish pancreas during four stages encompassing the period from β-cell destruction to the emergence of new insulin-expressing cells and the resolution of diabetes.
Materials and methods: We employed single-cell RNA sequencing (both 10X-Genomics and SmartSeq2), time-course live-imaging and -*in vivo* calcium imaging to study β-cell regeneration following near-complete destruction. Single-cell sequencing (10X-Genomics) was performed on dissected pancreata from adult zebrafish at 0 (β-cell destruction), 2, 7 (hyperglycemia) and 14 (glucose normalization) days post β-cell destruction.
Results: Using single-cell RNA sequencing, we classified the cell populations in the adult zebrafish pancreas. Among the pancreas population, we discovered stable populations of endocrine cells with hybrid identity, sharing the hormones and fate-determinants of both δ- and β-cells. Moreover, we revealed that upon β-cell destruction, a novel population of plastic δ-cells gives rise to such intermediate lineage cells. The hybrid cells serve as the chief-insulin expressers during diabetes reversal. These plastic δ-cell population also have a distinct expression of progenitor markers such as *pdx1, ppdpfa, ppdpfb.* These δ-cells also express the Wnt regulator *dkk3b* whose role has not been previously implicated in endocrine differentiation. We found that overexpression of *dkk3b* led to an increase in the pool of hybrid cells in the absence of injury. Finally, using *in vivo* calcium imaging, we show that the hybrid cells acquire glucose-responsiveness during the course of regeneration.
Conclusion: Our study has identified a novel population of δ cells in the zebrafish pancreas, which exhibit higher phenotypic plasticity compared to other pancreatic cells and rapidly acquire insulin expression and glucose-stimulated calcium influx post β-cell destruction.
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Disclosure: **P. Chawla:** None.

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An integrated analysis of human pancreatic islet single cells reveals autocrine and paracrine interactions

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Background and aims: The function of the endocrine pancreas requires a concerted communication between islet cells to achieve a closely regulated hormone secretion. However, with the exception of a few well-characterized paracrine interactions, a comprehensive picture of the human islet interactome is still missing. New developments in the analysis of single-cell transcriptomics may allow to overcome this. Here we used an integrated single-cell dataset of islets from diabetic (T2D) and non-diabetic (ND) donors to leverage islet cell interactions in health and disease.
Materials and methods: We integrated three single-cell transcriptome datasets of human islets using our recently published pipeline. The collection contains 3,046 single cells classified in 7 different cell types (alpha, beta, delta, PP, ductal, stellate and acinar) based on expression of marker genes. The cells were divided according to donor clinical state. Next, each group was analysed with CellPhoneDB to identify significant ligand-receptor interactions occurring across cell types. The interactions were modelled using a multi-edge, multi-partite directed network with 3 distinct layers corresponding to cell types, ligands and receptors. With this data structure, each interaction corresponds to a path going from a cell type to another, through a ligand and its receptor.

Results: The most abundant cell types were alpha and beta cells (731 and 386 in ND, 807 and 414 in T2D), but, surprisingly, the least abundant stellate cell type showed the highest number of interactions. Considering the topological features of the networks, the T2D interactome displayed, as compared to ND, an increased number of interactions, with 11% more nodes (333 vs 301) and edges (2528 vs 2272). Of such interactions, the most prominent change in T2D was the establishment of 20% novel ligand-receptor pairs (346 vs 289), whereas the addition of new cell types to existing interactions was less relevant. The analysis of beta cell interactions in T2D (2709) revealed an extensive interactome rewiring, with 591 newly emerging interactions and 404 disrupted ones. Examining the most markedly altered connections in T2D, we identified ligand-receptor pairs of potential pathophysiological relevance, such as the emergence of EphA-Efrin A communications between beta and exocrine cells, or the loss of C5AR1-RPS19 between delta and beta cells.
Conclusion: This analysis represents the first reconstruction of the human islet interactome at the single-cell level. The comparison between ND and T2D islet cells allowed us to identify signatures potentially relevant for T2D pathophysiology and to generate hypotheses that merit further investigation.
Supported by: RHAPSODY, INNODIA-HARVEST
Disclosure: **E. Bosi:** None.

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The transcription factor CEBPG regulates beta cell function

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Background and aims: Impaired beta cell function is central for the development of type 2 diabetes (T2D). However, the underlying molecular mechanisms have been hard to determine due to the complexity of the pancreatic islets. We previously identified T2D-affected Gene Regulatory Networks (GRNs) in human beta cells by a comprehensive analysis of data from single-cell RNA sequencing (sc-RNA-seq). This analysis pinpointed CCAAT Enhancer Binding Protein Gamma (*CEBPG*) as a node gene in a GRN of unfolded protein response (UPR) in the beta cells. The aim of our study was to investigate the regulatory and biological functions of CEBPG in pancreatic beta cells.
Materials and methods: We performed siRNA-mediated *Cebpg* knockdown (KD) and RNA-seq in INS-1 832/13 cells to assess affected pathways and genes. Moreover, we used cell stressors and glucose-stimulated insulin secretion after KD in INS-1 832/13 cells to examine the role of *Cebpg* in beta cell function. Also, CRISPR/Cas9 gene editing was used to generate *Cebpg* KO INS-1 832/13 cells. *CEBPG*-mediated KD was performed in human islets to investigate if our findings in cell lines can be translated to humans. Further, we performed a chromatin immunoprecipitation assay followed by sequencing (ChIP-Seq) in INS-1 832/13 cells to identify genes regulated by CEBPG in beta cells.
Results: *Cebpg* KD in INS-1 832/13 cells stimulated insulin mRNA expression (*Ins1* 1.3-fold, $p<0.001$; *Ins2* 1.4-fold, $p<0.001$). Glucose-stimulated insulin secretion (GSIS) was increased 72 h after *Cebpg* KD at 16.7 mM glucose with IBMX (2-fold, $p<0.001$), L-arginine (2.0-fold, $p<0.001$) and palmitate (1.7-fold, $p<0.001$), and at 2.8 mM glucose with α-KIC (2.1-fold, $p<0.001$). Moreover, the ER stressor thapsigargin, increased *Cebpg* (3.7-fold, $p<0.001$), *Atf6* (1.8-fold, $p<0.05$), *Atf4* (3.0-fold, $p<0.001$) and *Xbp1s* (2.9-fold, $p<0.001$) mRNA expression, and decreased insulin mRNA expression by 85% (*Ins1* and *Ins2*, $p<0.01$). Glucotoxicity (GTX, 20 mM glucose) decreased *Ins1* (51%, $p<0.001$), *Ins2* (55%, $p<0.001$), and *Cebpg* (29%, $p<0.001$) expression. *Cebpg* KD suppressed the stress response produced by thapsigargin and GTX treatments on *Ins1* and *Ins2* mRNA expression and blocked thapsigargin-induced *Atf6* and *Atf4* mRNA expression. CRISPR/Cas9 *Cebpg* KO increased GSIS at 16.7 mM glucose with IBMX (1.5-fold, $p<0.001$). Moreover, *CEBPG* silencing in human islets led to decreased *ATF6* (80%, $p<0.01$), *ATF4* (79%, $p<0.01$), *DDIT3* (70%, $p<0.01$) and *XBP1* (78%, $p<0.05$) mRNA expression. Our RNA-seq studies provides the first genome-wide identification of CEBPG target genes in rat beta cells. CEBPG-bound genes in the promoter region in the control group analyzed by KEGG pathway clearly point to insulin secretion, while in GTX conditions an enrichment in genes related to maturity onset diabetes of the young (MODY) was seen. A comprehensive bioinformatic KEGG analysis using a combined approach of CEBPG-bound and *Cebpg* KD differentially expressed genes in INS-1 832/13 cells pointed to a dysregulation of insulin secretion, insulin resistance and insulin signaling pathways.
Conclusion: Our findings show that CEBPG is a novel regulator of insulin secretion and transcription, as well as UPR in beta cells, indicating an important role for CEBPG in beta cell function.
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A long non-coding RNA that harbors a SNP associated with insulin levels regulates TGM2 gene expression in pancreatic beta cells

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Background and aims: The Transglutaminase 2 (TGM2) is a multifunctional enzyme which catalyzes transamidation reactions that acts as a G-protein in intracellular signaling. TGM2 is expressed in human pancreatic islets and has been implicated in insulin secretion in rodent pancreatic beta cells. Recently, it has been shown that the expression of a lncRNA (named *LOC107987281*) that is transcribed from an intron of the *TGM2* coding gene, correlates with the expression of *TGM2* in several human cell lines. Against this background, the aim of the present study was to characterize the potential role of *LOC107987281* in pancreatic beta cell function and in the development of diabetes.
Materials and methods: The SNP rs2076380, located within *LOC107987281* sequence but intronic for *TGM2*, was genotyped in 557 individuals (46±10 y; 30% men) using a Taqman Genotyping assay. *LOC107987281* and *TGM2* expression were determined in a set of human tissues and in the human pancreatic beta cell line EndoC-βH1. Cellular localization of *LOC107987281* was assessed by analyzing its expression in nuclear and whole cell RNA fractions. *LOC107987281* overexpression and knockdown experiments in beta cells were performed by transfection of an overexpression vector or specific siRNAs. Promoter activation assays were performed using a luciferase plasmid under the control of the *TGM2* gene promoter (promTGM2).
Results: Genotyping of rs2076380 revealed that this SNP was associated with basal and glucose-stimulated (120 min of an oral glucose tolerance test) insulin levels in women (p=0.0016), as well as with the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) (p=0.001) and fasting glucose levels (p=0.005). The expression of *LOC107987281* and *TGM2* was correlated across human tissues (Spearman's R= 0.87 (0.59-0.9); p<0.001)). Regarding the localization of *LOC10798728* in pancreatic beta cells, our results demonstrated that this lncRNA was preferentially nuclear, suggesting a potential role in transcriptional regulation. Indeed, inhibition of *LOC107987281* in EndoC-βH1 cells using siRNAs led to a significant decrease in *TGM2* expression. In contrast, overexpression of *LOC107987281* induced a nearly 2-fold increase in *TGM2* mRNA

expression. Finally, cells co-transfected with the *LOC107987281* overexpressing vector and the promTGM2 plasmid presented higher luciferase activity than control cells, suggesting that *LOC107987281* binds to the promoter of *TGM2* to induce its expression.
Conclusion: Our study reveals that *LOC10798728* harbors a SNP that is associated with several parameters related to pancreatic beta cell function and type 2 diabetes (T2D). In addition, our results show that *LOC10798728* regulates *TGM2* expression in pancreatic beta cells. Taking into account the role of TGM2 in insulin secretion, it is plausible to think that *LOC10798728* might be implicated in the regulation of insulin production and release.
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Disclosure: **I. González-Moro:** None.

OP 03 Many faces of diabetic pregnancy

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Sweet pregnancy: digital solutions for an effective management of diabetic pregnancies

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Background and aims: Hyperglycemia in Pregnancy (HIP) is a global concern and closely associated with adverse outcomes in mothers and offspring, such as pregnancy and birth complications respectively comorbidities, as well as metabolic and cardiovascular diseases. Influences during the pre- and perinatal period play a decisive role for health and illness in the course of later life - for mothers and offspring. In short and in view of the offspring, mechanisms of transgenerational programming ("fetal programming") may lead to "mal-programming" in organ functions and metabolic regulations. In particular HIP may contribute to such "mal-programming" respectively short- as well as long-term consequences. To optimize the management of diabetic pregnancies, sustainable strategies are needed. Technological solutions present innovative opportunities to improve clinical care for women with diabetes mellitus (DM) in pregnancy. The aim of this review was to examine the clinical effectiveness of current diabetes technologies in diabetic pregnancies. We considered maternal glycemic outcomes as well as pregnancy and birth-related outcomes, and neonatal outcomes.
Materials and methods: Empirical analysis were performed by investigating clinical studies in relevant databases including MEDLINE (PubMed), Cochrane Library, EMBASE, CINAHL and Web of Science Core Collection (2008 - September 2020). Study quality was assessed using "Effective Public Health Practice Project" (EPHPP) tool. Of m=974 records, we analyzed m=15 randomized controlled trials (RCT), m=3 randomized crossover trials (RcrT), m=2 cohort studies, and m=2 controlled clinical trials (CCT) [excluded: duplicates, wrong topic/population/intervention]. We assessed m=9 studies with strong, m=11 with moderate, and m=2 with weak quality.
Results: Overall, the various diabetes technologies seem to have a particularly positive effect on maternal glycemic control in all types of diabetes, as shown by studies of strong and moderate quality. In detail, we found: T1D (m=9 studies; n=1,142 patients): Improvements towards HbA1c (P<0.05), maternal and neonatal hypoglycemia, insulin dose, fewer caesarean sections, birth weight, large-for-gestational age (LGA) using continuous glucose monitoring (CGM; n=435). Continuous subcutaneous insulin infusion (CSII; n=643) significantly improved HbA1c values (P=0.002) and insulin dose (P=0.02). GDM (m=10 studies; n=1,164 patients): Positive effects regarding HbA1c (P=0.006), fasting blood glucose (FBG), birth weight, macrosomia, LGA, neonatal hypoglycemia using CGM (n=301). Significant Improvements (P<0.05) by mHealth-Apps (n=813) in HbA1c (P<0.001), FBG (P<0.001), off-target blood glucose measurement (P<0.001), compliance (P<0.001) as well as positive trends regarding hypertension, preeclampsia, preterm birth. T1D/T2D (m=3 studies; n=267 patients): Improvements regarding HbA1c (P=0.007), caesarean section, neonatal hypoglycemia by CGM (n=225). Improving trends in terms of HbA1c, birth weight, estimated fetal weight through CSII (n=42).
Conclusion: Digital solutions in the management of diabetic pregnancies showed clearly potential for remotely improving diabetic metabolic conditions in pregnant women and therefore in their offspring. Against this background short- and long-term outcomes in mothers and offspring may be improved, subsequently. Further research is urgently required.
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Disclosure: **M. Löhnert:** None.

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Decreased insulin sensitivity during pregnancy after assisted reproductive therapy in women in the Stork cohort

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Background and aims: Assisted reproductive therapy (ART) seems to increase the risk of gestational diabetes (GDM) during gestation and glucose intolerance after pregnancy, although the nature of the association remains unclear. Older age and the treatment per se could be primary determinants of altered glucose metabolism. To gain more insight in this association we have investigated levels of glucose and insulin, beta-cell function (BCF) and insulin sensitivity (IS) in singleton pregnancies, comparing women with normal glucose tolerance (NGT), ART and GDM from the STORK cohort.
Materials and methods: 1031 healthy pregnant Norwegian women attended OGTTs at gestational week (GW) 14-16 and 30-32. In the women that had available blood samples and OGTT results, 75 women received ART, 104 women were diagnosed with GDM (WHO 2013 criteria), and 847 women served as controls (NGT). Eleven subjects that were diagnosed with GDM in the ART group were removed for data analysis (n= 64 women in the ART group). None of the participating women required antidiabetic medication during the study period. Glucose was measured using the hexokinase method at an accredited clinical chemistry laboratory. Insulin levels were measured using radioimmunoassay. We calculated indices of BCF (ISSI-2) and IS (Matsuda) from the 5-point OGTTs, and performed linear regression analysis to adjust for BMI and age.
Results: The ART women were 32.8 years (SD 3.5) at inclusion, ie. 1.8 years older than the NGT women (p<0,001), but had similar age to the GDM women. The reasons for ART were: 30% male factors, 21% endometriosis, primary/unspecified 21%, 11% PCOS, and 15% combined etiologies. Although fasting glucose and fasting insulin levels in the ART women were at an intermediate level compared to NGT and GDM women, only fasting insulin levels at GW 14-16 differed significantly. Both BCF and IS were significantly lower than in NGT women in GW 14-16, but at GW 30-32 the difference was significant only for IS. BCF levels in ART women were higher at both time points compared to the GDM women. The influence of ART persisted for IS after adjustment for age and BMI.
Conclusion: The women with ART demonstrated reduced IS in pregnancy compared with the NGT women, and a similar trend for BCF in early pregnancy. In late pregnancy insulin measurements and indices of BCF or IS in the ART subjects were more similar to the NGT women than to the GDM women. Our data suggest that ART treatment exerts a negative influence on insulin sensitivity already during gestation, which persists after adjustment for age and BMI. However, ART did not result in high rates of glucose intolerance, indicating compensatory effects such as increased insulin secretion.

	NGT n= 847	ART n= 64	GDM n= 104
BMI GW 14-16 (kg/m2)	24,2 (3,7)	25,2 (4,6)	26,1 (4,9) c*
F-glucose GW 14-16(mmol/l)	4,58 (0,36)	4,64 (0,35)	4,74 (0,36) c*
F-insulin GW 14-16 (pmol/l)	25 (18;37) a#	34 (20;51)	33 (26;54) c*
F-glucose GW 30-32(mmol/l)	4,54 (0,41)	4,58 (0,37) b*	4,95 (0,57) c*
F-insulin GW 30-32 (pmol/l)	39 (25;57)	45 (31;61)	58 (37;79) c*
Insulin sensitivity GW 14-16	213 (149; 298) a*	144 (100;223)	140 (88;208) c*
Insulin sensitivity GW 30-32	116 (80; 171) a*	85 (63;116) b*	71 (48;99) c*
Beta-cell function GW 14-16	1146 (917;1497) a*	910(727;12349) b#	833 (621;1077) c*
Beta-cell function GW 30-32	929 (722;1187)	783 (621; 1047) b*	568 (454; 684) c*
*GW gestational week, a NGT vs ART, b ART vs GDM, c NGT vs GDM, #: p<0.05, *p<0.01*			

Table 1 Descriptives presented as mean (SD) or median (25p; 75p).

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Maternal C-peptide reappearance in type 1 diabetes pregnancy: Evidence of beta cell regeneration or fetal hyperinsulinism?

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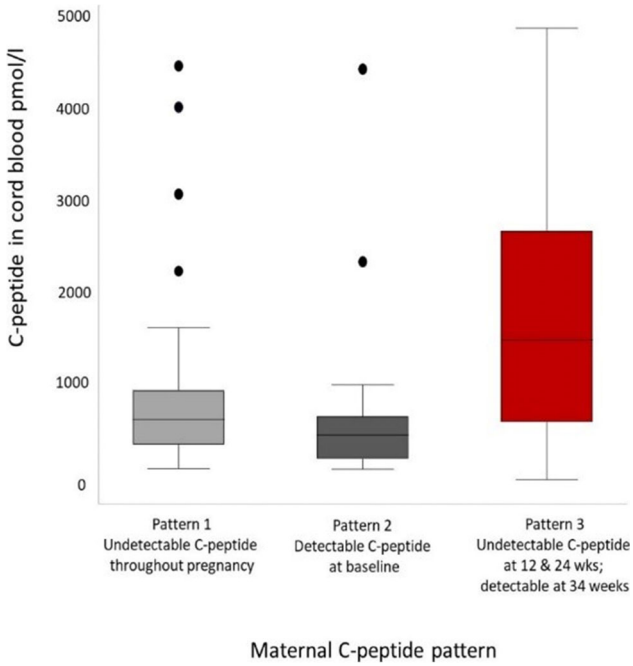
Background and aims: Reports of increasing endogenous insulin secretion have occurred in pregnant women with established type 1 diabetes. However, the cause of this phenomenon, and its relevance to maternal-fetal pregnancy outcomes are unclear. The aim of this study was to assess longitudinal patterns of maternal C-peptide concentration using a highly sensitive electrochemiluminescent assay to examine the hypothesis of pregnancy-induced beta cell regeneration in women with type 1 diabetes.
Materials and methods: The Continuous Glucose Monitoring in Women with Type 1 Diabetes in Pregnancy Study (CONCEPTT) was a multinational randomised controlled trial to assess the effects of real-time CGM in comparison to standard care (capillary blood glucose monitoring) in pregnant women with type 1 diabetes. Women with type 1 diabetes who delivered a liveborn singleton infant and gave samples for the biorepository were included in this analysis. Highly-sensitive direct and solid-phase competitive electrochemiluminescent immunoassays were used to measure C-peptide in maternal serum (12, 24, 34 wks; n=127) and cord blood (n=85). Associations were made with adjudicated pregnancy outcomes using adjusted and unadjusted logistic regression.
Results: Three discrete patterns of maternal C-peptide trajectory were identified (see figure 1): Pattern 1 undetectable throughout pregnancy (n=74; 58%); Pattern 2 detectable at baseline (n=22; 17%); Pattern 3 undetectable C-peptide (12 & 24 weeks), became detectable at 34 weeks (n=31; 24%). Women in patterns 1 and 3 had comparable baseline characteristics. Women in pattern 2 had shorter duration of diabetes (median 10.6 years vs 16.9 years overall; p<0.001). Offspring of women in pattern 3 had higher rates of neonatal hypoglycemia (42% vs 14%; p=0.001), large-for-gestational-age (90% vs 60%; p=0.002) and elevated cord blood C-peptide (see figure 1; geometric mean 1319 vs 718 pmol/l; p=0.007) compared to offspring from women in pattern 1, despite comparable 34-week glycemia.
Conclusion: Increased C-peptide in maternal serum at 34 weeks suggests fetal hyperinsulinism with fetal-to-maternal transfer not improved maternal beta cell function. First appearance of C peptide in late pregnancy could identify pregnancies at highest risk of neonatal complications.



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Disclosure: **C.L. Meek:** None.

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Maternal efficacy, safety, and pregnancy outcomes with degludec vs detemir in the treatment of pregnant women with type 1 diabetes: an international, multicentre, randomised trial

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Background and aims: To compare the efficacy and safety of insulin degludec (degludec) versus insulin detemir (detemir) in pregnant women with type 1 diabetes (T1D).
Materials and methods: EXPECT was an open-label, randomised trial of women aged ≥18 years with T1D and previously treated with insulin, who were at 8-13 weeks' gestation or planned to become pregnant within 52 weeks. Women were randomised to degludec once daily or detemir 1-2 times daily, both with insulin aspart 2-4 times daily. The primary analysis aimed to demonstrate the non-inferiority (margin of 0.4%) of degludec to detemir with respect to the last planned glycated haemoglobin (HbA_{1c}) measurement prior to delivery (>16 weeks' gestation) using ANCOVA. Secondary endpoints were maternal efficacy, safety, and pregnancy outcomes.
Results: In total, 225 women (degludec: 111; detemir: 114) were randomised including 144 who were, and 81 who planned to become, pregnant. Mean (±SD) HbA_{1c} at treatment baseline was 6.8% (±0.7%) and 6.7% (±0.8%) with degludec and detemir, respectively. Over the trial, 188 women (degludec: 92; detemir: 96) with singleton pregnancies were included and there were 171 live-born infants (degludec: 86; detemir: 85), with no perinatal deaths (between ≥20 week's gestation and <1 week after delivery). Estimated mean HbA_{1c} prior to delivery was 6.23% with degludec and 6.34% with detemir (estimated treatment difference: −0.11% $[-0.31; 0.08]_{95\%\ CI}$, confirming non-inferiority). See **Table** for supportive maternal safety and pregnancy outcomes.
Conclusion: In pregnant women with T1D, degludec was non-inferior to detemir with respect to HbA_{1c} prior to delivery. Hypoglycaemia rates and pregnancy outcomes were comparable between insulins.

Table: Supportive maternal safety and pregnancy outcomes in women with type 1 diabetes randomised to degludec or detemir.

		Degludec				Detemir			
		n/N	%	Events	Rate	n/N	%	Events	Rate
Maternal safety outcomes	Overall hypoglycaemia	89/91	97.8	5431	11,845	91/94	96.8	5982	13,401
	Nocturnal hypoglycaemia†	71/91	78.0	712	1553	69/94	73.4	759	1700
	Adjudicated severe hypoglycaemia‡	6/91	6.6	8	–	3/94	3.2	4	–
	Adverse events	78/91	85.7	429	936	76/94	80.9	328	735
	Pre-eclampsia	12/86	14.0	–	–	7/85	8.2	–	–
Pregnancy outcomes	Fetuses/infants with major abnormalities§	8/92	8.7	–	–	8/96	8.3	–	–
	Pre-term delivery (<37 weeks' gestation)¶	29/86	33.7	–	–	19/85	22.4	–	–
	Infants born large for gestational age	55/86	64.0	–	–	43/85	50.6	–	–
	Infants with neonatal hypoglycaemia††	20/86	23.3	–	–	19/85	22.4	–	–

Maternal safety outcomes were assessed over the pregnancy period and based on all randomised women who were pregnant during the trial and received ≥1 dose of trial product. Additionally, for pre-eclampsia, only women who delivered an infant at ≥20 weeks' gestation were considered. Pregnancy outcomes were based on all randomised women who delivered an infant at ≥20 weeks' gestation, except for major abnormalities, where all randomised women who were pregnant during the trial were considered. The analyses of maternal safety and pregnancy endpoints were pre-specified to provide a descriptive summary only.
‡Events occurring between 00:01 and 05:59 (both inclusive); †events requiring third-party assistance; §EUROCAT classified; ¶after 20 weeks' gestation; ††defined as blood glucose 1.7 mmol/L (≤31 mg/dL) and assessed over the first 24 hours after birth.
%, percentage of women with ≥1 event; degludec, insulin degludec; detemir, insulin detemir; EUROCAT, European Concerted Action on Congenital Abnormalities and Twins; n, number of women with ≥1 event; N, total number of women in treatment group and analysis set; rate, rate of events per 100 years of exposure.

Clinical Trial Registration Number: NCT03377699
Supported by: Novo Nordisk A/S
Disclosure: **E.R. Mathiesen:** Grants; Novo Nordisk. Lecture/other fees; Novo Nordisk, Sanofi, Lilly.

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Epigenetic alterations in offspring born to mothers with type 1 diabetes (the EPICOM study)

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Background and aims: Offspring born to women with pregestational type 1 diabetes (T1D) are exposed to an intrauterine hyperglycaemic milieu and has later in life an increased risk of metabolic disease. We hypothesize that in utero exposure to T1D induces epigenetic and transcriptome changes that may alter the offspring's risk of metabolic disorders later in life.
Materials and methods: EPICOM (Epigenetic, Genetic and Environmental Effects on Growth, Cognitive Functions and Metabolism) is a Danish prospective nationwide follow-up study in 278 offspring of women with T1D and a control group of 303 offspring of mothers without diabetes. In the present study, we performed whole-blood DNA methylation profiling using the 450K-Illumina Infinium assay in addition to RNA-Seq transcriptome profiling among the oldest participants of the EPICOM cohort (n=40, mean age 19.8 (SD 0.9)). Besides fulfilling the matching criteria for inclusion in the EPICOM study, we made sure that they did not differ in respect to body mass index, glucose tolerance, HbA_{1c}, cholesterol or triglyceride levels.

Results: We identified 1,043 differentially methylated positions (DMPs, p<0.005) between controls and offspring born to women with T1D. Of these DMPs, 14 showed a substantial change in methylation (9 hypermethylated and 5 hypomethylated, |deltaM|>1). In the transcriptome analysis, we found 38 up-regulated genes and 1 down-regulated gene (p<0.005 and absolute logFC≥0.3). One of the up-regulated genes, *ARHGDIA,* was found close to, and possibly under epigenetic control of one of the hypomethylated DMPs (p<0.005). However, no association between the level of methylation and *ARHGDIA* expression was found in the correlation analysis (rho=-0.13, p=0.42). This gene has previously been described to be associated with ß-cell dysfunction in metabolic disease. Furthermore, using Gene Set Enrichment analysis on gene expression data, we observed enrichment in ontologies or pathways relating to diabetes, carbohydrate metabolism, glucose metabolism disease and pathways including MAPK1/MAPK3 and MAPK family signalling and genes relating to type 1 diabetes, obesity and atherosclerosis.
Conclusion: Our result could indicate methylation changes as a possible pathway between the intrauterine environment, later life methylation status and changes in RNA expression.
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Disclosure: **S. Knorr:** None.

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Maternal exercise in gestational diabetes has sex-specific effects on offspring adiposity and beta cell function

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Background and aims: Adult offspring from dams with gestational obesity and diabetes have greater adiposity and insulin resistance. The goal of this study was to determine if maternal exercise mitigates the adverse effects of maternal gestational diabetes with obesity on offspring metabolic health.
Materials and methods: Female (C57BL/6N) mice were fed from weaning a control (MC; 10%kcal fat) or western (MW; 45% kcal fat) diet to induce excess adiposity and glucose intolerance (obese dams). At 13 weeks, mice were bred and maintained on the diets, with or without access to a running wheel (exercise), throughout pregnancy and lactation. Male and female offspring were studied at two timepoints; i) postnatal day 7 (P7); and ii) adulthood (age 4-5months, fed the control or western diet from weaning). At P7, pancreatic islets were isolated for *ex vivo* assessment of insulin secretion and β cell mass was quantified by immunohistochemistry. In adult offspring, physiological assessments of glucose tolerance, β cell function and insulin sensitivity were conducted. DNA methylation was assessed by reduced representation bisulfite sequencing of mature adipocytes from male offspring.
Results: At P7, islets from male and female pups from exercised dams fed the control diet (MCE) had greater insulin secretion when stimulated by low glucose (2.6mM; $p<0.01$) and KCl (40mM; $p<0.05$) compared to pups from sedentary dams (MCS). Islets from female MCE offspring had greater insulin secretion when stimulated with high glucose (16.7mM); no differences were observed in male offspring. This was accompanied by increased ($p<0.01$) β cell mass in both male and female P7 offspring from exercised control and obese dams compared to those from sedentary dams. Adult male offspring fed the control diet from obese dams that exercised (MWE) had greater ($p<0.05$) retroperitoneal, mesenteric, and subcutaneous adipose tissue than those from obese sedentary dams (MWS). Adult female offspring from MWE dams had greater retroperitoneal adipose tissue ($p<0.05$).
Despite an increase in adiposity in offspring from exercised dams, the offspring did not have impaired glucose tolerance, insulin sensitivity or β cell function. We observed 371 differentially methylated CpG sites (FDR<0.05 and delta beta>10%); 95 CpG sites had increased methylation and 276 CpG sites had decreased methylation in MWE male offspring compared to MWS male offspring. This included sites involved in adipocyte differentiation (*Wnt10b, Sirt4*), fatty acid transporter (*Slc25a29*), adipocyte cell proliferation (*Mybl2*), cell adhesion (*Cdc181*), and lipid synthesis (*Sirt4*). We also challenged some of the adult offspring with a western diet from weaning. This diet exacerbated the effect of maternal obesity on male offspring glucose intolerance. However, maternal exercise improved glucose tolerance ($p<0.05$) from MWE offspring compared to offspring from MWS dams.
Conclusion: Our findings suggest that the effect of maternal exercise on offspring metabolic health is sex-specific. Islets from female, but not male, offspring have improved β cell function in response to high-glucose, which appear to protect the female offspring against obesogenic diet-induced glucose intolerance later in adulthood.
Supported by: CIHR
Disclosure: **N. Boonpattrawong:** None.

OP 04 GLP-1 receptor agonism: higher dose, combination therapy, or both?

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Effect of semaglutide 2.4 mg on glucose metabolism and body weight in adults with overweight or obesity and type 2 diabetes in the STEP 2 trial

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Background and aims: The STEP 2 trial showed mean weight loss of 9.6% with semaglutide 2.4 mg vs 7.0% with semaglutide 1.0 mg and 3.4% with placebo (both p<0.0001). We present detailed glucose metabolism outcomes.
Materials and methods: Adults with BMI ≥27 kg/m^2, type 2 diabetes and HbA_{1c} 7-10% (53-86 mmol/mol) were randomised to 68 weeks' once-weekly s.c. semaglutide 2.4 mg (N=404), 1.0 mg (N=403) or placebo (N=403). Glucose metabolism outcomes were secondary or exploratory endpoints. Treatment comparisons were done using an ANCOVA model with treatment and stratification factors as fixed effects and baseline endpoint values as covariate (all patients in the full analysis set).
Results: At baseline, patients had a mean age of 55 years, body weight 99.8 kg, HbA_{1c} 8.1% (65.3 mmol/mol) and diabetes duration 8.0 years; 88% were on 1-2 oral antihyperglycaemic drugs. From weeks 0 to 68, semaglutide 2.4 mg and 1.0 mg reduced HbA_{1c} to a greater extent vs placebo (estimated treatment difference -1.2% [-13.5 mmol/mol] and -1.1% [-11.8 mmol/mol]; both p<0.0001) and improved fasting plasma glucose, HOMA-IR and -β, and reduced oral antihyperglycaemic drug use (Table). With semaglutide 2.4 mg, more patients achieved HbA_{1c} <7.0% and ≤6.5%, and composite endpoints of HbA_{1c} <7.0% with weight loss ≥10% or ≥15% vs semaglutide 1.0 mg or placebo (Table). Exploratory analyses showed that a greater proportion of patients receiving semaglutide at either dose decreased their oral antihyperglycaemic medication intensity vs placebo by week 68.
Conclusion: Weight loss with semaglutide 2.4 mg was accomplished with improvements in insulin resistance and β-cell function, two key mechanistic drivers and pathophysiologic abnormalities that cause type 2 diabetes and fuel diabetes progression. With semaglutide 2.4 mg, more patients achieved HbA_{1c} <7.0% and weight loss ≥10% (composite endpoint) vs placebo or semaglutide 1.0 mg, and reduced oral antihyperglycaemic drug use vs placebo.

Table. Glucose metabolism endpoints (full analysis set). A) Baseline values and estimated mean change from baseline to week 68 or estimated ratio to baseline at week 68; B) categorical endpoints

A) Baseline and change	Semaglutide 2.4 mg Baseline	Semaglutide 2.4 mg Change	Semaglutide 1.0 mg Baseline	Semaglutide 1.0 mg Change	Placebo Baseline	Placebo Change	Semaglutide 2.4 mg vs placebo	Semaglutide 2.4 mg vs 1.0 mg
HbA_{1c}, mean %	8.1	-1.6	8.1	-1.5	8.1	-0.4	ETD: -1.2 [-1.4, -1.0]***	ETD: -0.2 [-0.3, 0.0]
FPG, mean mmol/l	8.5	-2.1	8.6	-1.8	8.8	-0.1	ETD: -2.0 [-2.4, -1.7]***	ETD: -0.3 [-0.7, 0.0]
HOMA-IR, geometric mean	4.83 (N=398)	0.67 (N=368)	4.99 (N=399)	0.72 (N=358)	5.27 (N=402)	0.90 (N=360)	ETR: 0.73 [0.65, 0.81]***	ETR: 0.89 [0.79, 1.00]
HOMA β, geometric mean	59.2 (N=398)	1.73 (N=368)	57.8 (N=399)	1.66 (N=358)	58.6 (N=401)	1.00 (N=358)	ETR: 1.69 [1.53, 1.87]***	ETR: 1.05 [0.95, 1.16]
B) Observed proportion of patients achieving categorical endpoints at week 68 (semaglutide 2.4 mg, N=381; semaglutide 1.0 mg, N=376; placebo, N=374)								
HbA_{1c} <7.0%	78.5%		72.3%		26.5%		OR: 9.8 [6.9, 13.9]***	OR: 1.4 [1.0, 2.0]*
HbA_{1c} ≤6.5%	67.5%		60.1%		15.5%		OR: 10.5 [7.5, 15.0]***	OR: 1.4 [1.0, 1.9]*
HbA_{1c} ≤6.5%, no OADs or metformin	77.0% (N=191)		61.4% (N=207)		17.0% (N=185)		OR: 17.2 [9.9, 30.0]***	OR: 2.0 [1.3, 3.2]*

*p<0.05; **p<0.001; ***p<0.0001. ETD: estimated treatment difference; ETR, estimated treatment ratio; FPG: fasting plasma glucose; OAD: oral antihyperglycaemic drug.

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Tirzepatide, a dual GIP/GLP-1 receptor agonist, is effective and safe when added to basal insulin for treatment of type 2 diabetes (SURPASS-5)

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Background and aims: Tirzepatide (TZP) is a novel dual glucose-dependent insulinotropic polypeptide/glucagon-like peptide-1 (GIP/GLP-1) receptor agonist in development for treatment of type 2 diabetes (T2D). Efficacy and safety of TZP vs placebo was assessed in people with T2D as an add-on to titrated insulin glargine with or without metformin.
Materials and methods: In this double-blind, placebo-controlled, 40-week Phase 3 study, 475 people with T2D (mean baseline age 60.6 y; T2D duration 13.3 y; HbA_{1c} 67.4 mmol/mol [8.31%]; BMI 33.4 kg/m^2) were randomised (1:1:1:1) to TZP (5 mg, 10 mg, 15 mg) or placebo, as an add-on to their existing therapy. Primary efficacy measure was mean change in HbA_{1c} from baseline at 40 weeks. Secondary measures included change in fasting serum glucose (FSG) and body weight (BW), and proportion of people achieving HbA_{1c} and BW loss goals.
Results: All three TZP doses were superior to placebo in mean change from baseline in HbA_{1c}, FSG, BW, and percentage patients achieving all HbA_{1c} and BW loss targets at week 40 (Table). LSM treatment difference vs placebo (95% CI) in HbA1c for all TZP groups were statistically significant (p<0.001 all doses): for TZP 5 mg (-14.2 [-16.6,-11.7] mmol/mol or -1.30 [-1.52, -1.07] %); TZP 10 mg (-18.1 [-20.6,-15.7] mmol/mol or -1.66 [-1.88, -1.43] %); and TZP 15 mg (-18.1 [-20.5,-15.6] mmol/mol or -1.65 [-1.88, -1.43]%). The mean insulin glargine dose change from baseline was 4.3, 2.3, -3.9, and 25.6 U/day for TZP 5, 10, 15 mg, and placebo groups, respectively. TZP was generally well tolerated and the most common adverse events were gastrointestinal and a vast majority were mild-to-moderate in severity. Diarrhoea, nausea, and vomiting were reported in 12-21%, 13-18%, and 7-13% of patients treated with TZP vs 10%, 3%, and 3% in the placebo arm, respectively. Incidence of hypoglycaemia (blood glucose <3.0 mmol/L) or severe hypoglycaemia did not differ between TZP and placebo groups (14-19% vs 13%). Three episodes of severe (level 3) hypoglycaemia were observed in 2 episodes in TZP 10 mg group and 1 in TZP 15 mg group.
Conclusion: In conclusion, TZP demonstrated superior and clinically meaningful improvements in glycemic control and BW loss without increasing hypoglycaemia vs placebo in patients with T2D when added to titrated basal insulin.

Key Efficacy and Safety Endpoints at Week 40	TZP 5 mg (N=116)		TZP 10 mg (N=119)		TZP 15 mg (N=120)		Placebo (N=120)	
	Baseline	CFB	Baseline	CFB	Baseline	CFB	Baseline	CFB
HbA_{1c} (mmol/mol)[a,b]	67.1 ± 0.86	-24.4 ± 0.80**	67.7 ± 0.87	-28.3 ± 0.80**	66.4 ± 0.85	-28.3 ± 0.01**	68.2 ± 0.85	-10.2 ± 0.86
HbA_{1c} (%)[a,b]	8.20 ± 0.08	-2.23 ± 0.08**	8.34 ± 0.08	-2.59 ± 0.08**	8.22 ± 0.08	-2.59 ± 0.08**	8.39 ± 0.08	-0.93 ± 0.08
Bodyweight (BW) (kg)[a,b]	96.5 ± 2.02	-6.2 ± 0.58**	95.4 ± 2.03	-8.2 ± 0.58**	99.2 ± 2.00	-10.9 ± 0.59**	94.1 ± 1.99	1.7 ± 0.57
Fasting serum glucose (mmol/L)[a,b]	9.02 ± 0.27	-3.41 ± 0.14**	9.04 ± 0.27	-3.77 ± 0.14**	8.91 ± 0.26	-3.76 ± 0.15**	9.13 ± 0.26	-2.16 ± 0.14
Insulin glargine dose (U/day)[a,c]	34.3 ± 1.45	4.3 ± 2.46	32.0 ± 1.34	2.3 ± 2.33	35.0 ± 1.46	-3.9 ± 1.96	32.9 ± 1.36	25.6 ± 3.75
Patients with HbA_{1c}[a]: <7.0%[b] / ≤6.5%[d] / <5.7%[e] (%)	93** / 80** / 26**		97** / 95** / 48**		94** / 92** / 62**		34 / 17 / 3	
Patients with BW loss[a]: ≥3%[f] / ≥10%[f] / ≥15%[f] (%)	54** / 23** / 7*		65** / 47** / 27*		85** / 51** / 32**		6 / 1 / 0	
Incidence of Diarrhoea / Nausea / Vomiting (%)[a]	12 / 13 / 7		13 / 18 / 8		21 / 18 / 13		10 / 3 / 3	
Incidence of hypoglycaemia (BG<3.0 mmol/L) (%)[g]	16		19		14		13	

Data presented are model estimates [estimate ± SE at baseline and change from baseline at 40 weeks, unless otherwise noted]. *p<0.05 and **p<0.001 vs placebo. Percent patients discontinuing the study treatment were: 9.5%, 11.8% 18.3%, and 3.3% in TZP 5, 10, 15 mg and placebo groups.
[a]On treatment data prior to initiating rescue therapy from mITT population (all patients who administered at least 1 dose of study drug) excluding patients discontinuing study drug due to inadvertent enrolment (Efficacy estimand).
[b]Tested for superiority, controlled for Type 1 error.
[c]Insulin glargine was titrated throughout the study using a treat-to-target algorithm to reach target fasting blood glucose <5.5 mmol/L (<100 mg/dL).
[d]Tested for superiority, not controlled for type 1 error.
[e]Tested for superiority, TZP 10 mg and 15 mg vs. PBO was controlled for type 1 error, while TZP 5 mg was not controlled for type 1 error.
[f]Observed data is presented without statistical comparisons using all available data from mITT patients including safety follow up regardless of adherence to study drug or use of rescue therapy. Hypoglycaemia data censored after start of new anti-hyperglycemic therapy.
CFB=change from baseline; HbA1c=glycated haemoglobin A1c; mITT=modified intent-to-treat; N=Total number of randomised patients in each group; SE=standard error.

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Semaglutide reduced cardiovascular events regardless of metformin use: a post hoc exploratory subgroup analysis of SUSTAIN 6 and PIONEER 6

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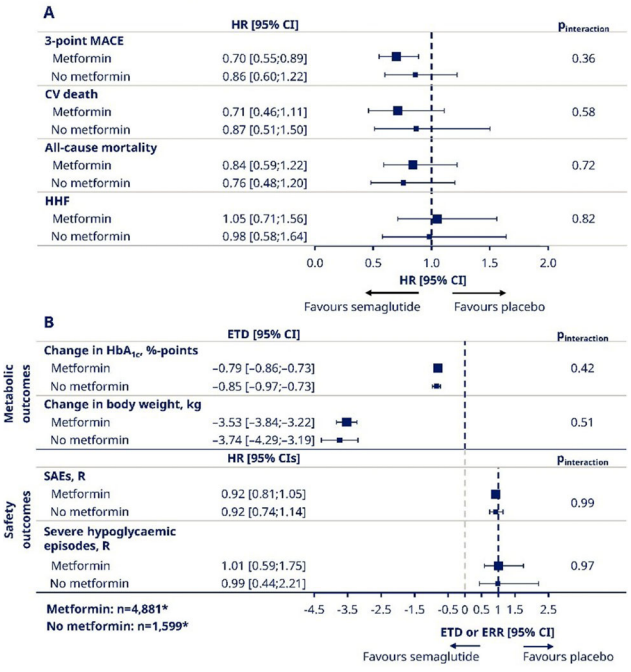
Background and aims: *Post hoc* analyses of liraglutide, dulaglutide and empagliflozin cardiovascular outcomes trials (CVOTs) showed reductions in major adverse cardiovascular events (MACE) vs placebo regardless of baseline metformin use. The aim of this *post hoc* analysis of the SUSTAIN 6 and PIONEER 6 CVOTs was to determine if semaglutide provided cardiovascular (CV) benefits independently of baseline metformin use in adults with type 2 diabetes at high risk of a CV event.
Materials and methods: Pooled data from the SUSTAIN 6 and PIONEER 6 trials were used to assess the following CV and metabolic outcomes in the two MET subgroups (baseline metformin, yes/no): time to first CV outcomes (3-point MACE [a composite of CV death, nonfatal myocardial infarction or nonfatal stroke], CV death, all-cause mortality and hospitalisation for heart failure), metabolic outcomes (change in glycated haemoglobin and body weight from baseline to week 80 for SUSTAIN 6 and week 83 for PIONEER 6) and safety outcomes (serious adverse events and severe hypoglycaemic episodes). CV analyses were adjusted for baseline characteristics in a Cox proportional hazards model. Consistency of treatment effects across subgroups was assessed using interaction p-values, with $p_{interaction}$ <0.05 indicating a statistically significant difference.
Results: Compared with subjects with baseline metformin (n=4,881; 75%), those without (n=1,599; 25%) were at a higher CV risk, were older (64.8 vs 67.1 years), had lower eGFR (79.4 vs 61.9 mL/min/1.73 m^2) and higher body weight (90.8 vs 93.6 kg) at baseline. Fewer subjects with than without baseline metformin received concomitant insulin (48.2% vs 70.7%), and more received sulphonylureas (40.5% vs 28.7%). Semaglutide reduced the risk of 3-point MACE vs placebo in both subgroups (HRs with/without metformin: 0.70/0.86; 1-year absolute risk reduction with/without metformin: 1.05%/0.78%). There was no significant interaction between treatment effect (semaglutide vs placebo) on MACE ($p_{interaction}$: 0.36) or other CV outcomes and subgroup (metformin, yes/no; **Figure A**). Glycated haemoglobin and body weight reductions from baseline with semaglutide vs placebo were similar in both metformin subgroups (**Figure B**).
Conclusion: No significant differences in outcomes could be detected between those subjects receiving metformin and those who were not. These findings indicate that the CV and metabolic benefits of semaglutide can be observed regardless of metformin use at baseline.

Cardiovascular (A) and efficacy and safety (B) outcomes by metformin use at baseline in pooled data from SUSTAIN 6 and PIONEER 6



*n-numbers are based on the FAS; the number of subjects included for each endpoint analysis differed according to data availability. Data estimated at week 80 for SUSTAIN 6 and week 83 for PIONEER 6. In panel A, the analyses were based on a Cox proportional hazards model with treatment by subgroup as fixed factors and stratification by trial, adjusting for the following baseline characteristics: sex, smoking status, antihyperglycaemic treatments, prior CV events, geographic region, age, T2D duration and eGFR. In panel B, the efficacy outcomes analyses were based on a mixed model for repeated measurements, with treatment by subgroup adjusted for baseline value and trial nested within visits; for the safety outcome analyses, number of events per 100 patient-years was analysed using a negative binomial regression model with a log link and the logarithm of the observation time (100 years) as offset, with treatment by subgroup as fixed factors adjusted by trial. HR compares semaglutide with placebo within each subgroup. ETD compares semaglutide vs placebo. ERR compares semaglutide:placebo. Dashed vertical lines represent lines of null effect (0 for ETDs and 1 for HRs and ERRs). All data points are proportional to subgroup size.
CI, confidence interval; CV, cardiovascular; ERR, estimated rate ratio; ETD, estimated treatment difference; FAS, full analysis set; HHF, hospitalisation for heart failure; MACE, major adverse cardiovascular event; R, events per 100 patient-years; SAE, serious adverse event; T2D, type 2 diabetes.

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Disclosure: **M. Husain:** Employment/Consultancy; Dr. Husain has served as a paid consultant for Novo Nordisk on advisory boards. Grants; Dr. Husain has previously received investigator-initiated research grants from Novo Nordisk. Honorarium; Dr. Husain has received honoraria from Novo Nordisk for crafting and delivering educational content related to diabetes, obesity, cardiovascular disease and GLP-1RA.

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Efficacy and safety of tirzepatide versus semaglutide once weekly as add-on therapy to metformin in people with type 2 diabetes (SURPASS-2)

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Background and aims: The novel dual glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 receptor agonist tirzepatide (TZP) is in development for type 2 diabetes (T2D). Efficacy and safety of once weekly TZP vs semaglutide (SEMA) was assessed in people with T2D on background metformin.

Materials and methods: In this open-label, 40-week Phase 3 study, people with T2D (N=1879; mean baseline HbA_{1c}: 67 mmol/mol [8.28%], age: 56.6 years, T2D duration: 8.6 years, body mass index: 34.2 kg/m^2) were randomised (1:1:1:1) to once weekly TZP (5, 10, 15 mg) or SEMA (1 mg). Primary efficacy objective was noninferiority of TZP 10 and/or 15 mg vs SEMA for mean change in HbA_{1c} from baseline at 40 weeks. Secondary objectives included noninferiority (TZP 5 mg) for HbA_{1c} change and superiority (all TZP doses) for change in HbA_{1c}, body weight and fasting serum glucose, and proportion of patients with HbA_{1c} <53 mmol/mol (<7%), ≤48 mmol/mol (≤6.5%) and <39 mmol/mol (<5.7%; except TZP 5 mg) and body weight loss ≥5%, ≥10%, ≥15% at 40 weeks.

Results: At 40 weeks, all TZP doses were superior to SEMA in mean HbA_{1c} change from baseline (Table). LSM treatment differences vs SEMA (95% CI) were -2.5 mmol/mol (-3.9, -1.1) (-0.23% [-0.36, -0.10]) for TZP 5 mg, -5.6 mmol/mol (-7.0, -4.1) (-0.51% [-0.64, -0.38]) for TZP 10 mg, and -6.6 mmol/mol (-8.0, -5.1) (-0.60% [-0.73, -0.47]) for TZP 15 mg ($p<0.001$, all TZP doses). TZP 5, 10 and 15 mg were also superior to SEMA in achieving all HbA_{1c} targets. Fasting serum glucose significantly decreased from baseline with TZP 5, 10 and 15 mg vs SEMA (-0.41 mmol/L [-7 mg/dL], -0.72 mmol/L [-13 mg/dL], and -0.82 mmol/L [-15 mg/dL]; $p\leq0.001$, all TZP doses). Significant body weight loss was achieved with TZP 5, 10 and 15 mg vs SEMA (-1.7 kg [-2.6, -0.7], -4.1 kg [-5.0, -3.2], -6.2 kg [-7.1, -5.3]; $p<0.001$, all TZP doses). A greater proportion of patients achieved body weight loss ≥5%, ≥10% and ≥15% with TZP vs SEMA ($p\leq0.002$, all TZP doses). TZP was well tolerated. The most common adverse events were gastrointestinal and mostly mild to moderate in severity (nausea: 17-22% vs 18%, diarrhoea: 13-16% vs 12%, vomiting: 6-10% vs 8%). Hypoglycaemia <54 mg/dL or severe hypoglycaemia events were reported in 4 (0.9%), 1 (0.2%) and 8 (1.7%) patients treated with TZP vs 2 (0.4%) with SEMA. Two patients (TZP 5 and 15 mg) had one episode of severe hypoglycaemia.

Conclusion: In conclusion, all TZP doses demonstrated superior and clinically meaningful improvement in glycaemic control and substantial weight loss vs once weekly SEMA 1 mg in people with T2D treated with metformin.

Primary and Secondary Endpoints[a], Week 40	TZP 5 mg (N=470)	TZP 10 mg (N=469)	TZP 15 mg (N=469)	SEMA (N=468)
Baseline HbA_{1c}, mmol/mol	67.5 ± 0.52	67.3 ± 0.52	66.7 ± 0.52	66.6 ± 0.52
Change from baseline in HbA_{1c}[b], mmol/mol	-22.84 ± 0.52	-25.9 ± 0.52	-26.9 ± 0.52	-20.3 ± 0.52
Baseline HbA_{1c}, %	8.33 ± 0.048	8.31 ± 0.048	8.25 ± 0.048	8.24 ± 0.048
Change from baseline in HbA_{1c}[b], %	-2.09 ± 0.047	-2.37 ± 0.048	-2.46 ± 0.048	-1.86 ± 0.048
% of patients achieving HbA_{1c} <53 mmol/mol (7.0%)[b] / <48 mmol/mol (6.5%) / <39 mmol/mol (5.7%)[b]	86* / 74* / 29**	89** / 82** / 45**	92** / 87** / 51**	81 / 66 / 20
Baseline fasting serum glucose, mmol/L	9.67 ± 0.13	9.69 ± 0.13	9.56 ± 0.13	9.49 ± 0.13
Change from baseline in fasting serum glucose, mmol/L	-3.11 ± 0.09	-3.42 ± 0.09	-3.52 ± 0.09	-2.70 ± 0.09
Baseline body weight, kg	92.6 ± 1.02	94.9 ± 1.02	93.9 ± 1.02	93.8 ± 1.02
Change from baseline in body weight[b], kg	-7.8 ± 0.35	-10.1 ± 0.34	-12.4 ± 0.34	-6.2 ± 0.35
% of patients achieving body weight loss ≥5% / ≥10% / ≥15%	69* / 36** / 15*	82** / 53** / 28**	86** / 65** / 40**	58 / 25 / 9

Data are LSM ± SE at baseline predicted from ANCOVA and change from baseline at 40 weeks predicted from MMRM analysis, unless otherwise noted. Note: proportion of patients achieving HbA_{1c} and weight loss goals was obtained by dividing the number of patients reaching respective goals at Week 40 by the number of patients with baseline value and at least one non-missing postbaseline value. Missing value at Week 40 was predicted from MMRM analysis. *p<0.05 and **p<0.001 vs semaglutide 1 mg.

[a]mITT-Efficacy Analysis Set: on treatment data prior to initiating rescue therapy from mITT population excluding patients who discontinued study drug due to inadvertent enrolment (efficacy estimand).

[b]Tested for superiority, controlled for Type 1 error (except for HbA1c <5.7% for TZP 5 mg).

ANCOVA=analysis of covariance; HbA_{1c}=glycosylated haemoglobin; LSM=least squares mean; mITT=modified intent-to-treat (all randomised patients who took at least 1 dose of study drug); MMRM=mixed model repeated measures; N=number of patients in specified dataset; n=number of subjects with events meeting specified criteria; SE=standard error.

Clinical Trial Registration Number: NCT03987919

Supported by: Eli Lilly and Company

Disclosure: **M.J. Davies:** Employment/Consultancy; Novo Nordisk, Sanofi-Aventis, Eli Lilly and Company, Merck Sharp & Dohme, Boehringer Ingelheim, AstraZeneca, Janssen. Grants; Novo Nordisk, Sanofi-Aventis, Eli Lilly and Company, Boehringer Ingelheim, AstraZeneca, Janssen. Lecture/other fees; Novo Nordisk, Sanofi-Aventis, Eli Lilly and Company, Merck Sharp & Dohme, Boehringer Ingelheim, AstraZeneca, Janssen, Napp Pharmaceuticals, Mitsubishi Tanabe Pharma Corporation, Takeda Pharmaceuticals International. Other; Novo Nordisk, Sanofi-Aventis, Eli Lilly and Company, Merck Sharp & Dohme, Boehringer Ingelheim, AstraZeneca, Janssen, Servier, Gilead Sciences.

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Efficacy and safety of GLP-1RAs with or without baseline SGLT-2i: post hoc analysis of the SUSTAIN 10 trial

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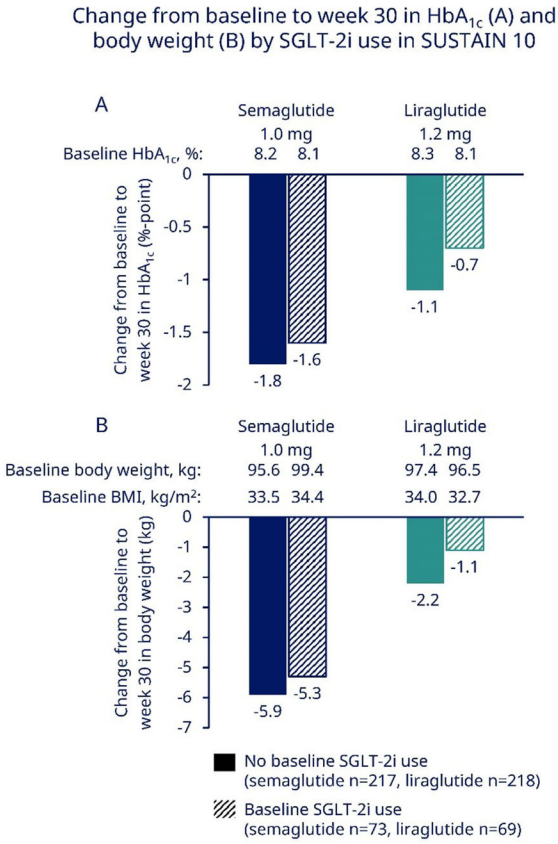
Background and aims: As glucagon-like peptide-1 receptor agonists (GLP-1RAs) and sodium-glucose cotransporter-2 inhibitors (SGLT-2is) are often used together in clinical practice, data on the safety and efficacy of these two classes in combination are valuable to clinical decision-making, yet such data are sparse. The SUSTAIN 10 trial analysed the efficacy and safety of the GLP-1RAs semaglutide and liraglutide in subjects with type 2 diabetes. This *post hoc* analysis of SUSTAIN 10 examined clinical outcomes with these GLP-1RAs in subjects not receiving an SGLT-2i and in those on background SGLT-2i treatment.

Materials and methods: Randomisation in the SUSTAIN 10 trial was stratified based on background medication. In this *post hoc* analysis, treatment effects with once-weekly semaglutide 1.0 mg or once-daily liraglutide 1.2 mg on HbA_{1c} and body weight (BW) without the confounding effect of rescue medication use ('on-treatment without rescue medication') were assessed in subjects without or with SGLT-2i use at screening. Effects on systolic blood pressure (SBP) were also assessed in these subgroups, as was safety (premature treatment discontinuation due to adverse events [AEs]) ('on treatment' data).

Results: Regardless of SGLT-2i use (without SGLT-2i: n=435; with SGLT-2i: n=142), in the semaglutide 1.0 mg and liraglutide 1.2 mg treatment arms, baseline use of metformin (without SGLT-2i: 97.2% and 94.0%; with SGLT-2i: 93.2% and 91.3%, respectively) and sulphonylureas (without SGLT-2i: 50.7% and 51.4%; with SGLT-2i: 35.6% and 31.9%) was similar. Regardless of SGLT-2i use, there were reductions from baseline to week 30 with semaglutide 1.0 mg and liraglutide 1.2 mg in HbA_{1c} (without SGLT-2i: 1.8 and 1.1 %-points, respectively; with SGLT-2i: 1.6 and 0.7 %-points, respectively; Figure 1A), BW (without SGLT-2i: 5.9 and 2.2 kg; with SGLT-2i: 5.3 and 1.1 kg; Figure 1B) and SBP (without SGLT-2i: 5.2 and 3.6 mmHg; with SGLT-2i: 2.4 and 3.2 mmHg). There were no unexpected safety concerns in subjects receiving both a GLP-1RA and SGLT-2i; premature treatment discontinuations due to AEs remained relatively low with semaglutide 1.0 mg and liraglutide 1.2 mg in both SGLT-2i subgroups (without SGLT-2i: 13.4% and 6.4%; with SGLT-2i: 5.5% and 7.2%).

Conclusion: Our results suggest that addition of a GLP-1RA to SGLT-2i treatment was associated with further reductions from baseline in HbA_{1c}, BW and SBP without additional safety concerns. As SGLT-2is and GLP-1RAs are often used together in clinical practice, our data on the safety and efficacy of combining these two classes are meaningful to clinical decision-making.



'On-treatment without rescue medication' data. The responses were analysed using an ANCOVA with treatment, subgroup and treatment by subgroup interaction as fixed factors and baseline value as covariate. For each subgroup, the reductions are estimated from a common baseline value for both semaglutide and liraglutide. Before analysis, missing data were imputed using observed data from subjects within the same group defined by randomised treatment, using a regression model including stratification factor and region as categorical effects and data from baseline and all previous visits as covariates. SGLT-2i, sodium–glucose cotransporter-2 inhibitor.

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Adherence and persistence in patients with type 2 diabetes initiating once-weekly versus once-daily injectable GLP-1 RAs in US clinical practice (STAY study)

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Background and aims: Drugs with prolonged action allow for a reduced dosing frequency, which may decrease treatment burden and improve adherence and persistence. We assessed persistence and adherence in patients with type 2 diabetes (T2D) initiating once-weekly or once-daily injectable glucagon-like peptide-1 receptor agonists (GLP-1 RAs) in US clinical practice.

Materials and methods: Adults (≥ 18 years) with T2D in the MarketScan Explorys Claims-EMR Data Set who were GLP-1 RA- and insulin-naïve at first claim for once-weekly or once-daily injectable GLP-1 RA were included. Patients required ≥ 180 days' enrolment pre-index and ≥ 365 days' enrolment post-index; the index period was 1 July 2012-31 January 2019, and follow-up was index date + 365 days. Individuals with type 1 diabetes, gestational diabetes or secondary diabetes were excluded from the analysis. Patients were propensity score matched 1:1 by baseline age, sex, baseline Charlson Comorbidity Index score, baseline HbA1c and weight (90 days pre-index), and use of sulfonylureas, metformin, dipeptidyl peptidase-4 inhibitors, and sodium–glucose co-transporter-2 inhibitors (180 days pre-index). Discontinuation was defined as ≥ 60 days not covered by medication. Persistence was defined as stay time (patients who had not discontinued within 12 months were censored at 360 days) and assessed using Kaplan–Meier analysis and Cox proportional hazard models. Adherence was defined as proportion of days covered (PDC) ≥ 0.8.
Results: At baseline, the matched cohorts (n = 784 each) had similar mean age (once-weekly vs once-daily, 54.6 years vs 54.4 years), percentage of women (50% vs 51%), mean body mass index (36.5 kg/m^2 vs 36.7 kg/m^2), mean HbA1c (8.5% vs 8.4%) and mean Charlson Comorbidity Index score (1.0 each), and received similar antidiabetic medications (biguanides, 46% vs 48%; dipeptidyl peptidase-4 inhibitors, 18% each; sodium–glucose co-transporter-2 inhibitors, 12% vs 5%; sulfonylureas, 23% each). Once-weekly injectable regimens were associated with significantly higher persistence compared with once-daily injectable treatments (median stay time: 333 days vs 269 days; HR 0.80 [95% CI, 0.71-0.90], $p < 0.01$). Once-weekly GLP-1 RAs were also associated with higher adherence, relative to once-daily regimens, at 6 months (+23%) and 12 months (+35%), irrespective of glycaemic control and weight change. Improvement in glycaemic control was greater with once-weekly than once-daily treatments at 6 months (mean HbA1c change, -1.1% vs -0.9%) and 12 months (-0.9% vs -0.7%). Over 12 months, adherent patients experienced greater mean changes in HbA1c with both once-weekly (-1.1%) and once-daily (-1.0%) regimens than patients with PDC < 0.8 (-0.6% each).
Conclusion: Our results suggest that, in a real-world setting, once-weekly injectable treatments are associated with better persistence and adherence than once-daily regimens, irrespective of glycaemic or weight control.
Supported by: Novo Nordisk A/S
Disclosure: **W.H. Polonsky:** Employment/Consultancy; Consultant for Eli Lilly, Novo Nordisk and Sanofi.

OP 05 Epidemiology of diabetes complications

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The natural history of 786 episodes of diabetic ketoacidosis in adults with type 1 and type 2 diabetes

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Background and aims: Although diabetic ketoacidosis is traditionally associated with type 1 diabetes, it is increasingly recognised that people with type 2 diabetes can also develop this condition. Currently, there is limited evidence studying the differences between these groups. We aimed to explore the differences in the demographics, presentation and management of diabetic ketoacidosis in adults with type 1 diabetes and type 2 diabetes.
Materials and methods: This cohort study included all episodes of diabetic ketoacidosis from April 2014 to September 2020 in a tertiary centre in the United Kingdom. Diabetes was classified into type 1 diabetes and type 2 diabetes in accordance with previously established diagnoses, autoantibody status, and/or phenotypic features. Data for diabetes type, demographics, biochemical and clinical features at presentation and management of diabetic ketoacidosis were collected. As data were skewed, Wilcoxon sum rank test was applied to compare the two groups on various parameters.
Results: From 786 consecutive diabetic ketoacidosis episodes, 583 (75.9%) type 1 diabetes and 185 (24.1%) type 2 diabetes episodes were included in the final analysis. The median (IQR) age for overall cohort was 38.2 years (23.8-56.8) with male: female ratio of 1:1.04. People with type 2 diabetes were older (type 1 diabetes: 28.97 years [21.9 - 48.7] vs type 2 diabetes 61.54 years [52.0-75.1]; p<0.0001) and had more ethnic minority representations than those with type 1 diabetes (non-white: type 1 diabetes 19.7% vs type 2 diabetes 26.5%; p=0.030). Intercurrent illness (35.4%) was the most common precipitating causes for diabetic ketoacidosis in both cohorts. Severity of diabetic ketoacidosis as assessed by pH (type 1 diabetes 7.22 [7.09-7.29] vs type 2 diabetes 7.24 [7.11-7.30]; p=0.3266), glucose (type 1 diabetes 28.0 mmol/l [20.5-34.8] vs type 2 diabetes 13.2 mmol/l [7.8-17.8]; p=0.4496) and lactate (type 1 diabetes 2.6 mmol/l [1.8-4.3] vs type 2 diabetes 2.6 mmol/l [2.0-4.2]; p=0.6532) at presentation was similar in both groups. However, urea was higher in those with type 2 diabetes (type 1 diabetes 7.1 mmol/l [5.1-10.6] vs type 2 diabetes 8.9 mmol/l [6.4- 16.8]; p=0.0001). Insulin requirements (type 1 diabetes 91.6 units [43.5 - 143.9] vs type 2 diabetes 90.2 units [53.6 - 157.4]; p=0.5551) and total duration of diabetic ketoacidosis (type 1 diabetes 13.9 hours [9.1-21.9] vs type 2 diabetes 13.9 hours [7.7-21.1]; p=0.4638) were similar between the two groups. People with type 2 diabetes had longer hospital stay (type 1 diabetes 3.0 days [1.7-6.1] vs type 2 diabetes 11.0 days [5.0-23.1]; p<0.0001).
Conclusion: A quarter of diabetic ketoacidosis episodes occur in people with type 2 diabetes who were older and with greater ethnic minorities proportion compared to type 1 diabetes. However, the severity of presentation and management is similar in both groups suggesting that the same protocol is equally effective in either type of diabetes. People with type 2 diabetes admitted with diabetic ketoacidosis have a longer hospital admission, perhaps reflecting a more complex need of care.
Disclosure: **K. Nash:** None.

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Burden of established cardiovascular disease in people with type 2 diabetes and matched controls: hospital-based care, days absent from work, costs, and mortality

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Background and aims: Established cardiovascular disease (eCVD) is associated with need for healthcare interventions, reduced work capacity and excess mortality. People with type 2 diabetes have increased risk and earlier onset of eCVD compared to people in general. The objective was to assess the burden of hospital-based care, days absent from work, associated costs, and excess mortality for people with type 2 diabetes with and without eCVD with comparison to matched controls.
Materials and methods: The study used a Swedish retrospective database cross-linking longitudinal individual-level data (2007-2016) from national population-based health, social insurance, and socio-economic registers for 454,983 people with type 2 diabetes and their matched controls (5:1 on year of birth, sex, and region of residence). Status eCVD (coronary artery disease, stroke, amputation, periphery vascular disease, non-fatal cardiac arrest, or related interventions) was derived from retrospective data 1997-2006 and updated at change of status 2007-2016. Use of hospital-based care, days absent from work (calendar days) and mortality used data 2007-2016. Regression analysis accounting for individual-level clustering was used for comparison to controls and to attribute costs of hospital-based care and days absent from work to eCVD and to individual complications considering multimorbidity. Excess mortality adjusted for age, sex, and educational level was attributed to eCVD using Cox proportional hazards.
Results: Thirty percent (n=136,135) of people with type 2 diabetes up to age 70 years were observed with eCVD ≥1 observation year in 2007-2016 (women 24% n=43,847; men 34% n=92,288). The mean annual costs of hospital-based care for diabetes complications were EUR 2,758 (95% CI 2,729 to 2,787) of which EUR 2,461 (95% CI 2,432 to 2,490) were attributed to people with eCVD (89%). Main drivers of costs of hospital-based care for people with eCVD were acute myocardial infarction, angina pectoris, and stroke; but also end-stage renal disease (ESRD) and eye disease confirming that eCVD is associated with an increased burden from other complications. eCVD was a leading cause behind work absence for both diabetes and controls. People with type 2 diabetes <66 years had on average 146 days absent (95% CI 145-147) of which 68 days (47%) were attributed to eCVD. Controls had 106 days absent of which 63 days (59%) were attributed to eCVD. The annual cost of eCVD work absence was EUR 9,337 (95% CI 9,150 to 9,523) per individual. The highest work absence was attributed to ESRD, stroke, and heart failure. Type 2 diabetes without eCVD did not differ from controls regarding mortality risk, but type 2 diabetes with eCVD had a four-fold risk of death hazard rate 4.13 (95% CI 4.10 to 4.18) adjusting for age, sex, and educational level.
Conclusion: This study assessed the size of the burden of eCVD-status in people with type 2 diabetes in three measures: 1) eCVD was associated with excess mortality; 2) 9 out of 10 EUR spent on hospital-based care for diabetes complications; and 3) even higher costs of days absent from work in the long-run. Reducing the risks of living with eCVD and postponing the onset of eCVD remain central goals to reduce the burden of type 2 diabetes on the individual and on society.
Supported by: Boehringer Ingelheim AB, Stockholm, Sweden, to the Swedish Institute for Health Economics, Lund, Sweden
Disclosure: **S. Persson:** Grants; Research grant Boehringer Ingelheim.

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One-year trajectories of impaired hypoglycaemia awareness in young people with type 1 diabetes

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Background and aims: Impaired hypoglycemia awareness has mainly been studied in the era of capillary blood glucose monitoring and in adults

with diabetes. It is less known in youth. The aim of this study is to describe impaired hypoglycemia awareness and its evolution during a one-year follow-up in children with type 1 diabetes using a flash glucose monitoring system.

Materials and methods: This 1-year follow-up study included people with type 1 diabetes using flash glucose monitoring, aged 4–20 years. Impaired hypoglycemia awareness was diagnosed using the Gold method, which consists of a 7-point Likert scale that we adapted for pediatric usage, with a cut-off level of ≥ 3 to diagnose impaired hypoglycemia awareness.

Results: Of the 283 participants included, 48% were diagnosed with impaired hypoglycemia awareness. Participants diagnosed with impaired hypoglycemia awareness were younger at diagnosis (p = 0.045), more often C-peptide negative (p = 0.026) and performed more scans (p = 0.045) than participants with normal awareness. They also reported worse quality of life in usual activity (p = 0.035). They had an increased time below 70 mg/dl (p = 0.003), an increased time below 54 mg/dl (p = 0.003) and an increased Low Blood Glucose Index (p = 0.012) compared to participants with normal hypoglycemia awareness and were 3.2 times more likely to experience severe hypoglycemia (p = 0.002). When defining impaired hypoglycemia awareness using the alternative cut-off of 4 on the Gold scale (as validated for adults), 90 participants (30.9%) remained with an impaired hypoglycemia awareness diagnosis, but this threshold was less discriminating in terms of the occurrence of severe hypoglycemia. After 1-year follow-up, 41% of participants diagnosed with impaired hypoglycemia awareness had reverted to normal awareness, while 22% of participants classified as normal awareness at baseline were newly diagnosed with impaired hypoglycemia awareness at follow-up.

Conclusion: Impaired hypoglycemia awareness is highly prevalent in youth using Flash Glucose Monitoring but affects people differently over time and is associated with an increased and consistent risk of (severe) hypoglycemia . Using the Gold method, in a pediatric population, a cut-off of 3 is more discriminating than the historical threshold of 4 (validated for adults) in terms of hypoglycemia risk. Frequent assessments of impaired hypoglycemia awareness in clinical follow-up may help to improve management of hypoglycemia risk in young people with type 1 diabetes.

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Disclosure: **A. Messaaoui:** None.

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Association and familial co-aggregation of type 1 diabetes with depression, anxiety and stress-related disorders: a population-based cohort study

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Background and aims: People with type 1 diabetes are known to be at heightened risk of common mental health problems. However, it remains unknown whether genetic liability contributes to the elevated risk. This study aimed to investigate the association and familial co-aggregation of type 1 diabetes with depression, anxiety and stress-related disorders.

Materials and methods: Using multiple Swedish nationwide registers, we obtained a population sample of individuals born 1973-2007 and still residing in Sweden at age 5. Individuals were linked to their biological parents, full-siblings, half-siblings, full-cousins and half-cousins. We obtained information from the National Patient Register (since 1973) on the diagnoses of type 1 diabetes, depression, anxiety and stress-related disorders using ICD codes and from the Prescribed Drug Register (since 2005) on the prescribed antidepressants and anxiolytics using ATC codes. Primary outcomes were any or specific diagnosis of 1) depression, 2) anxiety and 3) stress-related disorders. We examined a secondary outcome of using antidepressants or anxiolytics in those who resided in Sweden after 2005.

Results: In this cohort study of about 3.5 million individuals, 20005 (53.9% male) were diagnosed with childhood-onset type 1 diabetes (< 18 years of age, the median age at onset: 9.7). We used Cox models to estimate the association between type 1 diabetes and each outcome. Individuals were regarded as unexposed before diagnosis and exposed after. During a median follow-up of 22.2 years, individuals with type 1 diabetes were at a higher risk of all outcomes after adjusting for sex and birth year: any diagnosis (HR [95%CI]: 1.73 [1.67-1.80]), depression (1.93 [1.84-2.02]), anxiety (1.41[1.33-1.50]), stress-related disorders (1.75 [1.62-1.89]) and using antidepressants or anxiolytics (1.30 [1.26-1.34]). Familial co-aggregation was evaluated using Cox models, where an individual's relative was regarded unexposed before the individual's diabetes diagnosis and exposed after. Overall, higher risks of all outcomes were observed in relatives of individuals with diabetes and declined proportionally with decreasing genetic relatedness. Highest HRs were found in parents: any diagnosis (1.21 [1.16, 1.26]), depression (1.20 [1.13-1.26]), anxiety (1.22 [1.15, 1.30]), stress-related disorders (1.25 [1.17-1.34]) and using antidepressants or anxiolytics (1.18 [1.16, 1.21]). HRs decreased but remained significant in full-siblings after adjusting for sex and birth year of the sibling: any diagnosis (1.11 [1.05, 1.17]), depression (1.11 [1.03-1.19]), anxiety (1.10 [1.02, 1.1]), stress-related disorders (1.20 [1.08-1.32]) and using antidepressants or anxiolytics (1.05 [1.01, 1.09]). HRs decreased and were not significant in maternal and paternal half-siblings (HRs 0.90-1.10; 1.00-1.11), full-cousins (HRs 0.98-1.05) and half-cousins (HRs 0.80-1.02).

Conclusion: Our findings support existing evidence that individuals with childhood-onset type 1 diabetes were at higher risks of depression, anxiety, stress-related disorders and using antidepressants and anxiolytics and suggest that familial liability may contribute to these associations. The results highlight the importance of family support integrated with pediatric diabetes care.

Disclosure: **S. Liu:** None.

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High adherence to recommended diabetes follow-up procedures by general practitioners is associated with lower estimated cardiovascular risk

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Background and aims: The relation between diabetes quality indicators and diabetes outcomes is unclear. Our aims were to explore whether general practitioners' (GPs') performance of recommended processes of care were associated with estimated risk of cardiovascular disease (CVD) and poor glycaemic control in their type 2 diabetes patients.

Materials and methods: A cross-sectional study from Norwegian general practices including 6015 people with type 2 diabetes <75 years old, without CVD and their 275 GPs. The GPs were split into quintiles based on each GP's average performance of six recommended processes of care (measurements of HbA1c, LDL-cholesterol, albuminuria, blood pressure and foot examination the past 15 months; and recorded eye examination the past 30 months). We estimated the 10-year risk of cardiovascular events using the risk calculator NORRISK 2. To avoid excess confounding of strong non-modifiable risk factors, our main outcome was the modifiable fraction of estimated CVD risk. The exposure variable were

the quintiles of GPs in multilevel regression models with estimated CVD risk (total and modifiable fraction) and poor glycaemic control; HbA1c >69 mmol/mol (>8.5%) as outcome variables.

Results: The mean total and modifiable estimated 10-year CVD risk was 12.3% and 3.3% respectively. We found a difference in the mean estimated 10-year risk of CVD of 2.2 percent points between persons registered with GPs in the lowest (13.5%) and the highest (11.3%) quintile for the performance of procedures. Adjusted for confounders, those belonging to GPs in the lowest quintile had a 1.88 (1.17 - 2.60) percent point higher total CVD risk, representing a relative difference of 16.6% higher average risk among patients in quintile 1 compared with quintile 5. The mean modifiable CVD risk was almost 2 percent points higher in patients registered with GPs in the lowest (4.22%) compared with the highest quintile (2.30%). After adjusting for confounders, being registered with a GP in the lowest quintile was independently associated with a 1.78 (1.14 - 2.41, p<0.001) percent point higher modifiable CVD risk compared with those of GPs in the highest quintile. This represents a relative difference of 74.8% (59.0 - 85.8) higher average modifiable risk fraction among those in quintile 1 compared with quintile 5. In the lowest quintile, 14.1% of the people had HbA1c above 69mmol/mol (8.5%), compared with 7.8% in the highest quintile. For patients with GPs in the lowest-performing quintile, the adjusted odds of poor glycaemic control was 1.77 (1.27 - 2.46) times higher than for patients with a GP in the highest quintile.

Conclusion: We found a pattern of lower CVD risk and better glycemic control in patients of GPs performing more recommended diabetes processes of care.

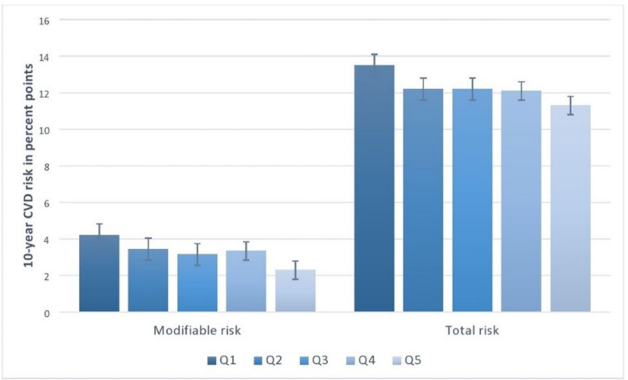

Figure 1. Mean estimated modifiable and total cardiovascular risk according to quintiles (Q1-Q5) of GPs' process performance.

Supported by: The Norwegian Research Fund for General practice funded the doctoral program of K.N.

Disclosure: **K. Nøkleby:** Grants; The Norwegian Research Fund for General practice funded the doctoral program of K.N., The Lillian and Werner Næss Fund and the Norwegian Diabetes Association granted this subproject funds.

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Assessment of the effect of the COVID-19 pandemic on HbA_{1c} testing: implications for diabetes management and diagnosis

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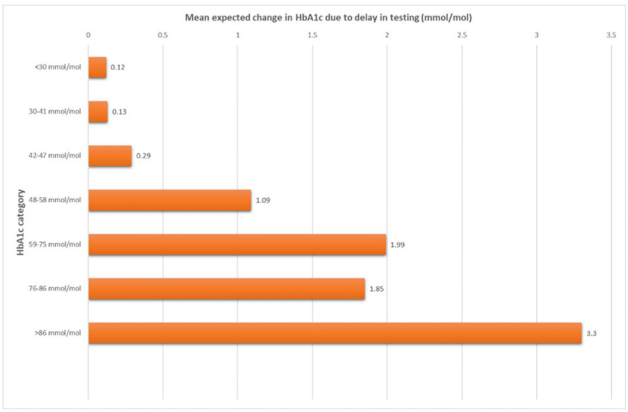
Background and aims: Since its arrival in 2020, the COVID-19 pandemic has challenged all healthcare systems across the world. The focus on mitigating the effects of the virus led to many routine healthcare services being disrupted. In the UK, services for the diagnosis and management of people with diabetes was one such area. Routine blood testing, a mainstay of diabetes diagnosis and management, became challenging, not least because of the potential risk it posted to facilitating transmission of the virus and associated concerns members of the public had regarding attending for tests.We aimed to quantify this effect of the COVID-19 pandemic on diabetes diagnosis and management, using HbA1c as a surrogate, across regions covered by six UK testing laboratories.

Materials and methods: We estimated the number of missed HbA1c tests, both for diagnostic and management purposes during the Covid Impact Period (CIP) between 23 Mar 2020 and 30 Sep 2020. We then examined the potential impact of these missed tests in terms of (i) effect on diabetes monitoring in people with or at risk of diabetes and, (ii) detection of new diabetes cases. The 6 sites represented mixed rural and urban populations with a range of deprivation scores. We categorised tests into three groups - 'monitoring', those already diagnosed with diabetes; 'screening' as part of general health and 'diagnostic' on those at risk.

Results: The 6 laboratories supported 3.7 million population or 6% of the UK population over the 3 years studied, with 3.6 million HbA1c tests on 1.7 million people. The change between 12 months pre-CIP and the 6 month CIP tests/month were 32k vs 19k monitoring; 46k vs 32k screening and 31k vs 12k diagnostic. 79,000 monitoring tests were missed. 28,500 of these for people with suboptimal control, this delay in monitoring was linked to 2-3 mmol/mol increase in HbA1c. Around 149,000 screening tests in high-risk groups were missed, including nearly 27,000 with HbA1c values within the pre-diabetes range. We also identified that 142,000 diagnostic tests were missed during the period, of which ~12,000 would be expected to be in the pre-diabetes range and 3,800 in the diabetes range. Across the whole UK, these data would equate to 2.54m missed/delayed diagnostic tests during the CIP (on average, 0.40m per month), including ~213,000 in the pre-diabetes range in whom lifestyle advice might be delayed, and 68,500 delayed new diagnoses of diabetes (~24,000 and ~11,000 per month, respectively).

Conclusion: Our findings illustrate the widespread collateral impact of implementing measures to mitigate the impact of COVID-19 in people with, or being investigated for diabetes Ironically, failure to focus of the wider implications for people with diabetes and other groups with long-term conditions, may place them at increased risk of poor outcomes from COVID-19 infection itself, irrespective of the implications for their longer-term health prospects.



Disclosure: **D. Holland:** None.

OP 06 Insights into diabetic retinopathy

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Evaluation of maculopathy deep learning prediction models using the SDRNT1BIO cohort

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Background and aims: Diabetic Retinopathy and Maculopathy are the leading cause of blindness for people with diabetes. Many countries including Scotland have screening programmes to enable early detection which currently screen people based on fundus image photography. In Scotland, it is found that many people are referred from the screening programme to a specialist with suspected maculopathy but later return to the screening programme - suggesting that maculopathy was not confirmed by the specialist. Accurate prediction of maculopathy from fundus images would reduce unnecessary visits to specialists for people. The objective of this study was to establish whether deep learning prediction models using retinal images could predict those people in the Scottish Diabetic Retinopathy screening programme who were referred to a specialist with suspected maculopathy and did not later return to the screening programme.
Materials and methods: We trained a RegNetY016 network to predict Diabetic Macula Edema using data from the Diabetic Retinopathy Clinical Research Network as well as the Kaggle-EyePACS and Messidor datasets using a combined 25557 fundus images. We evaluated our model and also the publicly-available DeepSeeNet and ARIANNA age-related macular degeneration prediction models. The evaluation data contained 68488 fundus images (886 referred and did not return) from 5539 people in the Scottish Type 1 Diabetes Bioresource who attended the Scottish National Screening programme between the 2005 and 2017. The evaluation outcome was defined as an image being graded as referable and the person having no subsequent screenings. Images within 2 years of the latest image in the dataset and images taken within 2 years of a patient's death were excluded from evaluation.
Results: The RegNetY016 model discriminated images graded as having suspected maculopathy who did not return to the screening programme with an AUROC of 0.85. The DeepSeeNet Drusen prediction model discriminated the same outcome with an AUROC 0.75 whereas the ARIANNA model only attained an AUROC 0.60.
Conclusion: Deep learning prediction models were able to discriminate between fundus images from patients referred for maculopathy in the Scottish screening programme and did not return to the programme subsequently. This suggests deep learning models may be an effective tool to help reduce unnecessary specialist appointments for maculopathy.
Supported by: JDRF Grant 2-SRA-2019-857-S-B
Disclosure: **J. Mellor:** Grants; 2-SRA-2019-857-S-B.

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Whole exome- and whole genome sequencing reveals novel rare genetic variants for severe diabetic retinopathy in type 1 diabetes

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Background and aims: Genetic factors play a role in the susceptibility to diabetic retinopathy but studies aiming to identify genetic factors associated with diabetic retinopathy remain scarce. The aim of the study is to discover rare variants associated with severe diabetic retinopathy (SDR) in type 1 diabetes (T1D) with whole exome- (WES) and whole genome sequencing (WGS) studies in the Finnish Diabetic Nephropathy (FinnDiane) Study.
Materials and methods: We performed WGS and WES on ~600 and ~500 individuals with T1D, respectively. WES was done on the Illumina HiSeq2000, and WGS on the Illumina HiSeq X platform with at least 30x coverage, and both were aligned to the GRCh38 reference panel. The SDR phenotype was based on data given by the attending physician, medical records, and the Finnish Hospital Discharge Registry until the end of 2017, and SDR was defined as the first laser treatment (photocoagulation) event, or a diagnosis code for proliferative diabetic retinopathy. Controls had at least a 20-year duration without SDR. After phenotypic ascertainment and quality control, the study population consisted of 490 individuals with WES (390 cases) and 583 individuals with WGS (405 cases). For exonic regions in WES and WGS, association was tested with the score test and meta-analysed with METAL; for other regions, the association was tested with the Firth regression for WGS. The SKAT-O gene aggregate test was performed with WES and WGS for protein altering or truncating variants with two minor allele frequency (MAF) thresholds, 5% and 1%, to improve the power to detect low frequency and rare variant associations. Covariates in the analysis included sex, age at onset, and sequencing data set principal components 1 and 2.
Results: Single marker meta-analysis revealed six variants with a p-value < 10^{-5} for SDR, with the strongest association for a synonymous variant rs372789789 on *LDB3* (MAF=0.2%; p=4.6×10^{-7}) and a synonymous variant rs56043607 in *PASK* (MAF=0.6%; p=8.2×10^{-7}). Outside exons, variants in 11 loci reached a p-value < 10^{-5} for SDR, with the strongest associations obtained for an intergenic variant rs9940767 (MAF=22%; p=5.7×10^{-7}). The strongest burden of protein truncating variants in WGS (both in MAF < 5% and MAF < 1%) was found for *UACA* (SKAT-O p=9.6×10^{-4}), and replicated in WES (p=0.02). *UACA* is an uveal autoantigen with coiled-coil domains and ankyrin repeats and is associated with panuveitis. In *CACNB2* - a gene previously associated with SDR - we found an intron variant associated with SDR (rs4747341, WGS p=8×10^{-4}). No signal for association was found with gene-aggregate tests for *CACNB2*, but protein altering variants on *CACNA1C*, which is part of the same calcium channel as the *CACNB2*, were associated with SDR in WGS (MAF < 1%, SKAT-O p=0.001).
Conclusion: Data from WES and WGS suggest that rare protein truncating variants in *UACA* contribute to the risk of SDR in individuals with T1D. Look-up of *CACNB2* revealed an intron variant association on *CACNB2* and protein altering variants on *CACNA1C*, which is part of the same calcium channel.
Supported by: Silmä- ja kudospankkisäätiö, Mary och Georg C. Ehrnrooths stiftelse
Disclosure: **N. Vuori:** None.

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Mapping the daily rhythmic transcriptome in the diabetic retina

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Background and aims: The eye is a rhythmic organ specifically evolved to function around the light cycle via functional circadian clocks. Diseases such as diabetes have been reported to disrupt circadian rhythms and circadian disruption emerges as an important factor in the prognosis of disease outcomes and treatment success. Herein, we mapped the rhythmic transcriptome in the mouse retina to understand the extent of circadian disruption due to diabetes.
Materials and methods: Healthy control and Ins2Akita/J diabetic mice were kept under physiological 12h:12h light dark cycle until 4 months of age. Deep mRNA sequencing was conducted in retinas collected every 4

hours around the day/night cycle. Computational approaches were used for the detection of rhythmicity (empirical JTK_CYCLE, $p<0.05$, $fc>1.25$), acrophase prediction (harmonic regression analysis), differential rhythmic patterns (DORD, $p<0.05$), phase set enrichment analysis ($p<0.05$) and upstream regulator predictions (Ingenuity Pathway analysis, Qiagen). Validation was done with in vitro experiments on human retinal endothelial cells.

Results: Almost 10% of the retinal transcriptome was identified as rhythmic with a clear 12hr axis of transcriptional activity, peaking at midday and midnight. Although the 12 hour transcriptional axis is retained in the diabetic retina, it is phase-advanced by approximately 1-3 hours. A total of 478 genes were identified as differentially rhythmic in diabetes compared to control. A higher number of genes identified as phase shifted (405 transcripts) compared to genes with altered amplitude (154 transcripts). Diabetes did not alter the phase of the circadian clock, but phase set enrichment analysis revealed that oxidative phosphorylation was phase advanced by almost 4 hours in the diabetic retina. Diabetic mice had a peak of glucose levels in the blood at that time, possibly driving the metabolic shifts in the retina. Downstream analysis identified oxygen sensing mechanisms and HIF1A as major predicted upstream regulators for the phase shifts.

Conclusion: To our knowledge, this is the first study mapping the effect of diabetes on the rhythmic output in the retina. Importantly, we have started to dissect the source of this rhythmic disruption in the diabetic retina and identified that in an entrained retina, the circadian clock is preserved but metabolic perturbations shift a set a genes within the retina that may contribute to an internal jet-lag within the tissue.

Supported by: JDRF 1-FAC-2019-878-A-N

Disclosure: **H.R. Winter:** None.

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Transcriptional and post-transcriptional impact of methylglyoxal in human retinal endothelial cells: New players in diabetic retinopathy?

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Background and aims: The accumulation of methylglyoxal (MGO) - a highly reactive byproduct of glycolysis - in diabetic patients has harmful effects on vascular function. MGO mediates the hyperglycemia-induced alterations in macrovascular endothelial cells, but the impact on human retinal endothelial cells (hRECs) has not yet been elucidated. Thus, we assessed the functional and molecular effects of MGO in hRECs, defining regulatory networks of endothelial damage in diabetic retinopathy (DR).

Materials and methods: Functional assays (i.e. MTT, transwell and tube formation) were performed on commercially available primary hRECs exposed to 500μM MGO for 72h. Transcriptome and miRNome analysis were performed by RNA-Seq (~60M paired-end reads/sample) and smallRNA-Seq (10M single-end reads/sample). TopHat, RNA-SeqGUI and iSMART were used for mapping and data normalization. Biological processes/pathways of differentially expressed (DE) genes (edgeR tool; FDR≤0.05) and miRNAs (NOISeq tool; Prob≥0.9) were identified by open-source databases/tools (KEGG, DAVID, Panther, miRPathDB, Reactome). ENCODE ChIP-Seq tool and miRPathDB were used for selecting transcription regulators (TRs) and miRNAs with enriched binding sites in DE genes. Protein-protein and miRNA-genes interactions were analyzed by STRING and miRnet.

Results: MGO significantly impairs cell migration and tube formation capacity of hRECs ($p\leq0.05$), also inducing a wide transcriptional perturbation of genes involved in gene expression regulation (i.e. 454 genes), cell cycle (i.e. 108), proliferation (i.e. 104), cell death (i.e. 95), adhesion (i.e. 79) and vasculature development (i.e. 44). The neuron-restrictive silencer factor (NRSF/REST) is one of TRs with enriched binding sites (Q≤0.05) in ~60% of DE genes (i.e. 1009). According to ENCODE ChiP-Seq data, NRSF/REST is predicted to regulate most of DE genes involved in gene expression regulation (i.e. ~70%), cell cycle (i.e. ~78%) and cell death (i.e. ~74%), revealing to be a reliable mediator of MGO-induced deregulation in hRECs. Of note, a restricted interaction network indicates NRSF/REST as hub connecting multiple deregulated factors with known roles in retinogenesis (e.g. Hipk, Ngfr), retinal neuronal cells (e.g. Jun, FosL2, Hes1) and/or in DR (e.g. p53, Vegfa, Sirt1). Moreover, our analysis revealed 83 miRNAs deregulated by MGO and targeting ~75% of DE genes (i.e. 1354). Notably, the upregulated factors involved in NRSF/REST restricted network are targets of 23 downregulated miRNAs. Thus, we reconstructed a deregulated mRNA-miRNA interaction network potentially mediating the MGO-induced transcriptional and post-transcriptional perturbation in hRECs.

Conclusion: Our work reveals that MGO induces functional and molecular effects in hRECs, representing a putative mediator of primary events in DR. MGO directly induces transcriptional and post-transcriptional alterations in retinal endothelial cells. The interaction network between NRSF/REST, other key altered protein-coding genes and deregulated miRNAs could sustain these effects. Experimental assays are in progress to address whether targeting the candidate mRNAs/miRNAs restrain MGO-induced detrimental effects in hRECs, revealing molecular mediators of glucotoxicity in microvascular retinal cells.

Supported by: EFSD/Boehringer Ingelheim European Research Programme in Microvascular Complications of Diabetes 2018

Disclosure: **M. Aprile:** None.

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Two-year progression of retinal neurodegeneration in paediatric patients with type 1 diabetes: the role of glycaemic variability

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Background and aims: Retinal neurodegeneration (RN) is considered an early marker of diabetic retinopathy (DR), which precedes vascular damage. Few data are available on the impact and predictive role of glycemic control and daily glycemic variability (GV) on early RN signs in the pediatric population with type 1 diabetes mellitus (T1DM). The aim of our study is to evaluate for two years the structural alteration of neuroretina and the predictive role of GV on RN in pediatric T1DM subjects without any complications.

Materials and methods: 25 patients with T1DM (ages 10-20 years), using Continuous Glucose Monitoring (CGM) and treated with Continuous subcutaneous insulin infusion, without any complication, and 18 healthy control subjects (C), comparable in age and gender, were enrolled and followed for 2 years. All subjects underwent an Optical Coherence Tomography Heidelberg Spectralis, with analysis of all macular neuroretinal layers measuring mean of subfoveal, inner and outer quadrants. In T1DM patients, glycated hemoglobin (HbA1c), GV indexes and standardized CGM metrics were calculated. All the data were collected at baseline (V0) and after 12 (V1) e 24 months (V2).

Results: At V0, Retinal Nerve Fiber Layer (RNFL) thickness was significantly thinner in inner sectors, (91.8 ± 6.8 μm vs 96.5 ± 5.5 μm; $p = 0.02$) in T1DM versus C. At V1 and V2, the Outer Plexiform Layer (OPL) was significantly thinner in the inner quadrants (152.8 ± 9.4 vs. 163.9 ± 12.8, $p < 0.01$) (150.3 ± 9.5 vs 163.5 ± 12.8, $p < 0.01$) and in the whole quadrants (257.1 ± 12.6 μm vs. 286.4 ± 66.5 μm, $p = 0.05$), (254.3 ± 10.2 vs. $289.6 \pm 67,6$, $p = 0.05$) in T1DM versus C. At V2, the Inner Retinal Thickness (IRT) was significantly thinner (1201.3 ± 40.5 vs. $1244.1 \pm$

61.6, p = 0.04) in T1DM versus C. In the T1DM, a progressive reduction in OPL and IRT was observed after the two-year follow-up (p<0.05). In T1DM patients, a negative correlation was observed between Continuous Overlapping Net Glycemic Action (CONGA-1h) and inner RNFL at V0 (r=-0.42, p=0.05). A negative correlation between Mean Absolute Glucose (MAG) and inner OPL (r=-0.53, p=0.04) was observed at V2. A negative correlation between the IRT delta thickness (V2-V1) and Lability Index (r=-0.64, p=0.01) and MAG (r=-0.61, p=0.02) were found in T1DM patients. No significant correlation between HbA1c and macular layers thickness was observed (p>0.05). Among metabolic parameters, a negative correlation between triglycerides levels and the IRT delta thickness (V2-V1) (r=-0.67, p<0.01) was found.

Conclusion: Very early morphological alterations of neuroretina are already present in pediatric T1DM patients without both vascular retinopathy and neuropathy, supporting the hypothesis that RN occurs early in the course of diabetes. GV seems to play a predictive role in the morphological abnormalities of neurosensory retina in T1DM pediatric population. Next step is a longitudinal evaluation to identify if neuroretinal nerve damage could be a predictive marker of diabetic neuropathy.

Disclosure: **M. Menduni:** None.

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Effect of calcium dobesilate in patients with subclinical diabetic macular oedema: the CADODIAME study

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Background and aims: Calcium dobesilate (CaD) is indicated and approved for the treatment of diabetic retinopathy (DR) in several countries. Several randomised placebo-controlled clinical trials demonstrated CaD efficacy on the progression of early DR. However, in the CALDIRET study, CaD did not reduce the risk of developing clinical significant macular edema (CSME). Overall the data of those studies suggest that CaD is beneficial in the very early stages of DR but more supportive clinical evidence is needed. Retinal thickness seems a good predictor of diabetic macular edema (DME) and, therefore, patients with subclinical DME represent a good target population to evaluate therapeutic strategies for preventing CSME. On this basis, we aimed to test the effect of CaD in early stages of DR, including the progression from subclinical DME to CSME.

Materials and methods: In this randomized, multicenter placebo-controlled study of parallel groups, 60 eligible patients with type 2 diabetes with subclinical DME were randomized to receive CaD (Calcium dobesilate 1000mg twice per day) or placebo for 1 year in a 1:1 ratio. The primary endpoint was to determine whether CaD is able to prevent the increase of retinal thickness (RT). The secondary endpoints were to assess whether CaD is able to: 1) arrest the progression from subclinical to clinically significant DME; 2) arrest the progression of the ETDRS level (≥ 1 step); 3) arrest the progression of neurodegenerative changes, 4) arrest the reduction of visual acuity, and 5) arrest the progression of foveal avascular zone (FAV).

Results: A total of 50 patients completed the 1 year follow-up. RT decreased by a mean of 2.5 μm (SD14) in patients treated with CaD, whereas in patients treated with placebo RT increased by 3.8 μm (SD24), but this difference did not reach statistical significance (p= 0.26). Regarding secondary endpoints the only difference was the effect of CaD on the progression of ETDRS. We found an improvement of ETDRS in 9 of patients under CaD in comparison with 3 in the placebo group (37.5% vs. 12%; p=0.05). In the logistic regression analyses adjusting by age, hypertension and the mean of HbA1C (four measurements), treatment with CaD was independently associated with an improvement of ETDRS level at the end of 1 year follow-up (P=0.03). We did not find any difference in adverse effects when comparing placebo and CaD groups.

Conclusion: In our study, the treatment with CaD did not have any significant impact on the progression of retinal thickness, at least after one year of follow up. However, it was a safe and useful treatment for early microvascular impairment in the setting of DR as shown by the improvement of ETDRS level. Further studies with a larger number of patients and longer follow-up seem warranted.

Clinical Trial Registration Number: 2017-000250-19
Supported by: OM-Pharma

Disclosure: **O. Simo-Servat:** Employment/Consultancy; Consultancy fees from OM-Pharma.

OP 07 When men are mice: the study of human physiology in humans

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Exogenous LEAP2 improves postprandial glucose tolerance and reduces ad libitum food intake in men

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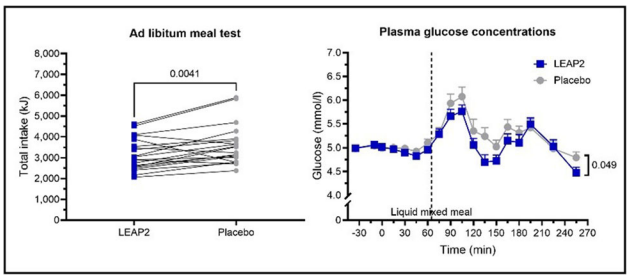
Background and aims: The gastric hormone ghrelin stimulates food intake and increases blood glucose through activation of the growth hormone secretagogue receptor (GHSR). Liver-expressed antimicrobial peptide 2 (LEAP2) has been proposed to inhibit actions of ghrelin through inverse effects on GHSR activity. In the present study, we investigated the effects of LEAP2 on postprandial glucose metabolism and *ad libitum* food intake in healthy volunteers.
Materials and methods: On two separate experimental days performed in randomised order, twenty healthy men (age 18-25 years, BMI 20-30 kg/m^2) underwent a liquid mixed meal test (3.0 ml/kg body weight; 1,010 kJ, 29.7 g carbohydrate, 9.6 g protein and 9.3 g fat per 100 ml) with double-blind i.v. infusions of LEAP2 (30 pmol/kg/min) and saline (placebo), respectively. After 260 minutes of infusion, an *ad libitum* meal of pasta Bolognese (565 kJ, 15.0 g carbohydrate, 5.3 g protein and 5.6 g fat per 100 ml) was served. Each experimental day was preceded by 12 hours of fasting including any liquids and 48 hours of abstinence from alcohol, strenuous physical activity, medicine and intermittent fasting and/or excessive eating.
Results: Compared to placebo, LEAP2 reduced postprandial peak plasma glucose (mean differences 0.36 (95% CI 0.13-0.60) mmol/l, $p = 0.0048$) and baseline-subtracted AUC for plasma glucose (mean differences 40.8 (95% CI 0.2-81.3) mmol/l × min, $p = 0.049$). LEAP2 significantly decreased *ad libitum* food intake assessed by total intake and intake per kg body weight, respectively, compared to placebo (mean differences 432 (95% CI 155-709) kJ, $p = 0.0041$; 5.4 (95% CI 2.1-8.7) kJ/kg, $p = 0.0029$). There was no statistically significant difference in visual analogue scale ratings of hunger, satiety, prospective food consumption, fullness, nausea, comfort or thirst.
Conclusion: Exogenous LEAP2 improves postprandial glucose tolerance during a standardised liquid mixed meal test and reduces food intake during an *ad libitum* meal test in healthy young men.



Clinical Trial Registration Number: NCT04621409
Supported by: Gubra Aps
Disclosure: **C.A. Hagemann:** None.

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Effect of a hypercaloric diet on brain insulin sensitivity and liver fat in normal-weight men

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Background and aims: Obesity and prediabetes are associated with brain insulin resistance with unfavorable effects on long-term weight and body fat composition. Whether short-term overfeeding can trigger brain insulin resistance in healthy humans is currently not known. Hence, we investigated the effect of a five-day hypercaloric diet on brain insulin responsiveness and body fat composition in healthy normal weight adults.
Materials and methods: Twenty-nine male volunteers (age range 19 - 27 years, BMI range 19 - 25 kg/m^2) were enrolled to participate either in a five-day hypercaloric diet (n= 18; increasing their daily caloric intake by 1500 kcal with high caloric snacks) or a control diet (n=11; no additional calories). Brain insulin sensitivity was assessed by functional magnetic resonance imaging (MRI) combined with intranasal administration of insulin to the brain before, directly after the hypercaloric diet and 2 weeks afterwards. Food intake and physical activity was recorded during the course of the study. Moreover, participants underwent two oral glucose tolerance tests, whole-body MRI for body fat distribution/ intrahepatic fat content and different behavioral and cognitive assessments.
Results: Paired t-test in the five-day hypercaloric diet group revealed a significant change in brain insulin action in the insular cortex from before to directly after the intervention ($p<0.05$, corrected for multiple comparisons). Two weeks after the diet, brain insulin action was restored. No change in brain insulin action was observed in the control group (n=11). Moreover, intrahepatic fat significantly increased in the intervention group directly after the five-day hypercaloric intervention (mean change of 53%; $p=0.001$), while no change was observed in the control group (mean change of 3%; $p=0.673$). High-caloric diet induced alterations in brain insulin responsiveness significantly correlated with increased liver fat ($r= 0.606$, $p=0.001$). No significant effects were observed on glucose metabolism, peripheral insulin sensitivity and body weight. To compare our results to a normal weight and obese control group without an intervention, we extracted the insular cortex response to intranasal insulin in twenty-nine age-matched participants from a previous study cohort. Interestingly, brain insulin action in the insula of the obese control group showed a similar response to intranasal insulin, as we observed in the current study after the high caloric diet. The normal weight control group, on the other hand, showed a response just as in our current study before the intervention.
Conclusion: In normal weight men, short-term overfeeding with high caloric snacks can trigger brain insulin resistance and liver fat accumulation. Brain insulin action after overfeeding was similar as observed in age-matched persons with obesity.
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Effect of lowering branched-chain amino acid levels in patients with type 2 diabetes using sodium-phenylbutyrate

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Background and aims: Patients with type 2 diabetes (T2D) are characterized by elevated branched-chain amino acid (BCAA) levels in plasma, which associate with insulin resistance. Recently, we showed that patients with T2D had reduced whole-body BCAA oxidation rates compared to controls, which may at least partly be explained by low mitochondrial BCAA oxidation. In the present study we aimed to pharmacologically accelerate BCAA oxidation in patients with T2D with use of sodium-phenylbutyrate (NaPB) and evaluated the effect on patients' metabolic health.
Materials and methods: Sixteen men and women with T2D (BMI: 29.6 ± 3.3 kg/m^2, age: 66 ± 6 y, 13m/3f) underwent a 2-week NaPB (4.8 g/m^2/day) treatment in a randomized, placebo-controlled, double-blind cross-over design with a wash-out period of 6-8 weeks. The primary outcome was peripheral insulin sensitivity measured with a 2-step hyperinsulinemic-euglycemic clamp, expressed as the change in insulin-stimulated glucose disposal rate minus baseline (ΔRd). Secondary outcomes were metabolic flexibility, defined as the change in insulin-stimulated respiratory exchange ratio minus baseline (ΔRER), and *ex vivo* mitochondrial oxidative capacity in skeletal muscle measured with high-resolution respirometry expressed as O_2-flux. Fasting blood samples were collected to determine levels of BCAA and glucose. In addition, phenylbutyrate levels were determined by LCMS to check compliance to the intervention.
Results: Plasma phenylbutyrate levels increased upon NaPB treatment compared to placebo (5.5-fold, p=2.08*10^-10) indicating compliance to the intervention. Fasting plasma BCAA levels (in μmol/l) decreased (valine 275.3 ± 10.3 vs. 304.7 ± 10.4, p=0.01; leucine 168.7 ± 5.1 vs. 181.5 ± 6.5, p=0.04; isoleucine 105.2 ± 3.5 vs. 112.1 ± 4.1, p=0.08) and fasting glucose levels tended to be lower (7.8 ± 0.4 vs. 8.2 ± 0.5 mmol/l, p=0.06) after 2 weeks of treatment with NaPB compared to placebo. Furthermore, peripheral insulin-sensitivity was 27% higher (ΔRd: 13.2 ± 1.8 vs. 9.6 ± 1.8 to μmol/kg/min, p=0.02) which appeared to be primarily accounted for by a higher peripheral insulin-mediated glucose oxidation (11.9 ± 0.7 vs. 10.8 ± μmol/kg/min, p=0.03) upon NaPB compared to placebo. In addition, metabolic flexibility tended to be higher after NaPB compared to placebo (ΔRER: 0.09 ± 0.01 vs. 0.08 ± 0.01, p=0.10). Also, maximally coupled mitochondrial respiration, fueled by the carbohydrate-derived substrate pyruvate was 10% higher after treatment with NaPB compared to placebo (O_2-flux: 74.0 ± 4.1 vs. 67.1 ± 4.3 pmol/(s*mg), p=0.05).
Conclusion: NaPB treatment for two weeks effectively reduced plasma BCAA levels in patients with T2D. This was paralleled by a higher peripheral insulin sensitivity and glucose oxidation. Our findings encourage future research to investigate the underlying mechanisms and whether stimulating BCAA oxidation could be a potential strategy to promote metabolic health in T2D.
Clinical Trial Registration Number: NTR7426
Supported by: Diabetes Fonds Nederland
Disclosure: **F. Vanweert:** None.

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Comparative effects of acute hypertriglyceridaemia with or without elevation of non-esterified fatty acids on glucose tolerance, insulin kinetics and beta cell function

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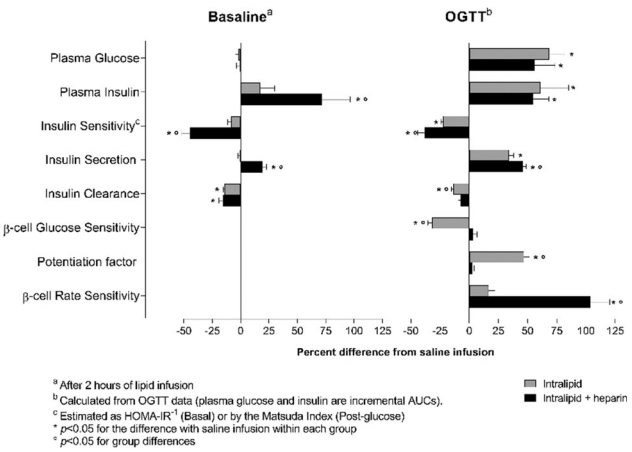
Background and aims: Chronic hypertriglyceridaemia is associated with glucose intolerance and type 2 diabetes risk in large population-based studies. Whether this association is directly mediated by triglycerides or their lipid metabolites (non-esterified fatty acids, or NEFAs), and which mechanisms are involved is unclear. Therefore, the aim of this study was to compare the glucometabolic effects of mild acute hypertriglyceridaemia alone or combined with elevation of circulating NEFAs in non-diabetic subjects.
Materials and methods: Twenty-two healthy lean volunteers underwent two 5-h intravenous infusions of either normal saline or Intralipid, without (n=12) or with heparin (I+H; n=10) to promote the release of NEFAs. Oral glucose tolerance tests (OGTTs) were performed during the last 3 h of infusion. Insulin sensitivity, insulin secretion, β-cell function parameters, and insulin clearance were analyzed by mathematical modeling of glucose, insulin, and C-peptide data at baseline (*i.e.* after 2 h of lipid infusion) and during the OGTTs.
Results: As for study design, plasma triglycerides reached similar levels during both lipid infusions (~2.5 mmol/L, p=0.40), while NEFAs were markedly higher during I+H (AUC 249 [130] vs 77 [20] mmol/L, p=0.0001). In fasting conditions, Intralipid alone reduced insulin clearance, while I+H increased insulin secretion and reduced insulin sensitivity and clearance, without significant changes in glucose levels. During the OGTT, the lipid infusions impaired glucose tolerance to a similar extent but via different mechanisms. Intralipid alone reduced insulin sensitivity and clearance, inhibited glucose-stimulated insulin secretion (*i.e.* β-cell glucose sensitivity), and stimulated β-cell potentiation. I+H induced a greater impairment in insulin sensitivity, enhanced the dynamic component of insulin secretion (i.e. β-cell rate sensitivity), and neutralized both the negative and positive effects of triglycerides on the β-cell.
Conclusion: This study dissected the pathogenetic mechanisms of glucose intolerance induced by mild acute hypertriglyceridaemia with or without a parallel increase in plasma NEFAs in non-diabetic individuals, providing new insight into plausible biological signals that generate and sustain insulin resistance and chronic hyperinsulinemia during diabetes progression.



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Disclosure: **D. Trico:** None.

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Pathophysiological changes during weight-loss induced remission of type 2 diabetes in non-obese people

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Background and aims: Classification of people according to BMI leads to the frequent assumption that weight loss is not indicated in non-obese individuals with type 2 diabetes. We have examined the pathophysiological changes during weight loss induced remission of type 2 diabetes in people with BMI <27kg/m^2.

Materials and methods: 25 people were studied to date, 2/25 were found to have MODY/ type 1 diabetes and were excluded [type 2 diabetes: n=12, age 58.3±7.2 years, BMI 24.5±2.0kg/m^2, diabetes duration 2.5±2.2years; non-diabetic controls: n=11, age 58.8±9.5 years, BMI 21.6±2.5kg/m^2]. Controls were age, sex and BMI-matched (after weight loss) to the type 2 diabetes group, and were studied on a single occasion. Dietary weight loss was achieved using a low-calorie diet (~800 kcal/day) followed by 4-6 weeks of weight stability. The cycle of weight loss plus maintenance was repeated 2-3 times (10-15% weight loss) until the clinical end point of remission was reached (HbA1c < 48mmol/mol). Intraorgan fat was quantified by 3-point Dixon magnetic resonance. Indices of insulin sensitivity (SI) and beta cell responsivity (Φ_{tot}) after a standard meal were estimated by mathematical modelling.

Results: After weight loss (Table 1), fasting plasma glucose and HbA1c decreased (7.3±0.3 to 6.3±0.3 mmol/l, and 53.8±2.1 to 48.0±1.5 mmol/mol, respectively, p<0.05 for both). Fasting plasma insulin normalised (52.2±9.5 to 23.7±3.6 pmol/L, p=0.007). Total body fat decreased (33.1±1.9 to 27.4±2.0%, p<0.001) as did plasma triglyceride (1.6±0.2 to 1.0±0.1mmol/L, p=0.002) with no changes in NEFA (0.67±0.04 to 0.68 ±0.07mmol/L, p=0.98). Liver fat at baseline was over twice that of controls (4.4±0.8 vs. 1.9±0.3%, p=0.02), and correlated negatively with HDL (r=-0.78, p=0.003). It decreased after intervention (1.4±0.1%, p=0.004 vs. baseline) becoming similar to the control levels. Pancreas fat fell (5.1± 0.6 to 4.5±0.6%, p=0.026) towards control levels (3.4±0.3%, p=0.13). Post meal SI, $\Phi_{tot,}$ and disposition index (DI) improved after weight loss. Weight loss induced remission (HbA1c <48mmol/mol off medication) was achieved in 67% (8/12) intervention participants.

Conclusion: 2/14 people diagnosed with type 2 diabetes in this BMI range were found to have other diagnoses. Remission and return of beta cell function with fall in liver and intrapancreatic fat content were observed in a similar proportion to previous studies in overweight or obese individuals with type 2 diabetes. Non-obese people with type 2 diabetes exhibit similar pathophysiological changes during remission of diabetes.

Table 1: Change in the main metabolic parameters after weight loss in comparison with controls

Parameters	Type 2 diabetes (n=12)				Controls (n=11)
	Baseline	Phase 1 (5% wt. loss)	Phase 2 (10% wt. loss)	Phase 3 (15% wt. loss)	
Weight (kg)	69.0±3.5	64.6±3.2***	61.8±3.0***	60.8±2.9***	62.2±3.4
Total body fat (%)	33.1±1.9	30.3±2.0***	27.8±1.9***	27.4±2.0***	25.4±1.6
Liver fat (%)	4.4±0.8	1.9±0.2***	1.5±0.1***	1.4±0.1***	1.9±0.3
Pancreas fat (%)	5.1±0.6	4.8±0.5	4.8±0.6	4.5±0.6*	3.4±0.3
Plasma TG (mmol/L)	1.6±0.2	1.4±0.1	1.0±0.1**	1.0±0.1**	0.9±0.1
HbA1c (mmol/mol)	53.8±2.1	49.8±1.5	49.2±1.6	48.0±1.5*###	37.2±1.0
Fasting insulin (pmol/L)	52.2±9.5	29.4±4.2*	26.8±3.2*	23.7±3.6**	21.4±3.0
Fasting glucose (mmol/L)	7.3±0.3	7.1±0.5	6.5±0.3*	6.3±0.3*###	4.8±0.1
NEFA (mmol/L)	0.67±0.04	0.57±0.05	0.56±0.04	0.68±0.07	0.63±0.04

Paired data were presented (Mean±SEM). *p<0.05 vs. baseline, **p<0.01 vs. baseline, ***p<0.001 vs. baseline. ###: p<0.001 vs. Controls

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Disclosure: **A. Al-Mrabeh:** None.

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The Single-Point Insulin Sensitivity Estimator (SPISE) in the prediction of abnormal glucose metabolism in obese children: a long term follow-up study

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Background and aims: Overweightand obesity in childhood are conditions epidemically spread worldwide; early onset obesity is an independent risk factor for the development of insulin resistance and type 2 diabetes, more than body mass index alone. The identification of accurate tools for risk stratification in obese/overweight children is a crucial step for designing strategies to prevent metabolic disease later in life. The value of insulin measurement and insulin-derived indicators for metabolic risk stratification in this young population is still debated, as their interpretation is not univocal. The Single-Point Insulin Sensitivity Estimator (SPISE) is a lipids and BMI-based index of insulin sensitivity recently validated in adolescents and adults. Aims of this study were to investigate the relationship between the SPISE index, glycometabolic profile and insulin sensitivity in a large population of children with and without obesity and to evaluate whether basal SPISE is predictive of the development of impaired glucose metabolism later in life.

Materials and methods: We analysed data from 909 overweight/obese children undergoing metabolic evaluations at University of Cagliari, Italy; SPISE (=600*HDL$^{0.185}$/Triglycerides$^{0.2}$*BMI$^{1.338}$), static and dynamic insulin-derived indicators of insulin sensitivity/resistance(i.e. ISI, Disposition Index (DI), HOMA-IR, HOMA-β%) were also assessed in 99 normal-weight, age-, sex-comparable children, selected as a reference group. Two-hundred out of 909 overweight/obese children were followed up between 2013 to 2016 (median [IQR] follow-up duration= 6.5[3.5-10] years), and data were used for longitudinal retrospective investigations.

Results: At baseline, 96 out of 909 (11%) overweight/obese children had impaired glucose regulation; in this subgroup, mean SPISE was significantly lower than in youths with normal glucose metabolism (mean±SD SPISE: 6.3 ±1.7 vs. 7±1.6, p<0.001). At the bivariate analysis, SPISE correlated positively with ISI and DI,and negatively with age, blood pressure, HOMA-IR, basal and 120 mins blood glucose and insulin (all p values <0.001). The presence of a significant association between SPISE and insulin-derived indexes of insulin-sensitivity, -resistance and -secretion,was also reported in normal-weight children. In this cohort, SPISE negatively correlated with age (r= -0.43, p<0.001) and FSI (r= -0.47, p<0.001) whereas no association with FBG was observed. At the 6.5-year follow-up, lower basal SPISE predicted the development of abnormal glucose metabolism with AUROC curve: 0.83(0.72-0.94), p<0.001, regardless of age, sex, fasting/120 mins glucose and insulin at the baseline.

Conclusion: Low SPISE in children is significantly associated with metabolic abnormalities and predicts the development of impaired glucose regulation later in life. In conclusion, our study shows that SPISE represents an easy, reliable and unexpensive surrogate of insulin sensitivity in overweight/obese children to be used as a screening tool for metabolic risk assessment on a large scale.
Disclosure: **S. Dule:** None.

OP 08 Bugs on fire

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Leukocyte counts and T cell frequencies differ between novel subgroups of diabetes

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Background and aims: Distributions of circulating immune cells are altered in type 1 and type 2 diabetes compared to healthy individuals and associate with insulin sensitivity, glycemic control and circulating lipid concentrations. This study aimed to determine whether total leukocyte counts and immune cell frequencies differ among the novel diabetes subgroups.
Materials and methods: We analyzed automated white blood cell counts (n=669) and flow cytometry data (n=201) of participants with recent-onset diabetes (known diabetes duration <1 year) from the German Diabetes Study, who were allocated to the five novel diabetes subgroups (severe autoimmune diabetes (SAID), severe insulin-deficient diabetes (SIDD), severe insulin-resistant diabetes (SIRD), mild obesity-related diabetes (MOD), mild age-related diabetes (MARD)) according to clustering analysis based on clinical variables (age, body mass index, HbA1c, homeostasis model assessment-2 estimates of β-cell function and insulin resistance (HOMA2-B, HOMA2-IR), and glutamic acid decarboxylase antibodies). Pairwise differences between subgroups were analyzed with linear regression models after log-transformation of variables, followed by Tukey-Kramer correction for multiple testing.
Results: Leukocyte numbers were higher in SIRD (median [IQR]; 7100 [6425; 8300] /μL), MOD (7200 [6225; 8400] /μL) and MARD (6500 [5500; 7675] /μL), but not SIDD compared to SAID (5700 [4800; 6900] /μL) and higher in SIRD and MOD vs. MARD (all P< 0.05). The percentage contribution of CD4+ T cells to the total T cell count was higher in SIRD (68.4 [64.0; 76.0] %) than in SAID (58.0 [52.7; 66.2] %), MOD (60.0 [55.5; 68.2] %) and MARD (63.8 [54.8,72.1]). The percentage of CCR4+ regulatory T cells of total regulatory T cells was higher in SIRD (74.8 [72.2; 78.6] %) vs. SAID (63.9 [55.0; 74.5] %) and MOD (67.2 [62.8; 77.3] %) and in MARD (77.1 [68.7, 83.0] %) vs. SAID (all P<0.05). After adjusting for the variables used to define the subgroups, none of these differences remained significant (P>0.05). Frequencies of CD4+ T cells were positively associated with age, BMI, HOMA2-B und HOMA2-IR, and frequencies of CCR4+ regulatory T cells with age, HOMA2-B and HOMA2-IR.
Conclusion: These results show differences in leukocyte profiles between novel subgroups of diabetes. Higher percentages of CD4+ T cells of total T cells in SIRD vs. MOD and MARD and higher percentages of CCR4+ regulatory T cells of total regulatory T cells in SIRD vs. MOD suggest the presence of distinct inflammatory processes in SIRD, MOD and MARD subgroups.
Clinical Trial Registration Number: NCT01055093
Disclosure: **J.M. Ratter:** None.

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Visceral adipose tissue-derived PD1-1+ Tconv from obese patients with type 2 diabetes are pro-inflammatory cells with the potential to recirculate to the blood stream

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Background and aims: Visceral adipose tissue (VAT) in obesity is featured by chronic low-grade inflammation leading to resistance to insulin action (i.e. insulin resistance). It is yet to be explored, however, why only a fraction of obese patients fails to control glucose metabolism resulting in the development of type 2 diabetes (T2D). Growing data from preclinical models of obesity indicate that, in the cascade of events leading to T2D, T cells are engaged early and precede macrophage recruitment in the VAT. Furthermore, we have shown that CD4 T cells from the VAT of obese patients with T2D display a pro-inflammatory phenotype and are resistant to TGF-β-mediated suppression. Here we aimed to assess profile and spatial dynamics of T cells localized in the VAT of obese patients with T2D.
Materials and methods: Characterization of T cell subsets in the stromal vascular fraction (SVF) obtained from the VAT of obese patients with prediabetes or T2D (OB-PreD&D) (n=21) and without T2D (OB-ND) (n=27) undergoing bariatric surgery was performed using unsupervised and supervised analysis of flow cytometry data. Whole transcriptome sequencing and T cell receptor (TCR) sequencing of VAT-derived PD-1-expressing CD4 conventional T cells (Tconv) was performed in comparison with the PD-1- counterpart (OB-PreD&D n=4, OB-ND n=4). For TCR sequencing a control group of lean donors (n=4) and peripheral blood (PB) samples were also included.
Results: Unsupervised and supervised analysis of flow cytometry data showed that a subset of resident CD4 Tconv cells expressing PD-1 is reduced in the VAT of OB-PreD&D compared to OB-ND (p-value=0.03). Increased frequency of TNF-α-producing PD-1+, but not PD-1-, CD4 Tconv were evident in OB-PreD&D (p-value=0.04). In line with this, transcriptomic analysis showed selective upregulation of pathways involved in inflammatory processes in PD-1+ CD4 Tconv from OB-PreD&D (adj p-value: 5×10^{-09}). Analysis of TCR sequencing indicates that clonotypes of PD1+ CD4 T cells from OB-PreD&D tend to be enriched in the peripheral blood compared to the VAT and that their clonality with the level of insulin resistance (p-value=0.04, R^2=0,5243).
Conclusion: These data indicate that impairment of glucose metabolism fosters the generation of pro-inflammatory PD1+ CD4 Tconv in the VAT with high potential to recirculate between blood and tissue(s). The PD1+ CD4 Tconv cell subset has the potential to be the mediator of "infectious" inflammation driving insulin resistance in insulin-sensitive tissues.
Supported by: Fit-The-Fat Marie Sklodowska-Curie grant agreement no 704779
Disclosure: **A. Giovenzana:** None.

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Plasma lipopolysaccharide levels and its relationship with glycaemic status and gut microbiota changes associated with H. pylori infection

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Background and aims: Apart from causing gastric disturbances, *Helicobacter pylori (H. pylori)* infection has been related to a number of extragastric diseases including diabetes and obesity. Notably, gut microbiota composition is affected by both *H. pylori* infection and *H. pylori* antibiotic-based eradication therapy (ET). Considering that gut microbiota dysbiosis has been widely related to metabolic dysregulation, modulation of the gut bacterial community emerges as a linking factor between *H. pylori* infection, antibiotic treatment and metabolic diseases. Indeed, our group has previously shown that changes in glucose homeostasis after *H. pylori* ET are related to gut microbiota. However, mechanisms underlying this relationship remains unexplored. Plasma gut-derived lipopolysaccharides (LPS) promote low-grade inflammation and metabolic diseases. The aim of this study was to analyse plasma LPS as the possible mediating factor between gut microbiota and glycemic changes related to *H. pylori* and its ET.
Materials and methods: 40 *H. pylori*-infected adult patients and 20 controls without infection were recruited. Infected subjects were evaluated before and two months after ET (omeprazole, clarithromycin, amoxicillin). Faecal microbiota composition, determined by 16S rRNA gene (V3-V4) sequencing using Illumina Miseq, glycemic status [glucose, glycosylated haemoglobin (HbA1c), HOMA-IR, GLP-1] and plasma LPS levels were analysed.
Results: Plasma LPS levels correlated positively and significantly with HbA1c patients (r=0.457; p=0.004) and GLP-1 (r=0.502; p=0.002) in *H. pylori*-infected patients. However, these correlations were not found after ET treatment or in controls. In addition, plasma LPS positively correlated with the genera *Roseburia* (r=0.339; p=0.037), *Megamonas* (r=-0.323; p=0.048), *Megasphaera* (r=0.446; p=0.05) and *Sutterella* (r=0.323; p=0.048) in *H. pylori*-infected patients. However, after ET correlations between LPS and these bacterial groups were abolished, except for *Megamonas* (r=-0.409; p=0.013).
Conclusion: Plasma LPS levels are related to glycemic status during *H. pylori* infection, but not after its eradication. Specific gut bacterial groups could be influencing plasma LPS levels in *H. pylori*-infected patients.
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Disclosure: **M. Clemente-Postigo:** None.

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TNFA mediates inflammation-induced effects on PPARG splicing in adipose tissue and mesenchymal precursor cells

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Background and aims: Low-grade chronic inflammation and reduced adipocyte differentiation capacity are hallmarks of hypertrophic adipose tissue (AT). These alterations correlate with low activity of PPARγ, the master adipogenic regulator. We recently identified the dominant-negative isoform PPARγΔ5 overexpressed in adipose tissue (AT) of obese/diabetic patients, able to impair PPARγ activity in hypertrophic adipocytes and differentiation capacity of adipocyte precursors. Our study investigates how inflammatory *stimuli* - abundant in hypertrophic AT - act on *PPARG* splicing, whose alteration could contribute to impaired neoadipogenesis and insulin resistance in obese individuals with increased risk to develop type 2 diabetes (T2D).
Materials and methods: Epididymal AT was isolated from LPS-injected C57BL/6J mice. Murine J774A.1 and human THP 1-derived macrophages (MΦ) were stimulated with LPS. Primary human monocytes were differentiated in LPS/IFN or IL-10 induced MΦ. Murine 3T3-L1 cells and human hTERT-immortalized adipose tissue-derived MSCs were treated with conditioned media of MΦ and recombinant cytokines. RNA from subcutaneous AT (SAT) biopsies were obtained from lean (BMI≤25 kg/m2; n= 14) and obese (BMI≥30 kg/m2; n= 24) individuals. Gene expression was analyzed by qPCR and P val by paired Student's t test.
Results: The expression analysis of canonical (cPparg) and dominant (PpargΔ5) Pparg transcripts in inflamed AT of LPS-treated mice revealed a marked repression of cPparg and 3.5-fold increase of PpargΔ5/cPparg *ratio* (n=6; p val<0.01), paralleled by reduced expression of *Pparg*-regulated genes (*Adipoq*, *Slc2a4* and *Cd36*). As high PPARγΔ5 levels impair adipogenic capacity of precursor cells and it is hallmark of hypertrophic obesity, we evaluated the effects of inflammatory factors on differentiation capacity of 3T3-L1 cells and on the expression and splicing pattern of *Pparg*. Interestingly, LPS-activated MΦ secretome impairs adipocyte differentiation markedly increasing PpargΔ5/cPparg *ratio* and impairing *Adipoq*, *Slc2a4 and Cd36* expression. Similar effects on *PPARG* expression and splicing alteration were observed in human MSCs exposed to conditioned media of pro-inflammatory MΦ. We identified TNFα as one of the pro-inflammatory factor able to increase PPARGΔ5/cPPARG *ratio* (2.5-fold; p val<0.01). This mechanism involves SRp40 phosphorylation through the PI3K/Akt signaling activation. According to *in vitro* data, *TNFA* is over-expressed in the SAT of obese (*vs* lean) patients and positively correlates with PPARGΔ5 levels (*r*=0.45, p val=0.027) only in obese patients and not in lean individuals.
Conclusion: Our study reveals for the first time the modulation of *PPARG* splicing in adipose precursor cells by pro-inflammatory *stimuli*. The alteration of the balance between canonical and dominant negative PPARG isoforms may contribute to impair PPARγ activity in hypertrophic AT. This process exacerbates the defective adipogenic capacity of precursor cells, hallmark of insulin-resistant adipose tissue driving T2D onset and progression.
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Disclosure: **S. Cataldi:** None.

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Empagliflozin-induced gut microbiota alternation reduces obesity in high-fat diet-fed mice

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Background and aims: Obesity has become a global epidemic, which can lead to many chronic complications. Empagliflozin,known as Sodium-glucose co-transporter-2 (SGLT2) inhibitors with putative anti-

obesity effects, but its anti-obesity mechanisms remain unclear. Gut microbiota plays a crucial role in obesity; however, whether empagliflozin is involved in gut microbiota alternation and improves obesity remains elusive. Here, we sought to determine the anti-obesity effects of Empagliflozin are related to modulations in the gut microbiota and metabolic functions.

Materials and methods: Six-week-old C57BL/6 mice were fed with a normal chow diet or an HFD for 12 weeks and then were treated with or without empagliflozin (10mg/kg) for another 8 weeks. Intestinal substance was taken as samples to estimate alternation of gut microbiota. DNA of intestinal substance was isolated, and whole-genome shotgun libraries were prepared and sequenced. The composition of the gut microbiota and related pathways were assessed by analyzing metagenomic sequences.

Results: HFD mice showed significantly increased body weight, fat mass, decreased glucose tolerance and insulin sensitivity (body weight, 45.4 ± 1.3 g vs. 29.0 ± 1.0 g; fat, 12.6 ± 1.9 g vs. 2.6 ± 1.3 g; $P < 0.05$), and with lower species diversity of the gut microbiota and down-regulated lipid metabolism. Treatment of HFD mice with empagliflozin reduced body weight, fat mass, improved glucose tolerance and insulin sensitivity (body weight, 40 ± 0.9 g vs. 45.4 ± 1.3 g; fat, 8.5 ± 1.5 g vs. 12.6 ± 1.9 g; $P < 0.05$). Compared with high-fat diet mice, gut microbiota of mice treated with empagliflozin has a higher species diversity and a lower evenness degree. Mice treated with empagliflozin show a remarkable alteration in microbiota composition compared with that of high-fat diet mice. This alteration is characterized by an enrichment of genus *Turicimonas*, *Cronobacter*, *Methanobrevibacter_A*, *Lawsonella* and *Parasutterella*. We further explored whether altered-microbiota has anti-obesity functions. We found these microbes could reduce sphingolipid metabolism, glycosphingolipid biosynthesis-globo and isoglobo series, galactose metabolism, glycerolipid metabolism, flavone and flavonol biosynthesis. These functions contributed to modulate lipid metabolism, reduce inflammation and improve intestinal barrier function.

Conclusion: Our study demonstrates that empagliflozin-induced microbiota plays a crucial role in controlling obesity development and brings new insights to a potential therapy based on host-microbe interactions.

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Disclosure: **J. Shi:** None.

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Heat shock 70 kDa protein 4 declines after bariatric surgery in association with markers of inflammation and glycaemia

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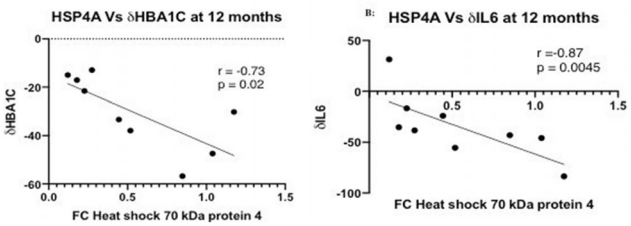
Background and aims: Bariatric surgery (BS) can have significant effects on multiple body systems. Heat shock 70 kDa protein 4 (HSPA4) functions as a cytosolic chaperone involved in facilitating protein folding, degradation, complex assembly, and translocation. Elevation of HSPA4 is associated with length of disease in type 2 diabetes mellitus (T2DM). HSPA4 has also been associated with inhibition of nitric oxide production in individuals with T2DM, correlating with CRP. Blood levels of HSPA4 correlate with the degree of microalbuminuria in T2DM.Aim: Identification of plasma proteomic biomarkers of inflammatory change in individuals achieving remission from T2DM following BS.

Materials and methods: Longitudinal plasma samples from 10 individuals who achieved remission of T2DM following Roux-en-Y gastric bypass (n=7) or Sleeve gastrectomy (n=3) were analysed. SWATH MS proteomics was performed on plasma samples taken at 4 months before, and 6 and 12 months after BS and concurrent analyses of a panel of inflammatory/metabolic parameters carried out. Change in absolute abundances of those proteins showing significant change at both 6 and 12 months was correlated with change in anthropometric and other parameters including body mass index (BMI), CRP, IL6, glycosylated haemoglobin (HbA1c) and fasting insulin.

Results: BMI ($p<0.001$) and HbA1C ($p<0.001$) declined significantly at 6 and 12 months. IL6, CRP HOMA -IR, HOMA-B also declined. Four proteins related to inflammation were identified that showed consistent change at 6 and 12 months post-BS; three showed negative fold changes (FC): HSPA4 (Figure) (6M FC -0.5, p = 0.026 and 12M FC = -0.39, p = 0.022), Proteoglycan 4 (6M FC -0.89, p = 0.011 and 12M FC = -0.78, p = 0.014), Serotransferrin (6M FC -1.06, $p < 0.001$ and 12M FC = -0.77, p = 0.002) and one showed positive FC: Sex hormone binding globulin (SHBG) (6M FC 1.48, p = 0.012 and 12M FC = 1.95, p = 0.003). Analysis at 12 months revealed HSPA4 to correlate inversely with IL6 (r =-0.87, p = 0.0045) (Figure), SHBG (r = -0.73, p = 0.04) and HBA1C (r = -0.73, p = 0.02)(Figure).

Conclusion: We have demonstrated a significant reduction in HSPA4 which correlated with change in IL6 levels between baseline and 12 months, and change in HbA1C. This is in keeping with previous work demonstrating reduction in both serum and liver HSPA4 in a murine model after BS, and accords with the narrative that HSPA4 appears to be induced by states of chronic inflammation such as T2DM. Using SWATH MS we have identified HSPA4 as a key protein implicated in inflammatory pathway changes, potentially involved in remission from T2DM after BS.

Figure



Disclosure: **H. Fachim:** None.

OP 09 SGLT2 inhibitor trials

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Safety and efficacy of SGLT2-inhibitors in over 70 years old type 2 diabetic patients: 1 year of follow up

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Background and aims: Sodium-glucose co-transporter-2 inhibitors (SGLT2i) may have important benefits for older people, however some safety concerns still limit the use of this treatment in older patients. This is a multicenter study, aimed to analyze efficacy and safety of SGLT2i in elderly in a real-life setting.
Materials and methods: We analyzed 450 adults (mean age 74,7±3,9 years, F/M 183/267) with T2D, that started SGLT2i-based treatment after the age of 70, with at least one year of follow-up. Patients were evaluated at baseline and after 6 and 12 month of SGLT2i treatment. Glycometabolic and antropometric parameters, adverse events and causes of drug discontinuation were recorded.
Results: 450 T2D aged≥70 years started SGLT2i (47.6% Empagliflozin, 41.6% Dapagliflozin, 9.1% Canagliflozin, 1.7% Ertugliflozin) as add-on therapy to Metformin in 79.6% of subjects, insulin treatment in 33.1% and other antidiabetic drugs in 16% (i.e. 38.9% sulfonylureas, 33.3% DPP-IV inhibitors, 16.7% pioglitazone, 8.3% GLP-1RA and 6.9% acarbose). At baseline arterial hypertension affected 88.1% of patients, while cardiovascular events occurred in 38.5% of subjects. The analysis of glucose metabolic parameters during follow-up, showed a statistically significant reduction of HbA1c and FPG during follow-up (*HbA1c*: 7.7±1.15 vs 7.4±1.0 vs 7.3±0.9%, p=0.001; *FPG*: 158±48 vs 146±41.3 vs 140±36.5%, p=0.001), while BMI reduced in non statistically significant manner (29,9±5,3 vs 29,2±5,2 vs 29,5±5,1 Kg/m2, p=0.17). Overall, eGFR values remains stable over time (74,5±15 vs 72.2±16 vs 72.4±16,1 ml/min/1,73m2, p=0.314). We then analyzed the subgroup of patients with eGFR decline over 20% (12,4%). At baseline they were characterized by higher percentage of cardiovascular events history (58,6% vs 32,5%, p=0,006), higher U-Albumin values (110,7±153,3 vs 43,9±78,7 mg/L, p=0,036) and higher serum creatinine values (0,95±0,15 vs 0,9±0,2, p=0,009) while age, BMI and HbA1c values were comparable. SGLT2i discontinuation occured in 7,4% of subjects after 6 months and in 7% after 12 months. Main reasons of discontinuation were intolerance in the 60,7% and UTI in the 25.5%. We recorded no cases of diabetic ketoacidosis or amputation. Cardiovascular adverse events during follow-up occured in a small proportion of patients: in the 1,4% of patients during first 6 months and 2,2% between 6 and 12 months of follow-up.
Conclusion: The SGLT2-inhibitor class is well-tolerated and safe in elderly though some caution is also suggested, expecially in frailer subjects.
Disclosure: **M.E. Lunati:** None.

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Cardiorenal outcomes with ertugliflozin by baseline cardiorenal medications: an analysis from VERTIS CV

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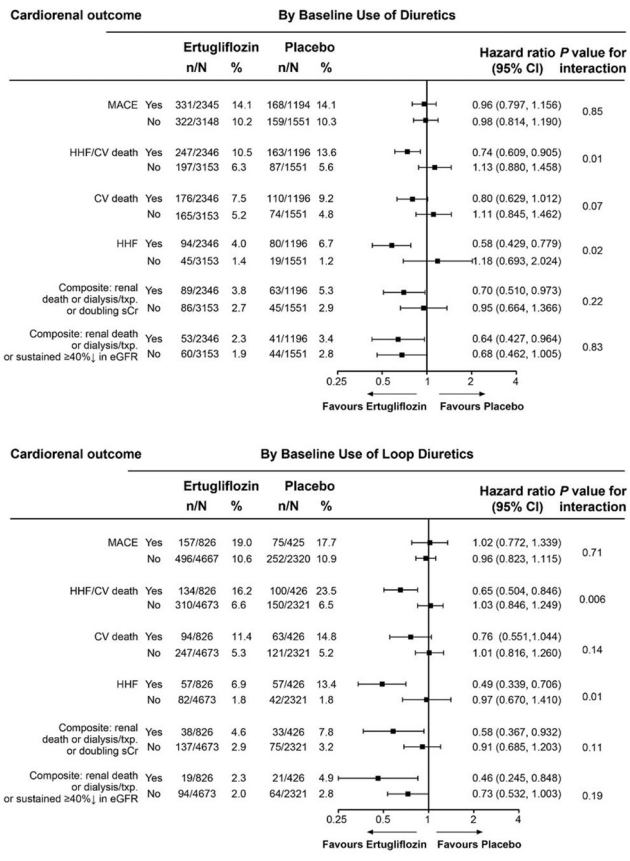
Background and aims: VERTIS CV was a placebo-controlled cardiovascular (CV) outcome trial evaluating the sodium-glucose cotransporter 2 inhibitor, ertugliflozin. The aim of the current analyses was to evaluate cardiorenal endpoints by baseline use of CV medications.
Materials and methods: A Cox proportional hazard assessment was used to analyse pre-specified CV and kidney outcomes according to use of renin-angiotensin-aldosterone system inhibitors (RAASi), diuretics and mineralocorticoid receptor antagonists (MRA) at baseline.
Results: Among 8246 randomised patients, at baseline 6686 (81%) were treated with RAASi, 3542 (43%) with diuretics, including 1252 (15%) with loop diuretics, and 674 (8%) with MRA. No significant interactions were observed for cardiorenal outcomes by baseline use of RAASi or MRA ($P_{interaction}$ >0.05 for all). Nominally significant interactions for first event of hospitalisation for heart failure (HHF) and HHF/CV death were observed with baseline use of diuretics, including loop diuretics, with an increased benefit of ertugliflozin vs placebo (Figure).
Conclusion: In VERTIS CV, baseline use of diuretics, including loop diuretics, appeared to be associated with greater benefit of ertugliflozin on first HHF and HHF/CV death, with no modification of effect based on baseline use of RAASi or MRA.

FIGURE. Cardiorenal outcomes with ertugliflozin vs placebo by baseline use of diuretics and loop diuretics



The analysis of the MACE outcome was performed using all patients who had received ≥1 dose of ertugliflozin or placebo and included events that occurred up to 365 days after the confirmed last dose. The analyses of other outcomes were performed on an intention-to-treat basis using all patients and all time on-study for each patient. For all analyses, pooled ertugliflozin (5 mg and 15 mg doses) was compared with placebo.

CI, confidence interval; CV, cardiovascular; HHF, hospitalisation for heart failure; MACE, major adverse cardiovascular events (the composite outcome of death from cardiovascular causes, nonfatal myocardial infarction or nonfatal stroke); sCr, serum creatinine; txp, transplantation.

Clinical Trial Registration Number: NCT01986881
Supported by: Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA, and Pfizer Inc., New York, NY, USA
Disclosure: **C.P. Cannon:** Grants; Amgen, Boehringer Ingelheim, Bristol-Myers Squibb, Daiichi Sankyo, Janssen, Merck, and Pfizer. Lecture/other fees; Aegerion, Alnylam, Amarin, Amgen, Applied Therapeutics, Ascendia, Boehringer Ingelheim, Bristol-Myers Squibb, Corvidia, Eli Lilly, HLS Therapeutics, Innovent, Janssen, Kowa, Merck, Pfizer, Rhoshan and Sanofi.

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Efficacy and safety of dapagliflozin on kidney and cardiovascular outcomes by baseline albuminuria: a secondary analysis of the DAPA-CKD trial

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Background and aims: Dapagliflozin reduced the risk of kidney failure and heart failure hospitalisations (HHF) in chronic kidney disease (CKD) patients, with or without type 2 diabetes (T2D), in the DAPA-CKD trial. Patients with CKD with higher levels of albuminuria are at higher risk of kidney failure and HHF. We assessed dapagliflozin according to baseline albuminuria.
Materials and methods: DAPA-CKD was a randomised, placebo-controlled trial. Adult patients with CKD (urinary albumin-to-creatinine ratio [UACR] 200-5000 mg/g; estimated glomerular filtration rate [eGFR] 25-75 mL/min/1.73m^2) were randomised to dapagliflozin 10 mg/d or placebo. Primary outcome was a composite of ≥50% reduction in eGFR, end-stage kidney disease (ESKD) and kidney or cardiovascular death. Secondary outcomes were the kidney outcome (≥50% reduction in eGFR, ESKD and kidney death), HHF and cardiovascular death and death from all causes. We used Cox proportional hazards regression analyses to assess relative and absolute effects of dapagliflozin across subgroups of UACR (≤1000, >1000 to ≤3500, >3500 mg/g).
Results: At randomisation, 2225 (51.7%), 1764 (41.0%) and 315 (7.3%) patients had an UACR of ≤1000, >1000 to ≤3500, >3500 mg/g, respectively. Dapagliflozin reduced relative risk of the primary and secondary outcomes consistently across subgroups of UACR (all P-interaction >0.59; primary outcome shown in Table). Absolute risk reductions for the primary outcome were greater in subgroups with higher UACR: the absolute risk reductions across ascending UACR categories were 3.5% (95%CI 1.6 to 5.4), 6.9% (95%CI 3.6, 10.1) and 13.8% (95%CI 3.0, 24.5; P-interaction 0.02). When patients with and without T2D were analysed separately, we observed that relative risk reduction with dapagliflozin on the primary outcome was consistent across UACR subgroups (P-interaction >0.43, Table). Proportion of patients with adverse events leading to study drug discontinuation and proportion of patients with serious adverse events were similar between the groups; this was consistent across UACR subgroups.
Conclusion: Efficacy and safety of dapagliflozin on kidney and cardiovascular outcomes was consistent across subgroups of baseline UACR. These results indicate dapagliflozin is effective and safe across a wide range of baseline UACR levels in patients with CKD with and without T2D.

Table: The relative effect of dapagliflozin on the primary outcome according to baseline UACR categories in the overall population and in patients with and without type 2 diabetes.

	Overall	With type 2 diabetes	Without type 2 diabetes
Overall	0.61 (0.51, 0.72)	0.64 (0.52, 0.79)	0.50 (0.35, 0.72)
UACR ≤1000 mg/g	0.54 (0.37, 0.77)	0.59 (0.38, 0.91)	0.43 (0.22, 0.85)
UACR >1000 to ≤3500 mg/g	0.59 (0.46, 0.76)	0.58 (0.43, 0.77)	0.60 (0.37, 0.97)
UACR >3500 mg/g	0.61 (0.43, 0.87)	0.67 (0.46, 0.99)	0.30 (0.10, 0.88)
P-interaction	0.93	0.90	0.43

UACR, urinary albumin-to-creatinine ratio

Clinical Trial Registration Number: NCT03036150
Supported by: Support received from AstraZeneca
Disclosure: **H.J. Lambers Heerspink:** Employment/Consultancy; AstraZeneca. Grants; AstraZeneca. Non-financial support; AstraZeneca.

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Ertugliflozin in older patients with type 2 diabetes: an analysis from VERTIS CV

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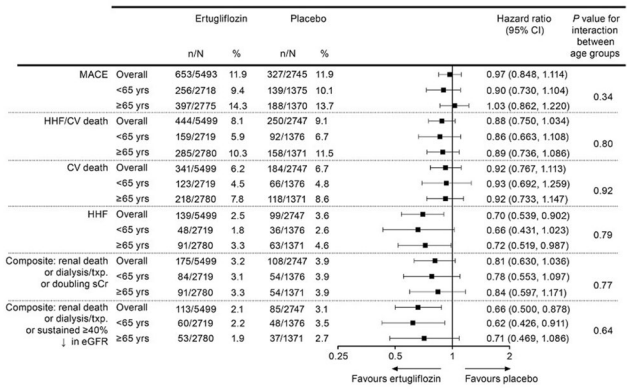
Background and aims: VERTIS CV was a cardiovascular (CV) outcome trial of ertugliflozin vs. placebo in patients (pts) with T2D and atherosclerotic CV disease.
Materials and methods: This analysis assessed the cardiorenal outcomes by Cox proportional hazard assessment in older vs. younger patients (<65 yrs and ≥65 yrs). The safety of ertugliflozin in older vs. younger patients was summarised descriptively.
Results: Among 8238 pts randomised and treated with ertugliflozin 5 mg, 15 mg, or placebo, 4093 (49.7%) were <65 yrs, 4145 (50.3%) were ≥65 yrs, with 903 (11.0%) ≥75 yrs. Baseline characteristics were similar across treatment groups within each age group; mean eGFR was lower in older pts (82.2, 69.9, and 63.6 mL/min/1.73m^2 for pts <65, ≥65, and ≥75 yrs, respectively). Cardiorenal outcomes for ertugliflozin vs. placebo did not differ between pts ≥65 and <65 yrs, with interaction *P* values >0.05 for all outcomes assessed (Figure). The incidences of adverse events (AEs), serious AEs (including deaths), and discontinuations due to an AE were higher in older vs. younger pts, but generally similar across treatment groups in pts ≥65 and ≥75 yrs. The incidence of renal-related AEs was similar across treatment groups in younger and older pts. The incidences of genital mycotic infection and urinary tract infection were higher with ertugliflozin vs. placebo in older pts. The incidences of hypovolemia AEs with ertugliflozin vs. placebo in pts ≥65 was 5.3% vs. 4.5% and in pts ≥75 yrs was 6.9% vs. 5.8%.
Conclusion: Cardiorenal outcomes were similar in pts ≥65 and <65 yrs. Ertugliflozin was generally well tolerated in pts ≥65 and ≥75 yrs.

FIGURE. Cardiorenal outcomes with ertugliflozin vs. placebo by age



The analysis of the MACE outcome was performed using all patients who had received ≥1 dose of ertugliflozin or placebo and included events that occurred up to 365 days after the confirmed last dose. The analyses of other outcomes were performed on an intention-to-treat basis using all patients and all time on-study for each patient. For all analyses, pooled ertugliflozin (5 mg and 15 mg doses) was compared with placebo.
CV, cardiovascular; eGFR, estimated glomerular filtration rate; HHF, hospitalization for heart failure; yrs, years; MACE, major adverse cardiovascular events (a composite of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke); sCr; serum creatinine; txp, transplantation.

Clinical Trial Registration Number: NCT01986881
Supported by: Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA, and Pfizer Inc., New York, NY, USA.
Disclosure: **R.E. Pratley:** Employment/Consultancy; AstraZeneca, Glytec, Janssen, Merck & Co. Inc., Mundipharma, Novo Nordisk, Pfizer, Sanofi, Sanofi US Services, Inc. Scohia Pharma Inc., and Sun Pharmaceutical Industries. Grants; Hanmi Pharmaceutical Co., Ltd, Janssen, Lexicon Pharmaceuticals, Inc., Novo Nordisk, Poxel SA, Sanofi. Lecture/other fees; Novo Nordisk.

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Empagliflozin versus dapagliflozin for type 2 diabetes in combination with metformin, dipeptidyl peptidase-4 inhibitor and sulfonylurea: 3-year prospective study

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Background and aims: Sodium glucose cotransporter 2 (SGLT2) inhibitor is a relatively new type of antidiabetic medication that has beneficial effects on glycemic control, cardiometabolic aspects, and has been increasingly used by proving not only cardiovascular outcome but also renal protection effect in recent years. However, there was no long-term efficacy and safety study for quadruple combination therapy with empagliflozin and dapagliflozin. Therefore, we aimed to evaluate the efficacy and safety of 3-year follow-up of empagliflozin and dapagliflozin as add-on in patients with type 2 diabetes mellitus (T2DM) inadequately controlled despite triple oral antidiabetic agents (OADs).
Materials and methods: A single center, 3-year, open-label, prospective observational study was started between March 2015 and March 2018. Eligible patients were T2DM aged 19years and older, had suboptimal glycemic control (HbA1c 7.5-12.0%) despite maximum tolerated doses of metformin, dipeptidyl-peptidase 4 inhibitor, and glimepiride with stable dosage for at least 3 months. Patients were treated adding empagliflozin (25mg/day) or dapagliflozin (10mg/day) as a fourth OAD to the existing triple combination therapy at the physician's discretion. Efficacy outcomes included the change in HbA1c, fasting plasma glucose (FPG), and other cardiometabolic variables at 3 years. Safety outcomes included adverse events (AEs), hypoglycemia, volume depletion, nocturia, genitourinary tract infections (GUTIs) and laboratory tests. All procedures were conducted in accordance with the Helsinki Declaration and the International Conference of Harmonization/Good Clinical Practice guidelines.
Results: A total of 362 patients were enrolled with empagliflozin (25 mg/day, n=185) and dapagliflozin (10 mg/day, n=177), respectively. At 3 years, the mean HbA1c reduction was -1.7% (SD 1.1) in the empagliflozin group versus -1.1% (1.3) in the dapagliflozin group (P=0.001). FPG also showed a similar trend (-59.3 ± 53.3 mg/dL vs -47.4 ± 61.7 mg/dL, empagliflozin and dapagliflozin, respectively, P=0.055). In terms of body weight, empagliflozin showed significantly greater reduction (-4.5 ± 2.5 kg vs. -1.0 ± 3.3 kg, P=0.024). Although the proportion of patients reporting total and serious adverse events was similar, volume depletion was reported higher in the dapagliflozin group and asymptomatic pyuria or bacteriuria was reported higher in the empagliflozin group.
Conclusion: The efficacy of addition of SGLT2 inhibitors as a quadruple combination therapy has been sustained for 3 years. In the respects of blood glucose control, empagliflozin (25 mg/day) was stronger than dapagliflozin (10 mg/day).
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Supported by: EJK/2021 Research grant from the Chungcheong Society of the Korean Society of Endocrinology
Disclosure: **E. Ku:** None.

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Effects of empagliflozin on uric acid levels and gout: observations from the EMPA-REG OUTCOME trial

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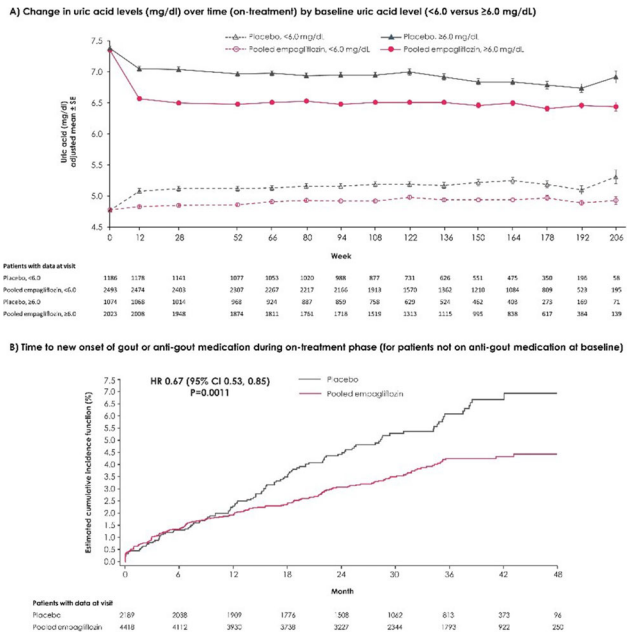
Background and aims: Higher serum uric acid (UA) levels are associated with gout and other adverse health outcomes in patients with type 2 diabetes (T2D). Sodium glucose co-transporter 2 inhibitors (SGLT2i) lower UA. Moreover, in the CANVAS program, the SGLT2i canagliflozin reduced gout flares in patients with T2D. Whether empagliflozin also positively impacts new-onset of gout or anti-gout medication in T2D patients has not been fully evaluated. We studied the effect of empagliflozin on UA levels, incident prescription of an anti-gout/UA-lowering medication or an episode of gout as an adverse event in the EMPA-REG OUTCOME trial.
Materials and methods: In EMPA-REG OUTCOME, 7,020 patients with T2D at high CV risk were treated with either empagliflozin (pooled n=4,687; 10 mg/day n=2,345; 25 mg/day n=2,342) or placebo (n=2,333). We assessed the effects of empagliflozin vs. placebo on serum UA levels using MMRM analyses and Cox proportional hazards models for the time-to-first occurrence of either an adverse event attributed to gout flare or initiation of a drug for the treatment of hyperuricemia or gout while patients were on-treatment.
Results: Of the 7,020 patients included at baseline, 413 (6%) were taking UA-lowering medications and 6,607 (94%) were not. Patients on UA-lowering medications were older, more frequently male, had a higher BMI, longer T2D duration, poorer kidney function, higher albuminuria, and more often treated with diuretics. At 12 weeks, mean serum UA level was lower in empagliflozin-treated patients vs. placebo, and this difference was maintained over all subsequent timepoints; by week 52 the adjusted mean (95% CI) treatment difference was −0.37 (−0.42, −0.31) mg/dL. The adjusted mean treatment difference (95% CI) in serum UA at week 52 was more pronounced with baseline UA levels ≥6 mg/dL (−0.46 (−0.55, −0.38) mg/dL, panel A). Among the 6,607 patients not taking UA lowering medications at baseline, 5.2% experienced a gout flare or initiated a drug for the treatment of gout in the placebo group vs. 3.6% in the pooled empagliflozin group, corresponding to incidence rates of 21.6 vs.14.1 events per 1,000 patient-years, and a hazard ratio (95% CI) of 0.67 (0.53, 0.85), P=0.001 (panel B). The

event rate reduction was similar with either empagliflozin 10 or 25 mg and in patients with baseline UA levels below or above 6.0 mg/dL as well as with vs. without a medical history of gout at baseline (interaction P=0.53 and 0.48, respectively).

Conclusion: Empagliflozin reduced mean UA, the need for UA lowering medications or gout flares vs. placebo. These data support further investigations into SGLT2i in the prevention of gout and reduction of anti-gout medication.



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Disclosure: **J.P. Ferreira:** Honorarium; Boehringer Ingelheim.

OP 10 Peripheral neuropathy: pathophysiology and intervention

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Advanced glycation end-products are associated with diabetic neuropathy in young adults with type 1 diabetes

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Background and aims: Cardiovascular autonomic neuropathy (CAN) and sensorimotor polyneuropathy (DSPN) are common diabetic complications. The formation of advanced glycation end-products (AGEs) as a consequence of hyperglycemia and lipid toxicity has been suggested to play a causal role in the pathogeneses of diabetic neuropathy. The aim was to investigate the association between AGEs and measures of CAN and DSPN in young adults with type 1 diabetes.

Materials and methods: In a cross-sectional study design of young adults with type 1 diabetes, CAN was assessed by three cardiovascular autonomic reflex tests (CARTs); lying-to-standing test, the deep breathing test (E/I) and Valsalva manoeuvre, and by 5-min resting heart rate variability (HRV) indices; the mean square of the sum of the squares of differences between consecutive R-R intervals and standard deviation of normal-to-normal intervals (SDNN), high (HF) and low frequency (LF) power, total frequency power and the LF/HF-ratio. DSPN was assessed by light touch and pain perception, vibration perception threshold (VPT), neuropathy questionnaires, electrochemical skin conductance and measures of sural nerve function. Associations between serum levels of AGEs and neuropathy outcomes were analysed by regression analyses by one unit increases of standardized z-scores of groups of AGEs categorized by origin; i. "Glycolytic dysfunction"; methylglyoxal-derived hydroimidazolone 1, N_{ε}-(carboxyethyl)-lysine, methylglyoxal-lysine dimers and argpyrimidine. ii. "Lipid peroxidation"; glyoxal-derived hydroimidazolone 1 and N_{ε}-(carboxymethyl)-lysine. iii. "Oxidative stress"; methionine sulphoxide. iv. "Glucotoxicity"; fructoselysine, glucosepane and 3-deoxyclucosone. The analyses were adjusted for relevant confounders; age, gender, HbA1c, diabetes duration, current smoking status, total cholesterol, triglycerides, systolic blood pressure and use of beta-blockers.

Results: One hundred and fifty-one patients were examined aged 22 years (SD 1.6) with a mean diabetes duration of 11 years (SD 5.1) and HbA1c was 67.0 mmol/mol (IQR 58.0;77.0). Higher z-scores in several AGE groups were associated with several outcomes: "Glycolytic dysfunction" was associated with a higher VPT (4.14% (95%CI 1.31;7.04), p = 0.004) and E/I (0.03% (95%CI 0.01;0.05), p = 0.005) with full adjustments. "Lipid peroxidation" was associated with higher VPT only in unadjusted models (12.90% (95%CI -2.30;30.45), p = 0.102), and a higher LF/HF ratio (37.72% (95%CI 1.12;87.57), p = 0.044) with full adjustments. "Glucotoxicity" was associated to lower values of several HRV indices and in adjusted models SDNN retained significance (-4.20% (95%CI -8.1416; -0.0896), p = 0.047).

Conclusion: In young adults with type 1 diabetes, increased levels of AGEs derived from glycolytic dysfunction and lipid peroxidation were associated with worse vibration perception threshold. Increased levels of AGEs from glucotoxicity were associated with autonomic dysfunction. We conclude that even in early stages of diabetes AGEs may play a diverse role in the pathogeneses of different types of diabetic neuropathy.
Disclosure: **E.F. Al-Saoudi:** None.

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Progression and regression of small and large nerve fiber pathology and dysfunction in recent-onset type 1 and type 2 diabetes: a 5-year prospective study

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Background and aims: It has been traditionally suggested that the early development of diabetic sensorimotor polyneuropathy (DSPN) is characterized by a predominant and progressive injury to small nerve fibres followed by large fibre impairment. We tested an alternative hypothesis that small and large fibre damage due to DSPN in type 1 and type 2 diabetes could develop in parallel and may not only be progressive but also reversible.
Materials and methods: Participants from the German Diabetes Study baseline cohort with recent-onset type 1/type 2 diabetes (n=350/570) and age-matched glucose-tolerant control individuals (control 1/control 2: n=114/190) were assessed by nerve conduction studies (NCS), thermal detection thresholds (TDT), vibration perception threshold (VPT), Neuropathy Symptom Score (NSS), Neuropathy Disability Score (NDS), and intraepidermal nerve fibre density (IENFD) in skin biopsies (type 1/type 2 diabetes: n=102/225; control 1/control 2: n=109/208). Subsets of participants with type 1/type 2 diabetes were followed for 5 years (n=184/307; IENFD subset: n=18/69). DSPN was defined by the Toronto Consensus criteria.
Results: At baseline, DSPN was present in 8.1% and 13.3% of the type 1 and type 2 diabetes groups, respectively. The most frequently abnormal tests in the lower limbs below or above the 2.5th and 97.5th centile of the controls were IENFD (13.7%) and individual NCS (up to 9.4%) in type 1 diabetes participants and IENFD (21.8%), malleolar VPT (17.5%), and individual NCS (up to 11.8%) in those with type 2 diabetes, whereas TDT abnormalities did not differ between the control and diabetes groups. The risk factors most consistently associated with impaired peripheral nerve tests across the groups studied were higher age, height, and weight. IENFD correlated variably with both small and large fibre function tests in the control and diabetes groups. After 5 years in the type 2 diabetes group, the highest progression rates from the normal to the abnormal range were found for IENFD (18.6%), malleolar VPT (18.4%), and NDS (15.0%), while vice versa the highest regression rates were observed for NDS (11.2%), sural nerve amplitude (9.1%), IENFD (8.7%), and NSS (8.2%). In type 1 diabetes participants, no major progression was seen after 5 years, but subclinical DSPN regressed in 10.3%.
Conclusion: These findings point to an early parallel damage to both small and large nerve fibres in well-controlled recent-onset type 2 and, to a lesser extent, type 1 diabetes. After 5 years peripheral nerve pathology and dysfunction progresses in type 2 diabetes, but initial nerve alterations are also reversible to a meaningful degree.
Disclosure: **G.J. Bönhof:** None.

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Dys- but not demyelination is the hallmark of diabetic neuropathic lesions

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Background and aims: Diabetic neuropathy (DN) remains the most prevalent complication of individuals with diabetes, affecting up to 50% of patients with either type 1 and type 2 diabetes. Magnetic resonance neurography (MRN) has shown that patients with DN have lesion on the proximal region of the sciatic nerve, which is associated with the clinical scoring. The nature of these lesions, which are visualized in MRN as bright focus spots within the fascicles of the nerve, remains unknown. The aim of this study was to understand the nature of these lesions by studying the differences at the protein level using a microproteomic approach.
Materials and methods: Formalin-Fixed and Paraffin-Embedded (FFPE) nerve material from type 2 diabetic patients were obtained following below the knee-amputation. The location of the lesion(s) was identified by ex vivo MRI. Fascicles, with and without damage were isolated by Laser Micro Dissection (Leica LMD 7000) microscopy. Following tryptic digestion, samples were analyzed by liquid-chromatography, tandem mass spectroscopy. MaxQuant software was used to analyze raw mass spectrometric files and Perseus software was used to perform further downstream statistical and bioinformatic analyses. A fold change cut-off of either -2 or +2 were used to identified proteins that were down- or up-regulated, respectively. Proteins that were found to be significantly changed ($P<0.05$) were subsequently validated by immunofluorescence.
Results: Using a microproteomic approach, an average of 1141 proteins could be identified in lesioned fascicles as compared to 1228 in healthy fascicles ($P > 0.05$). Bioinformatics analysis showed that in lesioned fascicles, a total of 12 proteins were significantly down-regulated with 4 proteins up regulated. The most notable of the down regulated proteins were associated with myelin sheath integrity such as Myelin Basic Protein (MBP) (log2 FC = -3.1; -log10 p-value = 5.4), Myelin Protein Zero (MPZ) (log2 FC = -2.6; -log10 p-value= 5.7) and Peripheral Myelin Protein 2 (PMP2) (log2 FC = -4.2; -log10 p-value= 5.3). Proteins associated with carbohydrate metabolism were also found to be downregulated such as Aldo-Keto Reductase Family 1 Member B (AKR1B1) (log2 FC = -3.1; -log10 p-value= 4.6). Pathway analysis showed that myelination deficiency was the most prevalent condition associated with the lesions observed in diabetic patients. This deficiency was associated with an increase in proteins involved in myelin clearance proteins as well as promoters of axon growth. Interestingly, this deficiency was associated with dysmyelination, the process of malformed or defective myelin sheath rather than the classical process of demyelination.
Conclusion: The preliminary results obtained from type 2 diabetic patients suggest that incorrect formation of the myelin sheath, rather than its loss is associated with the development of lesions in DN. The finding that AKR1B1, an enzyme which catalyzes the reduction of glucose to sorbitol as part of the polyol pathway, could suggest that this pathway may have a cause role in malformation of the myelin sheath
Disclosure: **M. Le Marois:** None.

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Morphometric abnormalities of the brain in diabetic peripheral neuropathy

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Background and aims: Advanced magnetic resonance imaging (MRI) techniques have enabled detailed interrogation of alterations in the brain in diabetic peripheral neuropathy (DPN). Small studies have demonstrated structural alterations in brain regions involved with the somatosensory and motor system in DPN and painful-DPN (pDPN). However, these preliminary findings have not been confirmed in a large cohort with detailed patient characterization. Moreover, such studies have not considered structural alterations in different pDPN phenotypes. Here we present the largest DPN neuroimaging study to date, which aims to examine morphological differences in brain structure in DPN and different phenotypes of painful-DPN.

Materials and methods: A total of 229 participants were enrolled, 177 with diabetes (46 no-DPN, 56 painless-DPN and 75 pDPN; 24 irritable [IR] and 50 non-irritable [NIR] phenotype), and 52 healthy volunteers underwent detailed clinical and neurophysiological assessments. All subjects underwent 3-dimensional T1-weighted brain MRI (3.0T, Phillips). Brain volume analysis was performed using Freesurfer (http://surfer.nmr.mgh.harvard.edu/). We used the German Research Network on Neuropathic Pain (DFNS) quantitative sensory testing (QST) profile to phenotype patients with pDPN.
Results: There was a significant group effect in the cortical thickness at the pre-central gyrus (ANOVA P=0.001), anterior cingulate cortex (ACC, p=0.036), insula cortex (p=0.021) and post-central gyrus (p=0.015), where there was also a group effect in grey matter volume (p=0.037). At the post-central gyrus, in comparison with HV (1.92mm±0.11) and NoDPN (1.90mm±0.14), there was a significantly reduced cortical thickness in DPN (1.85mm±0.14), in pDPN (1.87mm±0.12) cortical thickness was reduced compared with HV. At the precentral gyrus, both DPN (2.29mm±0.16) and pDPN (2.28mm±0.16) had significantly reduced cortical thickness compared with HV (2.37mm±0.14) and NoDPN (2.36mm±0.13). At the insula, compared with HV (2.93mm±0.15) the cortical thickness was reduced in DPN (2.85mm±0.18) and pDPN (2.84mm±0.17). The cortical thickness at the pre and postcentral gyrus, and insula correlated with measures of nerve conduction. At the ACC mean cortical thickness was reduced in the pDPN group (2.55mm±0.23) compared to DPN (2.62mm±0.25). Moreover, at the ACC the mean cortical thickness was significantly reduced in the IR- (2.40mm±0.23) compared to NIR-pDPN (2.58mm±0.20; p=0.003).
Conclusion: We present the largest study to examine cerebral morphometric alterations in patients with DPN and pDPN. We demonstrate that key somatomotor brain regions have a reduced cortical thickness in patients with DPN and pDPN, which correlate with neurophysiological measures suggestive of an ascending axonopathy. Moreover, we found that the cortical thickness at the ACC differentiated patients with the IR and NIR, suggesting neuroplasticity in this region may play a role determining different clinical phenotypes in pDPN.
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Disclosure: **G. Sloan:** None.

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Pain processing areas of the brain demonstrate altered microvascular perfusion during spontaneous neuropathic pain

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Background and aims: Painful diabetic peripheral neuropathy (painful-DPN) causes distressing neuropathic pain that is only partially responsive to treatment. A better understanding of CNS correlates of painful-DPN is vital to develop more effective therapeutics. We have previously reported increased thalamic vascularity in painful diabetic peripheral neuropathy (PDPN) but the microvascular perfusion of the other pain processing areas of the brain (Pain Matrix - PM), with or without pain at the time of scanning, is unknown. Thus, the aim of this study was to measure the cerebral perfusion characteristics of the PM areas using MR-Dynamic Susceptibility Contrast (MR-DSC).
Materials and methods: 55 T1DM subjects (20 PDPN, 23 painless-DPN, 13 no-DPN) and 19 healthy volunteers (HV) underwent clinical, neurophysiological and MR-DSC of the passage of IV-gadolinium-chelate through the cerebral vascular bed (3-Tesla, Philips, Netherlands) at rest. The PDPN group was further divided into those that had neuropathic pain during scan (P+) and no pain (P-) on a visual analogue scale (VAS). The intensity of the pain at the time of scanning was recorded. The Mean Transit Time -MTT and the time-to-peak -TTP concentrations of gadolinium in PM areas (Thalamus, Insular Cortex - IC, Anterior Cingulate Cortex - ACC) were measured.
Results: Participants experiencing spontaneous neuropathic pain (P+, n=10, VAS score≥4) during scanning had shorter mean TTP at the Right-Thalamus: P+ vs. painless-DPN p=0.017, P+ vs. HV p=0.033; Right-IC: P+ vs. painless-DPN p=0.048; and POWM: P+ vs. P- p=0.009, P+ vs. painless-DPN p=0.034, P+ vs. HV p=0.011. The MTT at the Right-Thalamus: P+ vs. HV p=0.043 and at the Left-IC: HV vs. No-DPN p=036, were also significant.
Conclusion: We demonstrate for the first time in PDPN that there are altered cerebral perfusion characteristics in the pain processing areas of the brain. We found shorter TTP during spontaneous pain at the time of scanning. This seems to be related to a bolus dispersal factor as MTT, and CBF do not match the TTP and the control area is also affected. However, whether this altered perfusion is primary or secondary to the experience of pain is yet to be determined. This novel finding may serve as objective marker of spontaneous neuropathic pain, and a target for the development of novel treatments.
Supported by: EFSD/Novo Nordisk A/S Programme for Diabetes Research in Europe 2012
Disclosure: **M. Greig:** None.

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Long-term high frequency (10 kHz) spinal cord stimulation in painful diabetic neuropathy: a randomised controlled trial

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Background and aims: The World Health Organization estimates there are 422 million people living with diabetes globally and approximately 20% will develop painful diabetic neuropathy (PDN). Current treatment options are ineffective for many patients; however, previous results suggest high frequency (10 kHz) spinal cord stimulation (SCS) relieves pain and may improve sensation in patients with refractory symptoms.
Materials and methods: Prospective, randomized controlled trial with 216 patients assigned 1:1 to 10 kHz SCS (Nevro Corp.) combined with conventional medical management (CMM) or CMM alone. Key inclusion criteria: PDN symptoms ≥12 months, lower limb pain ≥5cm (on 0-10cm visual analog scale [VAS]), and medically appropriate candidate for SCS procedure. Outcomes include pain (VAS), Brief Pain Inventory for Diabetic Peripheral Neuropathy (BPI-DPN), Pain and Sleep Questionnaire (PSQ-3), Patient Global Impression of Change (PGIC), and neurological examination. Patients could opt to crossover to the other treatment arm at 6 months if they had insufficient pain relief (<50%), were dissatisfied, and their physician deemed it was medically appropriate.
Results: Both the 10 kHz SCS and the CMM treatment arms were similar in baseline characteristics. The mean age of participants was 60.8 years (SD 10.7) with mean hemoglobin A1c of 7.4% (SD 1.2%). Median duration of diabetes was 10.9 years (IQR 6.3-16.4) with median 5.6 years (IQR 3.0-10.1) of peripheral neuropathy symptoms. Patients in the 10 kHz SCS group reported a mean 76% reduction in pain at 6 months as well as reduced pain interference with daily activities, and improvements in sleep (**Table**). On PGIC, 99% of patients reported improvement at 6 months compared to baseline. None of the patients in the 10 kHz SCS group chose to crossover to CMM at 6 months. Treatment benefits were sustained through 12 months. A majority of patients treated with 10 kHz SCS were noted to have improvement on neurological examination by investigators. Participants in the CMM group experienced no change in pain levels, pain interference with daily activities, or sleep quality over 6 months. Eighty-two percent opted to crossover to 10 kHz SCS at 6 months. Results post-crossover were similar to those in patients originally randomized to 10 kHz SCS: mean 70% reduction in pain as well as reduced pain interference with daily activities, improvements in sleep, and a majority with observed improvements on neurological examination. In total, 154 patients

received permanent SCS device implants, including those originally randomized to SCS and those who crossed over at the 6-month timepoint. There were 32 study-related adverse events in 26 (17%) patients. Five (3%) patients developed infections that required device removal.

Conclusion: This study is the largest RCT to-date of SCS management in PDN. Substantial improvements with high frequency (10 kHz) SCS were sustained over 12 months and support this treatment for PDN patients with symptoms refractory to conventional care.

	CMM Pre- and Post-crossover to SCS			10 kHz SCS		
	Baseline	6 Months (CMM only)	12 Months (6 mo CMM, 6 mo SCS)	Baseline	6 Months	12 Months
Lower Limb Pain VAS[1]	7.2 cm (6.8-7.6 cm)	7.4 cm (7.0-7.8 cm)	2.0 cm (1.6-2.4 cm)	7.6 cm (7.2-7.9 cm)	1.7 cm (1.3-2.1 cm)	1.7 cm (1.3-2.1 cm)
Pain relief from baseline	-	-6.7% (-15.0-1.7%)	70.3% (63.4-77.1%)	-	76.3% (70.7-81.9%)	77.1% (71.8-82.3%)
Pain interference with mood & daily activities (BPI-DPN)[2]	5.9 (5.4-6.4)	6.2 (5.6-6.8)	2.1 (1.6-2.6)	6.2 (5.8-6.7)	2.2 (1.7-2.6)	1.9 (1.5-2.3)
Impact of pain on sleep VAS (PSQ-3)[3]	6.7 cm (6.1-7.2 cm)	7.0 cm (6.3-7.6 cm)	2.7 cm (2.0-3.3 cm)	5.9 cm (5.4-6.5 cm)	2.1 cm (1.6-2.5 cm)	2.1 cm (1.6-2.6 cm)
Patient Global Impression of Change (PGIC)						
No change/almost the same	-	57/62 (92%)	1/58 (2%)	-	1/87 (1%)	4/84 (5%)
Improved since baseline	-	5/62 (8%)	57/58 (98%)	-	86/87 (99%)	80/84 (95%)

[1]10 cm visual analog scale measures pain from 0 (no pain) to 10 (worst pain imaginable)
[2]Brief Pain Inventory for Diabetic Peripheral Neuropathy questionnaire includes 7 items that measure interference of pain due to diabetes on an 11-point numerical rating scale with 0 representing "does not interfere" and 10 representing "completely interferes"
[3]Pain and Sleep Questionnaire Three-Item index uses 10 cm visual analog scales to measure how often pain interrupts sleep with 0 representing "never" and 10 representing "always"

Table: Data shown as mean (95% CI) for continuous variables, proportions for discrete variables

Clinical Trial Registration Number: NCT03228420
Supported by: Nevro
Disclosure: **E. Petersen:** Grants; Nevro.

OP 11 Cardiac complications: mechanisms and possible treatments

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Identifying myocardial insulin resistance by positron emission tomography combined with hyperinsulinaemic euglycaemic clamp: a new strategy for phenotyping type 2 diabetes

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Background and aims: The identification and clinical meaning of myocardial insulin resistance (mIR) in type 2 diabetic population are challenges to be met. In order to shed light to this issue we have conducted a pilot study using a methodology which might open up a new avenue in the phenotyping of type 2 diabetic population.

Materials and methods: The study comprised forty-seven patients with type 2 diabetes without history of previous cardiovascular event. Patients were prospectively recruited from February 2018 to July 2019 at the Outpatient's Diabetes Unit of tertiary university hospital. The study was conducted according to the tenets of the Helsinki Declaration. The Ethic Committee of our hospital approved all procedures. The assessment of mIR was performed by means of two [18F]FDG PET studies per patient within 2 days: one with at least 8 h of fasting conditions and after 24 h of draw out medication (baseline scan) and the other with the same conditions but after performing hyperinsulinemic euglycemic clamp (HEC scan). Myocardial [18F] FDG uptake was measured in SUVbw normalized by the max SUVbw voxel value. A cardiac CT scan was performed during the baseline PET to determine the Hounsfield units (HU) density of the myocardium and to calculate the coronary artery calcium score (CACs) in Agatstone units (AU). Statistics: Mann-Whitney U test and Spearman's correlation analysis.

Results: All patients showed a low left ventricle myocardial [18F]FDG uptake at the baseline scan closed to the background-to-noise signal. However, after performing HEC, 17 (39.5%) patients exhibited insulin sensitive myocardium (mIS) showing a strikingly enhancement of myocardial [18F]FDG uptake (1.59 SUV [1.42:1.92] vs.7.67 SUV [7.10-9.49]), whereas 26 (60.4%) showed a marginal increase of [18F]FDG uptake (1.18 SUV [1.06:1.59] vs. 2.27 SUV [1.62:2.93]), thus revealing the presence of mIR. Myocardial radiodensity (mRD) was higher in the mIR group than in mIS group (38.95 HU [33.81-44.06] vs. 30.82 HU [21.48-38.02]; p=0.03). A statistically significant correlation was found between mRD and either SUV_{HEC} (rho= -0.4649; p= 0.0024) or with ΔSUV (rho= -0.5238; p= 0.0005). Regarding the relationship between mIR with cardiovascular risk factors we found that patients with mIR did not presented a significantly higher levels of HbA1c, cholesterol or blood pressure but exhibited a significantly higher CACs than patients with mIS (CACs>400 AU: 52% vs. 29%; p=0.002). This result means that patients with mIR present a very high risk of cardiovascular events.

Conclusion: [18F]FDG PET combined with HEC is a reliable method that permits a clear identification of patients with mIR. This subset of patients with type 2 diabetes present structural changes in the myocardium and a high proportion of CACs>400 AU. Further prospective research is required, not only to confirm these findings but also to determine the global impact of mIR on myocardium remodelling, functionality and cardiovascular outcomes.

Clinical Trial Registration Number: NCT02248311
Supported by: Instituto de Salud Carlos III (PI16/02064, PI20/01588)
Disclosure: **R. Simo:** None.

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Do cardiovascular risk prediction models developed in primary care patients with type 2 diabetes perform better than the general population models? PREDICT cohort study

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Background and aims: There is only limited evidence that cardiovascular risk scores developed specifically in patients with diabetes estimate CVD risk more accurately. Studies comparing the predictive ability of CVD risk scores developed in diabetic populations, with those developed in general populations, showed that their performance varied considerably. However, comparing models developed from different studies is problematic because of between-study variation in methods and differences in populations' background risk. Large number of patients with diabetes in the PREDICT cohort provides an opportunity to compare models derived from the same patient population, using a standard set of performance measures, therefore resulting in a more valid comparison than has been possible previously.
Materials and methods: Using data from the PREDICT primary care cohort of 401,752 CVD-free patients aged 30 to 74 years, we developed general population and diabetes-specific prediction models for estimating the risk of first CVD event (coronary heart disease, ischaemic and haemorrhagic stroke, transient ischaemic attack, peripheral vascular disease, congestive heart failure). Identical definitions of the outcome and predictors, and identical approaches to model development were applied in both sets of models. Pre-specified clinically-relevant risk predictors were used to develop sex-specific models with Cox regression methods. For the PREDICT-1^{o} models developed in the total cohort of patients with and without diabetes, these were age, ethnicity, deprivation, smoking, diabetes status, family history of premature CVD, history of atrial fibrillation, systolic blood pressure, TC:HDL, and treatment with antithrombotic, blood pressure lowering and lipid lowering medications. For the PREDICT T2D models, developed in patients with type-2 diabetes, several relevant predictors were added to the above: duration of diabetes, glomerular filtration rate, albuminuria, haemoglobin A_{1c}, body mass index, and treatment with oral hypoglycaemic medications and insulin. We compared performance of the diabetes-specific models and the general population models, in T2D population, using a standard set of performance indicators.
Results: The diabetes-specific models were developed from 46,652 patients with type-2 diabetes. The median follow-up was 5.2 years (244,840 person-years), a total of 4,114 patents experienced an event (9% fatal). The general population models were developed from 401,752 patients, of whom 15,386 experienced an event (10% fatal) during 1,685,521 person-years follow-up. The T2D-specific models performed better than PREDICT-1^{o}, in terms of discrimination, calibration, explained variation and net benefit. Discrimination was good, with C statistic of 0.7 in both men and women, and Royson's D of 1.082 and 1.346, respectively. Calibration plots also indicated T2D models' excellent calibration. The net benefit analysis showed that diabetes-specific models were superior to the general population models at all clinically meaningful thresholds of risk.
Conclusion: Comparative assessment of the general population and T2D-specific models demonstrated that addition of clinically relevant predictors improves models' performance in patients with diabetes. Treatment with insulin, measures of renal function, and body composition contribute significantly to cardiovascular risk prediction in type-2 diabetes.
Supported by: Health Research Council of New Zealand
Disclosure: **R. Pylypchuk:** None.

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Autonomic dysfunction is associated with the development of arterial stiffness: the Whitehall II cohort

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Background and aims: The association between autonomic dysfunction and increased arterial stiffness is well documented among persons with diabetes. However, emerging evidence suggests that this association can be detected without the presence of diabetes. This study examined the association between level and change of autonomic nervous function and subsequent development of arterial stiffness in the general population.
Materials and methods: Data was obtained from 5,067 participants of the Whitehall II cohort in the period between 1997 and 2013. Autonomic nervous function was assessed by a heart rate variability index i.e. standard deviation of normal-to-normal heartbeat intervals (SDNN) measured three times between 1997-2009, while arterial stiffness was assessed by carotid-femoral pulse wave velocity (PWV) measured twice between 2007-2013 First, individual SDNN levels and their annual change were estimated. Then we modelled the development of PWV using the previous SDNN estimates as exposure using linear mixed effect models. We included age in the model and its interaction with SDNN. First, we adjusted the model for sex and ethnicity (model 1), and then for socioeconomic status, BMI, smoking status, alcohol use, physical activity, levels of cholesterol (total), triglycerides, HbA1c, systolic blood pressure, antihypertensive (e.g. beta-blockers) and glucose lowering medications (model 2). Similar analyses were performed in the subgroup without diabetes.
Results: The median (25^{th} & 75^{th} percentile) age at the first PWV measurement was 64.5 years (60.6; 70.0), while median PWV was 8.05 m/s (7.03; 9.45). SDNN level and annual change was 31.1 ms (26.1; 36.5) and -1.5% (-1.9; -1.2), respectively. A decrease in SDNN was associated with subsequent higher levels of PWV, but the effect of change in SDNN was less pronounced at higher age. A typical individual aged 65 years with a SDNN level of 30 ms and a 2% annual decrease in SDNN had 1.21 m/s (CI: 0.86; 1.56) higher PWV level and 0.09 m/s per year (CI: 0.05; 0.12) slower annual PWV increase, compared to one with the same age and SDNN level but with a 1% annual decrease in SDNN. The differences in the models were attenuated after further adjustments and exclusion of persons with diabetes.
Conclusion: Steeper decline of autonomic nervous function is associated with higher levels of arterial stiffness. However, the effect of a steeper decline in autonomic nervous function was less pronounced at higher age. Associations were diminished after exclusion of persons with diabetes.
Disclosure: **J.F.R. Schaarup:** None.

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Metabolically healthy obese and cardiovascular events in a nationwide cohort study

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Background and aims: Obesity is a risk factor for cardiovascular disease (CVD) and has been increasing globally over the past 40 years in many countries worldwide. Metabolic abnormalities such as hypertension, dyslipidemia and diabetes mellitus are commonly associated and may mediate some of the deleterious effects of obesity. A subset of obese individuals without obesity-related metabolic abnormalities may be classified as being "metabolically healthy obese" (MHO). We aimed to evaluate the associations among MHO individuals and different types of incident cardiovascular events in a contemporary population at a nationwide level.
Materials and methods: From the national hospitalization discharge database, all patients discharged from French hospitals in 2013 with at least 5 years or follow-up and without a history of major adverse cardiovascular event (myocardial infarction, heart failure [HF], ischemic stroke or cardiovascular death, MACE-HF) or underweight/ malnutrition were identified. They were categorized by phenotypes defined by obesity and 3 metabolic abnormalities (diabetes mellitus, hypertension, and hyperlipidemia). In total, 2,953,816 individuals were included in the analysis, among whom 272,838 (9.5%) were obese. We evaluated incidence rates and hazard ratios for MACE-HF, cardiovascular death, myocardial infarction, ischemic stroke, new-onset HF and new-onset atrial fibrillation (AF). Adjustments were made on age, sex and smoking status at baseline.
Results: During a mean follow-up of 4.9 years, obese individuals with no metabolic abnormalities had a higher risk of MACE-HF (multivariate-adjusted hazard ratio [HR] 1.22, 95% confidence interval [CI]: 1.19-1.24), new-onset HF (HR 1.34, 95%CI 1.31-1.37), and AF (HR 1.33, 95%CI 1.30-1.37) compared with non-obese individuals with 0 metabolic abnormalities. By contrast, risks were not higher for myocardial infarction (HR 0.92, 95%CI 0.87-0.98), ischemic stroke (HR 0.93, 95%CI 0.88-0.98) and cardiovascular death (HR 0.99, 95%CI 0.93-1.04). In the models fully adjusted on all baseline characteristics, obesity was independently associated with a higher risk of MACE-HF events (HR 1.13, 95%CI 1.12-1.14), of new-onset HF (HR 1.19, 95%CI 1.18-1.20) and new-onset AF (HR 1.29, 95%CI 1.28-1.31). This was not the case for the association of obesity with cardiovascular death (HR 0.96, 95%CI 0.94-0.98), myocardial infarction (HR 0.93, 95%CI 0.91-0.95) and ischemic stroke (HR 0.93, 95%CI 0.91-0.96). Hypertension and diabetes mellitus were independent predictors of all types of outcomes.
Conclusion: Metabolically healthy obese individuals do not have a higher risk of myocardial infarction, ischemic stroke or cardiovascular death than metabolically healthy non-obese individuals. By contrast they have a higher risk of new-onset HF and new onset AF. Even individuals who are non-obese can have metabolic abnormalities (including diabetes) and be at high risk of cardiovascular disease events. Our observations suggest that specific studies investigating different aggressive preventive measures in specific subgroups of patients are warranted.
Disclosure: **G. Fauchier:** None.

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Distribution of cardiovascular risk in type 2 diabetes: results of an analysis using data from CAPTURE study

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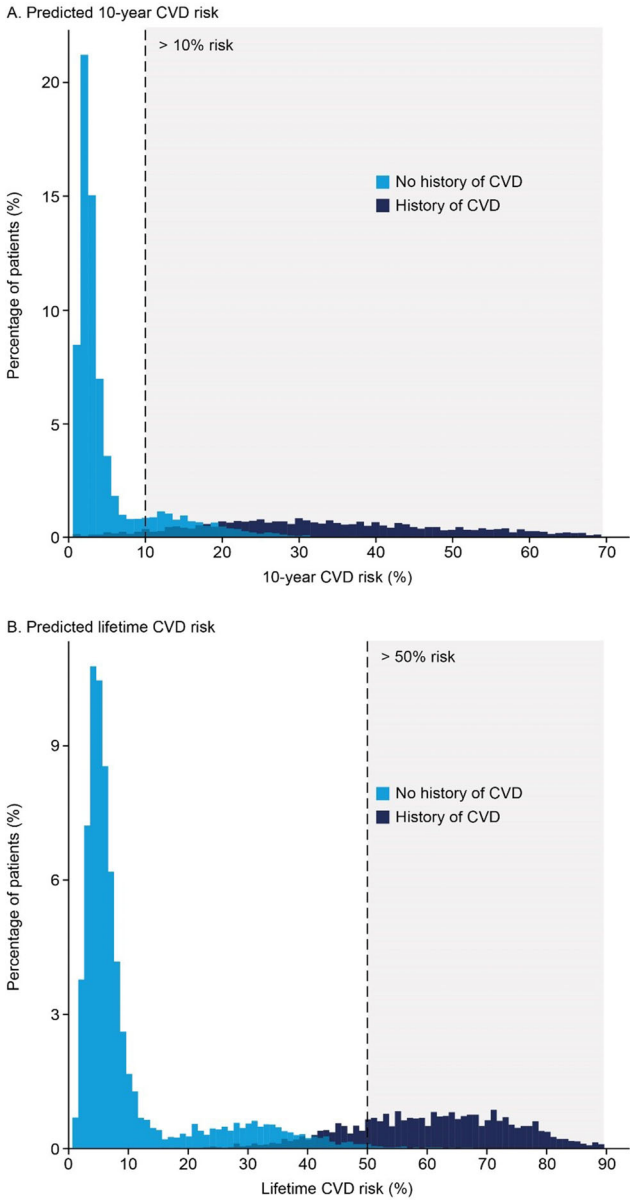
Background and aims: Cardiovascular disease (CVD) is the leading cause of mortality in type 2 diabetes (T2D). CAPTURE, a non-interventional, cross-sectional study across 13 countries in 2019, collected demographic and clinical data in almost 10,000 adults with T2D. Less than 25% of patients with established CVD treated with a glucose-lowering agent received an agent with demonstrated benefit in CV risk reduction. We used the Diabetes Lifetime-perspective prediction (DIAL) competing risk adjusted model to estimate CV risk distribution in the CAPTURE population, and to assess treatment patterns by CV risk. The model calculates absolute 10-year and lifetime risk of myocardial infarction, stroke or cardiovascular death, and life-expectancy free of a CVD event.
Materials and methods: Patient-level data from CAPTURE (age, sex, body mass index, smoking status, HbA_{1c}, CVD history, T2D duration, clinical parameters and treatment history) were used in the DIAL model; missing data were imputed by region. High risk was defined as 10-year risk > 10%, and lifetime risk > 50%.
Results: Data from 9457 patients with T2D aged 30-85 years were included. There was a wide distribution of 10-year and lifetime risk, with higher risk in patients with a history of CVD (n = 2914) than in those without (n = 6543). Among patients with a history of CVD, 96% had a 10-year risk of CVD > 10% and 81% had a lifetime risk of CVD > 50% (Figure). In patients with CVD and a high 10-year risk of recurrent CVD, 81% had a lifetime risk of recurrent CVD > 50%. In patients with no history of CVD, 14% had a 10-year risk > 10% and only 1% had a lifetime risk > 50% (Figure). Among patients with no previous CVD but a high 10-year risk of CVD, only 4% had a lifetime risk > 50%. Of the patients with CVD, 10% received a glucagon-like peptide-1 receptor agonist (GLP-1 RA) and 18% received a sodium-glucose co-transporter-2 inhibitor (SGLT-2i); of patients with CVD and a high 10-year risk of recurrent CVD, 10% received a GLP-1 RA and 17% received an SGLT-2i. Among patients with no CVD, 11% received a GLP-1 RA and 16% received an SGLT-2i; of patients without current CVD but a high 10-year risk of CVD, 12% received a GLP-1 RA and 16% received an SGLT-2i.
Conclusion: There is a wide distribution of CVD risk in the CAPTURE population, and only a minority of patients at high risk of CVD received a glucose-lowering agent with demonstrated benefit in CV risk reduction. Discussing these risks and the CV benefit to be gained from interventions with patients can enhance shared decision-making.

Figure. Predicted (A) 10-year and (B) lifetime risk of CVD for patients with and without CVD in the CAPTURE study.



Shading indicates patients with high risk: 10-year CVD risk > 10%, and lifetime CVD risk > 50%. CVD, cardiovascular disease.

Supported by: Novo Nordisk A/S
Disclosure: **J. Westerink:** None.

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Association of circulating metabolomic biomarkers with incident cardiovascular disease in type 2 diabetes: analysis from the Hong Kong Diabetes Biobank

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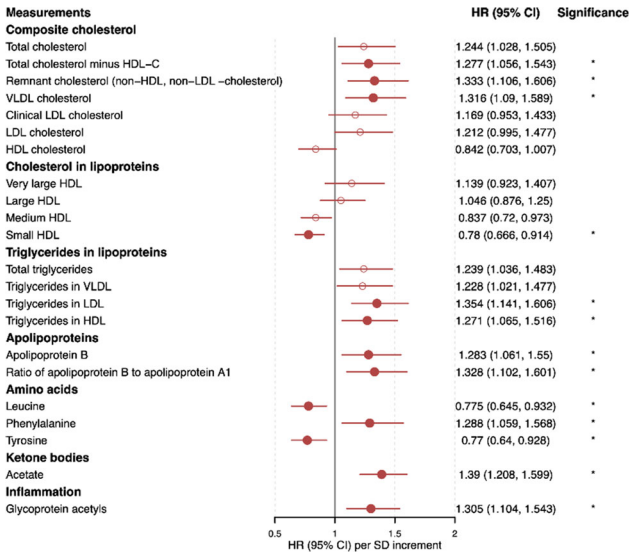
Background and aims: Metabolomics are emerging as promising biomarkers for cardiovascular disease (CVD). The current study aimed to investigate the metabolomic signature of CVD in participants with type 2 diabetes (T2D).

Materials and methods: Baseline serum samples from 1,991 participants with T2D were quantified for 250 metabolic measures using proton nuclear magnetic resonance spectroscopy. After excluding 544 subjects with prevalent CVD, 1,447 were included in the analyses. Cox regression was used to estimate hazard ratios (HRs) for a 1-SD increment in each measure, adjusting for established risk factors, and use of lipid-lowering drugs. False discovery rate (FDR) <0.05 was set as the significance threshold.

Results: Mean (SD) age was 59.3 (10.9) years, 815 (56.3%) were male, and mean (SD) diabetes duration was 10.7 (8.4) years. During a median (IQR) 5.2 (5.0-5.4) years of follow-up, 125 (8.6%) events were recorded. Cholesterol in triglyceride-rich, non-high-density, and very low-density lipoproteins (VLDL) were positively associated with CVD (HR: 1.28 to 1.33). Cholesterol in small high-density lipoprotein (HDL) was inversely associated with CVD (HR 0.78), while cholesterol in other HDL particles was not. Triglycerides in all lipoproteins, apolipoprotein B (apoB) and its ratio to apolipoprotein A1 were positively associated with CVD (HR: 1.27 to 1.35). Phenylalanine, acetate, and glycoprotein acetyls were positively associated with CVD (HR: 1.29 to 1.39); while leucine and tyrosine were inversely associated with CVD (HR: 0.78 and 0.77, respectively). A total of 66 metabolites were associated with CVD, including 9 which remained significant after Bonferroni adjustment. A metabolite score composed of concentration of very small VLDL, acetate, and albumin (selected based on backward Akaike information criterion [AIC] elimination) significantly improved the C-statistic (95% CI) (0.754 to 0.790 [0.013, 0.066]), integrated discrimination improvement (0.040 [0.015, 0.076]), and continuous net reclassification improvement (0.386 [0.124, 0.634]) over the RECODe (Risk Equations for Complications Of type 2 Diabetes) model.

Conclusion: Cholesterol and triglycerides in non-HDL and apoB were positively associated with CVD. Cholesterol in small HDL was inversely associated, whereas triglycerides in HDL were positively associated with CVD. Amino acids, ketone bodies, and glycoprotein acetyls were associated with CVD. Incorporating metabolomics could improve the prediction of CVD in T2D beyond an established prediction model.



Supported by: Research Grants Council Research Impact Fund (R4012-18)
Disclosure: **Q. Jin:** None.

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Predicting sensitivity and resilience to modifiable risk factors for cardiometabolic disease

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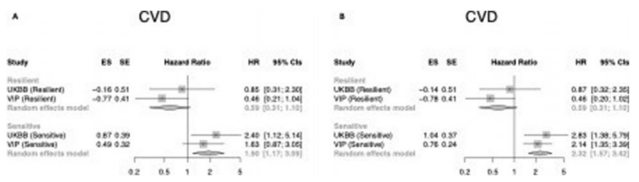
Background and aims: Lifestyle exposures play a major role in the development of disease, yet people vary in their susceptibility. Statistical prediction methods typically focus on population averages, with heterogeneity in effects treated as error. However, effects observed at the extremes of a population's distribution can be informative of underlying features that can help improve statistical inferences. Thus, we used a novel approach to identify population subgroups that are either especially sensitive or resilient to a range of lifestyle risk exposures and assessed the extent to which allocation to these groups predicted incident type 2 diabetes (T2D) or cardiovascular disease (CVD).
Materials and methods: From participants without T2D or CVD at baseline, in the Västerbotten Intervention Program (VIP, n= 35,440), blood samples were obtained after fasting and a 75-gram oral glucose load. Environmental exposure data (e.g. socio-economy, quality of life, tobacco use, alcohol consumption, diet, physical activity) were used to develop scores that predict levels of 9 intermediate cardiometabolic traits: obesity (BMI), dyslipidemia (triglycerides, LDL, HDL-C, total cholesterol), dysglycaemia (fasting and 2-hr glucose), and high blood pressure (SBP, DBP). 90% prediction intervals were determined using quantile regression forests, from which three exposure susceptibility groups were derived per cardiometabolic score ('sensitive' [>90% PI], 'resilient' [<10% PI], and 'normal' [11-89% PI]). Half the dataset was used for training and the remainder for validation. Incidence rates and Cox proportional hazards models were used to determine risk of developing T2D and CVD in the *sensitive* and *resilient* groups separately, using the *normal* group as reference. All analyses were undertaken in R software (v3.6.1).
Results: During a median of 9.7 y follow-up in the VIP cohort, the rate of developing T2D was ~4x higher in the *sensitive* compared with *normal* group for obesity risk factors (IRR=4.0; 95%CI 2.2, 7.2; p=0.02). For high blood pressure risk factors, the risk of CVD was >2x higher in the compared with the *normal* group (SBP: HR=2.4; 95%CI 1.12, 5.14; p= 0.02; DBP: HR=2.25; 95%CI 1.49, 3.4; p= 1.05E-04); conversely, the risk of CVD was more than halved in the *resilient* compared with *normal* group (HR=0.39; 95%CI 0.17, 0.87; p= 2.1E-02). For lipid risk factors, the *resilient* group had higher hazards for T2D, (HR=2.4; 95%CI 1.17, 4.99; p= 0.02) compared to the reference group. Similar results were seen in UKB (see Figure).
Conclusion: Individuals who are predicted to be sensitive to lifestyle risk exposures are generally at higher risk of developing T2D or CVD. This population subgroup may benefit from intensive preventive interventions focused on reducing the burden of adverse lifestyle exposures.



Supported by: Swedish Research Council, Swedish Heart-Lung Foundation, NASCENT
Disclosure: **H. Pomares-Millan:** None.

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Epidemiology of hypoglycaemic episodes leading to hospitalisations in Denmark over the last two decades

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Background and aims: Severe hypoglycaemia is a very unpleasant condition for diabetes patients and has been associated with increased mortality. Especially, episodes leading to hospitalisations, places a significant burden on patients and society. The aim of this study was to investigate the nationwide trends in incidence and associated risk factors with focus on blood glucose lowering medication for hypoglycaemic episodes leading to hospitalisations in Denmark among people with type 1 and 2 diabetes mellitus.
Materials and methods: A cohort study of all people with diabetes from 1977-2018 experiencing hypoglycaemic episodes leading to hospitalisation in 1998-2018 was established. Data were extracted from the Danish National Patient Registry. Trends in incidence rates were investigated descriptively and with linear regressions, and risk factors were investigated with Cox Proportional Hazards models using time-varying covariates.
Results: 66,453 hypoglycaemic episodes leading to hospitalisation in 1998-2018 were investigated among 644,009 people with type 1 (age 37 [SD:22] years) and type 2 diabetes (age 61 [SD:17] years). The incidence rate for hypoglycaemic episodes has declined since 2003 with 0.4 and 0.05 episodes per 100 person years per calendar year (p<0.0001) for type 1 and type 2 diabetes, respectively. Insulin glargine (HR: 1.20, 95% CI: 1.05-1.36, p=0.0059), insulin detemir (HR: 1.17, 95% CI: 1.04-1.32, p=0.0078) and insulin degludec (HR: 1.04, 95% CI: 0.81-1.33, p=0.7716) compared to human insulin (intermediate-acting HR: 1.38, 95% CI: 1.25-1.52, p<0.0001, combination: HR: 1.84, 95% CI: 1.65-2.05, p<0.0001) and especially SGLT2i (HR: 0.43, 95% CI: 0.33-0.57, p<0.0001), GLP-1-RA (HR: 0.50, 95% CI: 0.44-0.57, p<0.0001) and DPP-4i (HR: 0.44, 95% CI: 0.38-0.49, p<0.0001) compared to sulfonylureas (HR: 2.27, 95% CI: 2.18-2.37, p<0.0001), see figure 1 below, seemed safer with respect to hypoglycaemic episodes.
Conclusion: Incidence rates of hypoglycaemic episodes leading to hospitalisation are declining in Denmark, and the advent of new treatment alternatives may play a significant role in this decline. From a safety perspective, these findings are important and could be considered by clinicians when assessing treatment options for patients.

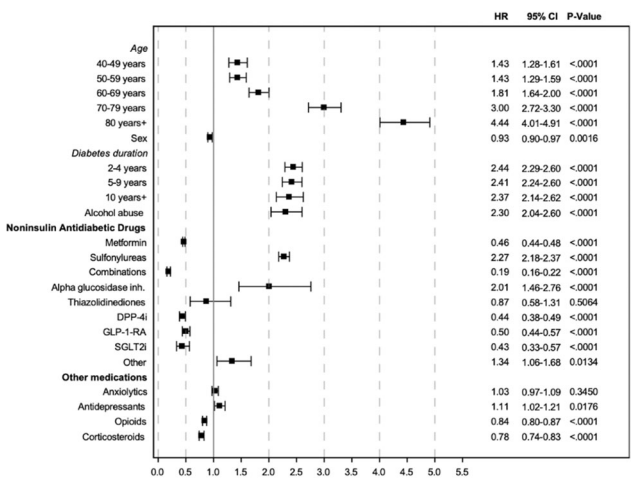

Figure 1: Forest plot showing adjusted hazard rate ratios and 95% confidence intervals for the effect of the time-varying covariates age, sex, diabetes duration, alcohol abuse, NIADs and other medications on time to hypoglycaemic episode for people with type 2 diabetes. The reference for age was 0-39 years, the reference for sex was male and the reference for diabetes duration was 0-1 years. NIADs, alcohol abuse and other medications were added as dichotomous yes/no variables to the model.

Disclosure: **M.H. Jensen:** None.

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The association of hypoglycaemia exposure with subsequent adverse events and severe hypoglycaemia: preliminary results from Hypo-RESOLVE

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University of Edinburgh, Edinburgh, UK.

Background and aims: Accurate characterisation of the association of hypoglycaemia with complications of diabetes is required for the prevention of their eventual occurrence. The Hypo-RESOLVE (Hypoglycaemia REdefining SOLutions for better liVEs) project brings together partners from different sectors to alleviate the burden that hypoglycaemia places on people with diabetes. A key aim for this project is an improved understanding of the clinical consequences of hypoglycaemia through the analysis of data pooled from dozens of clinical trials. We leveraged the quantity and quality of data collected during these clinical trials to estimate the association of hypoglycaemia with clinical outcomes.
Materials and methods: We used a mixed GLMs to estimate the association between hypoglycaemic events (HE) and subsequent severe hypoglycaemia (SH). We used proportional hazard models with time-updated HE exposure to estimate the association of several adverse events with HE exposure.Three definitions of HE severity were used: level 1 (events with an associated recorded blood glucose level < 3.9 mmol/L and ≥3.0 mmol/L), level 2 (< 3.0 mmol/L) and severe (need for third-party assistance). The adverse events of interest were CVD, subsequent SH, nephropathy, and neuropathy. Separate analyses were performed by type of diabetes and for each distinct definition of HE severity. All models were adjusted for age, sex and diabetes duration.
Results: Our study population consisted of 2,116 adults with available age, sex, diabetes type, diabetes duration, adverse event, and HE data. Among those with type 1 diabetes, the number of HE in the previous 45 days was associated with an increased probability of subsequent SH. The size of this association increased with the severity of HE exposure: odds-ratio = 1.02 [95% CI = 1.01, 1.04] for level 1 HE, 1.06 [1.03, 1.09] for level 2 HE, and 1.58 [1.25, 2.00] for SH (all $p < 0.01$). Similar patterns were observed for those with type 2 diabetes: OR=1.09 [1.05, 1.15], 1.20 [1.12, 1.29] and 4.30 [2.18, 8.49], respectively ($p < 0.01$ for all). An increase in cumulative SH events was associated with an increased hazard of CVD among those with type 1 diabetes (HR=1.28 [1.13, 1.44], $p < 0.01$) as well as an increased hazard of nephropathy (1.16 [1.06, 1.28], $p = 0.01$). We found no evidence for an association between HE and neuropathy in those with type 1 diabetes. An association between CVD and level 1 HE was observed in type 2 diabetes (1.01 [1.00, 1.02], $p = 0.03$). Estimates were 1.02 [0.99, 1.04] ($p = 0.17$) for level 2 HE, 1.37 [0.99, 1.89] ($p = 0.06$) for SH. Analyses suggested a potential association between level 1 HE and neuropathy (1.02 [1.00, 1.04], $p = 0.06$). There was no evidence of association between HE and nephropathy, although these outcomes were relatively rare in the analysed data.
Conclusion: In these preliminary analyses, increased HE exposure in the previous 45 days was associated with increased odds of SH, regardless of HE severity. Our results support an association between SH and subsequent CVD and provide some support for associations with subsequent neuropathy and nephropathy. These preliminary findings suggest that the consequences of HE can be profound, supporting the key role that prevention of HE plays in diabetes care. As the Hypo-RESOLVE project continues to accrue data, the statistical power, accuracy and precision of estimates will improve, allowing greater insight into the consequences of HE.
Supported by: This study was performed and funded as part of hypo-RESOLVE
Disclosure: **J.E. O'Reilly:** None.

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Heterogeniety in the effects of two anti-diabetic drugs evaluated using machine learning applied to registry data

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Background and aims: The vast majority of both randomized and epidemiological studies of the effect of anti-diabetic drugs are aimed at estimating the average treatment effect in some defined and often selected population. However, this approach will probably not be able to provide a well-informed basis for offering the right medicines to different patients, and thus calling for precision medicine. Precision medicine is commonly described as a means of exploiting genetic markers to predicate optimal effects, but such are rarely available in clinical practice. Therefore, we set out to assess the existence of, and if so, to characterize the heterogeneity of the treatment effect of two anti-diabetic drugs compared to SU, using state of the art machine learning combined with causal inference, utilizing data routinely available in a clinical setting.
Materials and methods: Based on data from the national diabetes register, the patient registry, the prescribed drug registry and the longitudinal integrated database for health insurance and labour market studies in Sweden we formed two cohorts. One cohort included 18587 persons who started treatment with liraglutide (n=2971) or sulphonyl urea (SU) (n=15616) added to metformin during 2010-2017, and one cohort included 10226 persons starting treatment with dapagliflozin (n=1051) or SU (n=9175) during 2013-2017. The average conditional treatment effect with respect to change in HbA1c, weight, systolic blood pressure (SBP) and kidney function after 6 months treatment was estimated using causal random forest based on 10.000 trees. The algorithm uses a propensity score adjusted doubly robust estimator in the evaluation of each split and mitigates overfitting by using half of the data to derive the split points and half of the data to estimate the treatment effects. The conditional average treatment effect was estimated conditional on age, gender, weight, smoking, HbA1c, SBP, DBP, LDL, HDL, triglyceride, eGFR, retinopathy, microalbuminuria, macroalbuminuria, physical activity, antihypertensive treatment, lipid-lowering treatment, education, income, marital status, geographic origin, and previous CHD, AMI, stroke, CVD, HF, AF, kidney failure, hyperglycaemia, psychiatric disease, alcohol or drug abuse, cancer or dementia.
Results: The average treatment effect on change in HbA1c was -2.57 [-3.20, -1.94] mmol/mol for liraglutide compared to SU, and -1.10 [-1.92, -0.27] mmol/mol for dapagliflozin vs SU. We found evidence of treatment effect heterogeneity in the effect of liraglutide on change in HbA1c ($p<0.001$) and SBP ($p=0.01$), and in the effect of dapagliflozin on change in HbA1c ($p=0.003$). Key drivers for the heterogeneity in the effect on HbA1c were baseline HbA1c, and to a lesser extent baseline weight, eGFR and age. There was also a marked difference in the effect of liraglutide with respect to change in HbA1c between the genders.
Conclusion: The effect of both liraglutide and dapagliflozin with respect to change in HbA1c after 6 months is heterogeneous and varies primarily by baseline HbA1c, and to a lesser extent also by baseline weight, eGFR and age. Heterogeneity of the effects of treatments should be examined in greater detail in order to better tailor diabetes care.
Disclosure: **S. Franzén:** None.

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Alendronate use and risk of type 2 diabetes: a Danish population-based case-control study

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Background and aims: There has been proposed a link between glucose homeostasis and bone metabolism. Bisphosphonates are first line treatment of osteoporosis and we aimed to investigate if the risk of developing type 2 diabetes was altered by previous use of alendronate.
Materials and methods: We conducted a population-based case-control study through access to all discharge diagnoses (ICD-10 system) from the National Danish Patient Registry and all redeemed drug prescriptions (ATC classification system) from the Health Service Prescription Registry. All cases with a diagnosis of type 2 diabetes between 2008 and 2018 were matched on sex and age with 3 randomly selected controls by incidence-density sampling. Exposure was defined as ever use of alendronate and further grouped as effective and compliant use. ORs were calculated by conditional logistic regression analysis with adjustment for several confounders and test for trend for dose-response relationship.
Results: A total of 163,588 patients with type 2 diabetes and 490,764 matched control subjects were included with a mean age of 67 years and 55% male subjects. The crude OR of developing type 2 diabetes after alendronate use was 0.93 (95% CI 0.90-0.96) and decreased further after adjustment (multiple adjusted OR: 0.64 [95% CI 0.62-0.66]). The adjusted OR decreased to 0.47 (95% CI 0.40-0.56) among those with more than 8 years of alendronate use. A test for trend suggested a dose-response relationship between longer effective use of alendronate and lower risk of type 2 diabetes (p=0.002).
Conclusion: These results suggest a possible protective effect of alendronate in a dose-dependent manner against development of type 2 diabetes with a potential 50% risk reduction after 8 years of alendronate use. We propose future clinical research to investigate if alendronate impacts on glucose homeostasis, e.g. insulin sensitivity and glycemic control, and if it differs among people with and without pre- or type 2 diabetes.
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Disclosure: **R. Viggers:** Grants; Novo Nordisk Foundation, Denmark (Grant no. NNF18OC0052064).

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Sex-specific differences in patients deceased after bariatric surgery: a retrospective, registry analysis

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Background and aims: Bariatric surgery is an effective treatment strategy for patients with obesity. The majority of patients undergoing bariatric surgery are female. Motivation and reasons for bariatric surgery are different in men and women. Thus, the aim of this study was to analyze sex-specific differences with emphasis on patients deceased with a history of bariatric surgery in a large, registry analysis.
Materials and methods: The Austrian health insurance provides service for about 99% of all Austrian inhabitants. Data from inpatient and outpatient services comprising reimbursed drug prescriptions based on Anatomical Therapeutic Chemical (ATC) codes, medical diagnoses based on International Classification of Diseases (ICD) and medical procedures as MEL (medical single procedure) were available. Overall, 19 901 patients with a history of bariatric surgery ((HF220 (Sleeve Gastrectomy - open), HF230 (Sleeve Gastrectomy - laparoscopic), HF240 (Gastric Bypass - open), HF250 (Gastric Bypass - laparoscopic), HF254 (Biliopancreatic Diversion - open), HF255 (Biliopancreatic diversion - laparoscopic), HF260 (Gastric banding - open) and HF270 (Gastric banding - laparoscopic)) from January 2010 to December 2018 with 107 806 patient years of observation were included. In deceased patients, comorbidities of patients were analyzed based on ICD-codes and ATC-codes. Comorbidities associated with obesity were categorized in 4 groups: Diabetes mellitus (DM), cardiovascular diseases (CV), psychiatric disorders (PSY) and malignancies (M).
Results: The mean age at operation of all was 40.6 ± 12.5 years (men: 41.8±12.6, women: 40.1±12.4; p=0.000). Within the observation period from January 2010 to April 2020, 367 (1.8%) patients deceased. The mean follow-up of the total cohort was 5.4 ± 2.6 years. The total rate of mortality per year of observation was 0.34%. The sex-specific rate of mortality was 2.7-fold higher in men compared to women (0.64% vs. 0.24%). The 30-days mortality was 0.2% and five-fold higher in men compared to women (0.5% vs. 0.1%, $p < 0.001$). In both men and women, cardiovascular comorbidities (48%, men: 53%, women: 44%, $p = 0.09$) and psychiatric disorders (47%, men: 44%, women: 50%, $p = 0.212$) were the most common comorbidities. Diabetes and malignant diseases were identified in 36% (men: 41%, women: 32%, $p = 0.07$) and 33% (men: 29%, women: 36%, $p = 0.145$), respectively.
Conclusion: This analysis demonstrates a five-fold higher 30-days mortality in men compared to women after bariatric surgery. The long-term mortality was also higher in men. Cardiovascular comorbidities and psychiatric disorders are independently of sex, frequently observed comorbidities in this special cohort.
Disclosure: **H. Beiglböck:** None.

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Dietary factors and risk of islet autoimmunity and type 1 diabetes: a systematic review and meta-analysis

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Background and aims: Numerous dietary components have been linked to the development of islet autoimmunity (IA) and type 1 diabetes (T1D); however, no associations have been firmly established. This systematic review and meta-analysis aims to synthesize current knowledge on the association between diet and incidence of IA and T1D.
Materials and methods: The literature search was performed in Medline, Embase, and Cochrane Library, from inception until October 2020. Eligible studies had IA or T1D as outcome, any dietary exposure, case-control, cohort, or randomized controlled trial design, and hazard ratios, risk ratios, or odds ratios as measures of association. Summary relative risks (RR) and 95% confidence intervals (CI) of IA and T1D were estimated with random-effects models. Heterogeneity was quantified with the I^2 statistic, risk of bias in individual studies with the ROBINS-I and RoB 2 tools and certainty of evidence with the GRADE tool.
Results: Among 5935 identified records, 152 were eligible and pooled estimates could be produced for 27 dietary components. The risk of T1D, but not IA, decreased with later introduction to cow's milk (≥2-3 vs <2-3 months, RR: 0.69, CI: 0.59-0.81, I^2= 0%, moderate certainty) and gluten (3-6 vs <3-5 months, RR: 0.46, CI: 0.25-0.84, I^2=11%, high certainty), while it increased with later introduction to fruits (4-6 vs <4-5 months, RR: 2.14, CI: 1.16-3.94, I^2=0%, moderate certainty). Higher consumption of cow's milk products during childhood was associated with increased risk of both IA (per 2-3 portions/day, RR: 1.25, CI: 1.06-1.47, I^2= 0%, moderate certainty) and T1D (≥2-3 vs <2-3 glasses of milk/day, RR: 1.78, CI: 1.36-2.33, I^2= 0%, moderate certainty). Lower risk of T1D was observed in relation to longer total (≥6-12 vs <6-12 months, RR: 0.39, CI: 0.26-0.58, I^2=43%, high certainty) and exclusive (≥2-3 vs <2-3 months, RR: 0.69, CI: 0.58-0.81, I^2=0%, moderate certainty) breast-feeding. Age at introduction to infant formula, cereal, meat and vegetables and maternal intake of gluten, iron and vitamin D were not associated with the risk of T1D.
Conclusion: Early dietary factors, including cow´s milk, gluten, fruit and breast-feeding might play a role in the development of T1D. Further well-designed studies are needed to better understand these associations.
Supported by: Swedish Research Council and FORTE
Disclosure: **A. Lampousi:** None.

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Alterations in biomarkers of carbohydrate and lipid metabolism up to 20 years before the diagnosis of type 1 diabetes: findings from the AMORIS cohort

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Background and aims: Type 1 diabetes (T1D) is described to have an acute onset, but autoantibodies can appear several years prior to diagnosis, suggesting a longer preclinical phase. We assessed whether elevations in metabolic and inflammatory biomarkers were associated with future T1D risk and the temporal relationships.
Materials and methods: We studied 591,239 individuals free of diabetes from the Swedish AMORIS cohort followed from 1985-1996 (baseline period) to 2012. Through linkage to national patient, diabetes, and prescription registers, we identified incident T1D cases based on ICD-code and insulin or age at first recording ≤30 years. We used Cox regression models to estimate hazard ratios for biomarkers at baseline and incident T1D. We additionally assessed trajectories of biomarkers during the 25 years before T1D diagnosis in a nested case-control design.
Results: We identified 1,122 T1D cases during follow-up. The biomarkers glucose, fructosamine, triglycerides, the apolipoprotein B/A-I ratio, uric acid, and alkaline phosphatase were positively associated with T1D risk. A higher apolipoprotein A-I was associated with a lower T1D incidence. These associations were independent of age and sex of the individuals. We obtained similar results for glucose and fructosamine in individuals with age-of-onset ≤ 30 years. Already 15 years before diagnosis, T1D cases had higher mean glucose, fructosamine, triglycerides, and uric acid levels compared to controls.
Conclusion: Alterations in carbohydrate and lipid metabolism are associated with T1D risk, and biomarkers may be elevated several years preceding diagnosis. These data suggest that disease processes leading to T1D may occur decades prior to disease onset.
Supported by: Jungner Foundation for Laboratory Medicine, Swedish Research Council, FORTE, Novo Nordisk Foundation
Disclosure: **K. Herzog:** None.

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Decreasing age-at-onset of type 1 diabetes in a unique multigenerational cohort

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Background and aims: Type 1 diabetes (T1D) is an autoimmune disease in which both genetic and environmental components play important roles. Heterogeneity is increasingly recognised as a key feature of the condition, and the importance of age-at-onset of T1D is well documented. Diagnosis in early childhood is associated with a distinct immune infiltration of islets and extensive beta-cell loss, a higher genetic risk score and decreased residual serum C peptide, all linked to poorer outcomes in later life. Understanding the drivers of early-onset disease is therefore of critical importance. In recent decades, the increasing incidence of T1D at a population level has been associated with more children developing the condition in early childhood but few family data have been reported. This study aimed to examine age-at-onset in a UK population-based cohort of families of children with type 1 diabetes recruited since 1985.
Materials and methods: The Bart's Oxford (BOX) family study has been registering cases of T1D diagnosed under the age of 21 years and their relatives since 1985. Families with a grandparent or parent and child diagnosed with T1D were selected from a total of 534 families with 2 or more effected family members. Families where both parents were affected were excluded. In total, 264 offspring/parent pairs and 44 grandchild/grandparent pairs were identified. In a cohort of offspring (incl. probands, siblings and proband children) aged between 0.9 to 28.1 years (y), differences in generational ages at onset of diabetes were examined.
Results: Of 264 parent/offspring pairs, 223(84.4%) of offspring had parents who were diagnosed older than them, with a mean difference in ages of -13.5y(SEM+/-0.8; paired T-test (p)<0.0001, Table 1). This trend was maintained irrespective of the sex of the affected parent [mother (-15.17(1.5)y; p<0.0001) or a father (-12.17(0.96)y; p<0.0001)]. This trend was even more striking in grandparent/grandchild pairs, where the mean of differences was -28.37(2.3)y (p<0.0001). More stringent analyses (Table 1) revealed that the only group of parents not significantly older than their children were those diagnosed under the age of 7y.
Conclusion: The vast majority of T1D is idiopathic, with only 3-5% of subjects having an affected first degree relative. In this observational study of generational T1D, we show that age-at-diagnosis is decreased significantly across successive generations. As islet autoimmunity is increased in children

diagnosed at the earliest ages, these data suggest that disease severity in subsequent generations of the same families will be exacerbated. The mechanisms underlying this decrease in age-at-onset between generations are unknown, but it is unlikely to be driven solely by genetic causes, suggesting increasing environmental effects. Further epigenetic and genetic studies may reveal hitherto unidentified age-associated interactions between genes and environmental triggers.

		Median AaD*; y**(IQR)***		Mean of differences y(+/-SEM****)	Paired T test (p)
	n	Parent	Offspring		
All offspring v parents	264	**21.3** (12.2,31.8)	**9.5** (5.2,13.3)	**-13.52** (0.8)	**<0.0001**
All offspring v mothers	95	**25** (13,35)	**11** (5.4,14)	**-15.17** (1.5)	**<0.0001**
All offspring v fathers	169	**21** (12,29)	**9.4** (5,13)	**-12.59** (0.96)	**<0.0001**
All diagnosed <21y	137	**13** (7.1,17)	**9** (3.4,13)	**-3.55** (0.54)	**<0.0001**
Mothers diagnosed <21y	46	**12** (4.5,17)	**8.4** (3.4,12)	**-2.93** (0.88)	**<0.001**
Fathers diagnosed <21y	91	**13** (8.8,17)	**9** (3.4,13)	**-3.87** (0.67)	**<0.0001**
All parents 12-21y	65	**17** (15,20)	**10** (7,14)	**-7.06** (5.45)	**<0.0001**
All parents <7y	31	**3.5** (2.4,5.3)	**5** (2.2,7.5)	**1.99** (0.3)	**<0.05**
G/parents vs G/children	44	**39** (21,49)	**8.9** (5.5,11)	**-28.37** (2.3)	**<0.0001**

*AaD=age at diagnosis** y = age(years) ***IQR=interquartile range
****SEM=standard error of the mean of differences

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Disclosure: **P. Leete:** None.

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Follow up of a French cohort of children with a family history of type 1 diabetes

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Background and aims: The underlying pathological processes of type 1 diabetes (T1D) commence several years before the appearance of clinical symptoms. The annual incidence of T1D is increasing, particularly in children younger than 15 years. Though T1D is sporadic in 90% of cases, individuals with a family history of T1D have a 20 times greater risk of developing diabetes than the general population and may thus benefit from screening.
Materials and methods: Children of parents with T1D were screened annually at the T1D screening centre in Lille (France) from 1996. Genetic markers (HLA class II) were studied and annual tests for autoantibodies (GAD, AAI, ICA, IA-2) were performed. The principal objective was to identify the proportion of children who developed autoantibodies and those who developed T1D. The secondary objective was to characterise the serological profile of those children who developed autoantibodies and evaluate their risk of developing T1D.
Results: Of the 587 children studied, 16.4% showed at least one persistant autoantibody and 2.7% subsequently developed diabetes. Children with an HLA DR3/4 genotype showed an increased likelihood of developing an autoantibody during the study period. Children who developed their first autoantibody at younger than 8 years of age more frequently showed multiple autoantibodies during the course of the study (61.5% vs 28.1%; p=0.001). 32.3% of children who seroconverted were no longer autoantibody positive at their last visit, and none of these children subsequently developed diabetes. The risk of presenting with diabetes within 5 years increased with the number of autoantibodies detected during the study (4.2%, 5.3%, 59.6% and 45.1% respectively for 1,2,3 and 4 antibodies). The presence of multiple autoantibodies at the time of seroconversion was associated with an approximately 14.5 times greater risk of developing type 1 diabetes (HR 14.46 [4.97-42.07].
Conclusion: Our study evaluated the risk of developing type 1 diabetes in a French cohort of children of parents with type 1 diabetes. Detection of multiple autoantibodies was associated with an increased risk of developing T1D. The presence of a single autoantibody and/or transient seroconversion was associated with a lower risk. Our study, on a French cohort, currently represents the only data of this type, and may be useful in establishing new diabetes prevention protocols.
Disclosure: **A. Vambergue:** None.

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Large socioeconomic differences in life expectancy and years spent with complications of diabetes: a cohort study in the Scottish population with type 1 diabetes, 2013-2018

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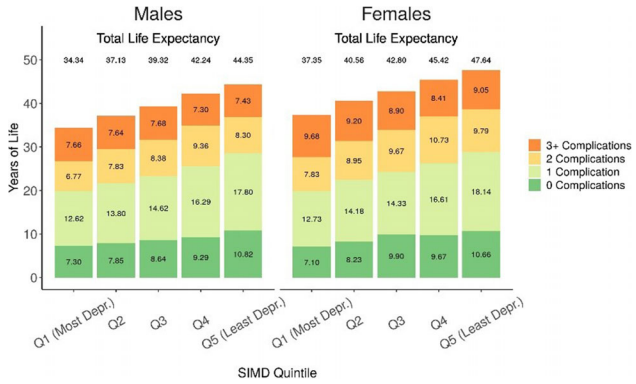
Background and aims: We aimed to examine current period life expectancy and life years spent with complications of diabetes among a national cohort of individuals with type 1 diabetes and to quantify socioeconomic differences in these measures.
Materials and methods: We studied 22370 individuals aged 30 and older with type 1 diabetes alive at any point in time between 1/1/2013 and 31/12/2018 in the national diabetes register. We used the clinical records in the register to capture prevalent and incident retinopathy/maculopathy, cardiovascular disease, nephropathy, and neuropathy, and also linked to mortality records. We captured socioeconomic status using the Scottish Index of Multiple Deprivation (SIMD) 2016. For each individual, we constructed a history of transitions to one or more of the above complications or death. We used parametric multistate survival models to estimate remaining life expectancy at an attained age of 30 and the expected number of years spent in particular health states.
Results: At an attained age of 30, remaining life expectancy was 42.18 years ((95% Confidence Intervals) 41.78-42.59) among females and 38.94 years (38.17-39.70) among males with type 1 diabetes, compared with 51.72 years and 48.00 years among the general population. Remaining life expectancy at an attained age of 30 was around 10 years lower among the most deprived quintile when compared with the least deprived quintile: 37.35 years (36.01-38.68) vs. 47.64 years (46.08-49.20) among females and 34.34 years (33.18-35.50) vs. 44.35 years (42.86-45.84) among males. Differences in the expected average number of years without complications were around 4 years lower among the most deprived quintile when compared with the least deprived quintile: 7.10 years (6.00-8.19) vs. 10.66 years (9.14-12.18) among females and 7.30 years (6.37-8.23) vs. 10.82 years (9.49-12.16) among males.
Conclusion: Differences in life expectancy between the population with type 1 diabetes and the general population have decreased in Scotland. However, our results indicate large socioeconomic differences in life expectancy and years of life spent without complications of diabetes. New initiatives to reduce these gaps are required.



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Disclosure: **A. Höhn:** None.

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Risk of incident obstructive sleep apnoea in patients with type 1 diabetes: a population-based matched controlled cohort study

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Background and aims: Type 2 diabetes (T2D) is associated with increased risk of incident obstructive sleep apnoea (OSA). In T2D, OSA is associated with a higher risk of diabetes-related vascular complications, poor quality of life, and mortality. Several cross-sectional studies showed high prevalence of OSA in type 1 diabetes (T1D). However, it is unknown whether patients with T1D are at increased risk of incident OSA. Therefore, we aimed to assess the incidence of developing OSA in patients with T1D compared to an appropriately matched control population, and identify the predictors of incident OSA in T1D. This is important in T1D considering the adverse outcomes associated with OSA and the impact on driving.

Materials and methods: We utilised the Health Improvement Network (THIN), which is a large UK primary care database, to conduct a retrospective cohort study. Each patient with T1D (exposed) was matched for age, sex, body mass index (BMI), and general practice to up to four persons without diabetes (control). Patients with OSA at baseline or a diagnosis of T2D at any time were excluded. The study period was 1st January 1990 to 31st August 2019. Cox regression was used to calculate hazard ratios using Stata IC version 15.

Results: 163,647 patients (34,147 exposed and 129,500 matched controls) were included. For the whole cohort, mean (SD) age was 38 (18) years, 93,082 (56.9%) were men, and BMI was 25.8 (4.3) kg/m^2. The median (IQR) follow-up was 5.43 (2.19-10.11) years. Compared to patients without T1D, the adjusted hazard ratio (aHR) of incident OSA in patients with T1D was 1.67 (95% CI 1.42-1.96; P < 0.001) after adjusting for age, sex, BMI categories, Townsend quintiles (social deprivation), smoking status, ethnicity, alcohol, cardiovascular disease, hypertension, and atrial fibrillation. In T1D, predictors of incident OSA were people in their forties, male sex, obesity, atrial fibrillation, depression, and being prescribed antihypertensive or lipid-lowering drugs (table 1).

Conclusion: Patients with T1D are at increased risk of incident OSA compared to the general population. Clinicians need to consider OSA in patients with T1D despite having lower prevalence of obesity than T2D, especially in those who have one or more of the incident OSA predictors identified in this study.

Table 1: Predictors of incident OSA in patients with type 1 diabetes

Predictors	Hazard ratio (95% CI); p-value
10 -20 years	0.11 (0.01 to 0.94); p=0.044
40 - 50 years	2.93 (1.04 to 8.31); p=0.043
Men	2.68 (1.94 to 3.69); p<0.001
Overweight	1.87 (1.21 to 2.88); p=0.005
Obese	6.16 (4.07 to 9.30); p<0.001
Depression	1.96 (1.39 to 2.75); p<0.001
Use Lipid-lowering drugs	1.91 (1.37 to 2.66); p<0.001
Using antihypertensive drugs	1.46 (1.01 to 2.10); p=0.042
Atrial fibrillation	3.15 (1.22 to 8.11); p=0.018

This model included the following variables: diabetes duration, age at diagnosis with T1D, hypoglycaemia, foot disease, depression, anxiety, retinopathy, age categories, sex, BMI categories, Townsend categories, drinking status, ethnicity, eGFR categories, HbA1c categories, ACR categories, heart failure hypertension, ischaemic heart disease, stroke and TIA, serious mental illness, atrial fibrillation, and using lipid-lowering or antihypertensive drugs.

Disclosure: **A.A. Tahrani:** None.

OP 14 "Humanomics" in diabetes

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Fasting lipidomic analysis: a tool to unveil type 2 diabetes heterogeneity

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Background and aims: Type 2 diabetes (T2D) is a heterogeneous condition whose greatest impact comes from its complications. However, just the gradient of glycemia does not explain them. Lipid and glucose metabolism are closely linked. Thus, plasma lipidomics will be an important tool to unveil diabetes phenotypes. We hypothesize that groups stratified by insulin resistance (HOMA-IR), secretion rate (ISR) and clearance (IC) are associated with distinct glucose and lipid profiles. Considering both glycemia and lipidomics will help understand T2D's heterogeneity and identify risk biomarkers associated with distinct mechanisms and new therapeutic targets, towards precision medicine.
Materials and methods: PREVADIAB2 includes 974 subjects profiled for glucose, insulin and C-peptide (OGTT) and surrogate indexes of dysmetabolism including a fatty liver score (NAFLD-FLS). For a sample of 145 women (68 NGT/NFG, 63 IGT and/or IFG, and 14 T2D), mean age 62y, lipidomic profile was performed by LC/MS on fasting serum samples with the quantification of triacylglycerols (TAG), ceramides (CER), sphingomyelin (SM) and phospholipids (PC, LPC). These subjects were then stratified using a hierarchical clustering algorithm informed by HOMA-IR, fasting ISR ($_f$ISR) and IC ($_f$IC). The resulting clusters (Cl) were profiled for several parameters and it was assessed whether there was a lipidomic pattern in tandem with the one from other dysmetabolic parameters. Statistical significance of mean differences was assessed with Bonferroni correction.
Results: The identified groups and their lipidomic profile are shown in Fig. Cl1 had the lowest HOMA-IR and $_f$ISR and highest $_f$IC ($p<0.001$). Also, had low glycemia (OGTT), the lowest NAFLD-FLS ($p<0.01$), TAG ($p<0.01$) and low CER. Consistently, it had high LPC18:2, that has been inversely correlated with IR. TAG48:0, a marker of *de novo* lipogenesis, was increased in Cl2, 3 and 4 ($p<=0.02$). Cl2 and 3 had lower $_f$IC and higher NAFLD-FLS, HOMAIR and $_f$ISR than Cl1 ($p<0.01$), however $_f$ISR was lower on Cl3 ($p<0.01$). Additionally, Cl3 had higher glycemia at 30' and 120'(OGTT) than Cl1 and 2 ($p<0.01$). Cl2 and 3 had higher TAG than Cl1 ($p<0.01$). Interestingly, Cl2 also had higher CER than Cl1($p<0.01$) whereas Cl3 had lower SM ($p=0.02$). Cl4 had a high proportion of dysglycemia, similar to Cl3. However, it had the highest HOMAIR, $_f$ISR and NAFLD-FLS ($p<0.01$), whereas $_f$IC was similar to Cl3 ($p=1$). Cl4 showed high levels of TAG and low levels of most SM species, as Cl3. Surprisingly, Cl4 CER levels were lower than Cl2 ($p=0.01$).
Conclusion: Clustering individuals by distinct metabolic defects identifies different degrees of glucose tolerance and lipidomic patterns.

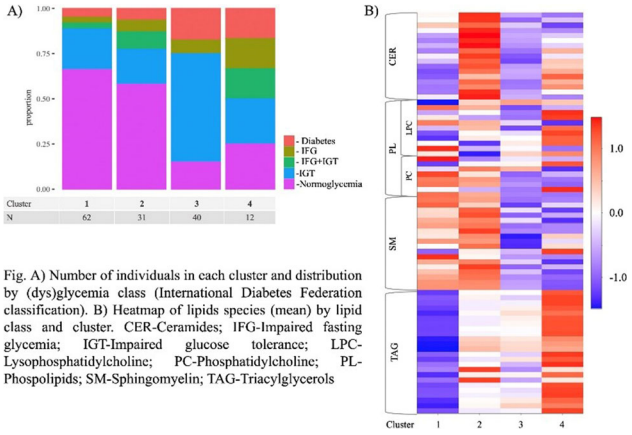

Fig. A) Number of individuals in each cluster and distribution by (dys)glycemia class (International Diabetes Federation classification). B) Heatmap of lipids species (mean) by lipid class and cluster. CER-Ceramides; IFG-Impaired fasting glycemia; IGT-Impaired glucose tolerance; LPC-Lysophosphatidylcholine; PC-Phosphatidylcholine; PL-Phospolipids; SM-Sphingomyelin; TAG-Triacylglycerols

Supported by: PDTC, FoieGras
Disclosure: **A.F. Pina:** None.

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Metabolite differences between type 2 diabetes and type 3c diabetes secondary to chronic pancreatitis based on an untargeted metabolomics approach

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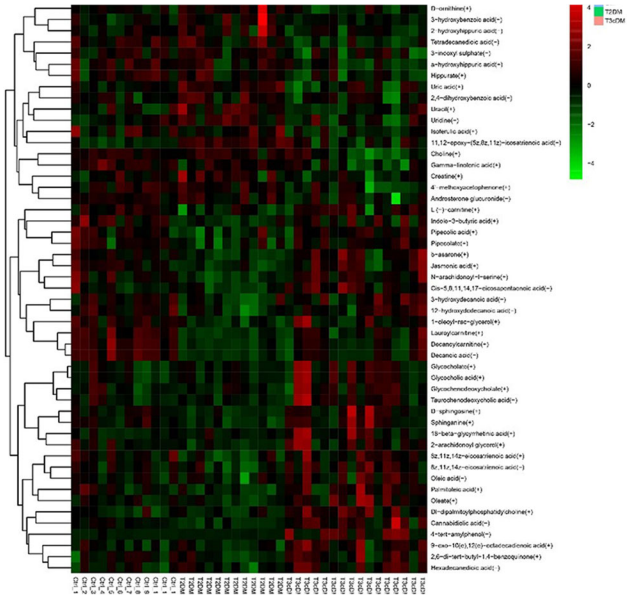
Background and aims: Type 3c diabetes mellitus (T3cDM) referred to diabetes secondary to exocrine pancreatic diseases, and chronic pancreatitis (CP) had proven to be one of the leading causes. In this study, a nontargeted metabolomics approach was established to characterize serum metabolic profile in T3cDM and compare with type 2 diabetes mellitus (T2DM).
Materials and methods: There were 16 patients with T3cDM, twelve gender and age-matched T2DM and 12 healthy controls recruited for metabolite analysis basing on liquid chromatography-mass spectrometry (LC-MS). Principal component analysis (PCA), partial least squares method-discriminant analysis (PLS-DA), differential metabolite volcanic plot, cluster heatmap, and KEGG metabolic pathway enrichment analysis were used to analyze the specific and differential metabolites.
Results: Fifty-one metabolites including lipids, carnitine, bile acid and hippuric acid were found to be different between T2DM and T3cDM, mainly enriched in fatty acids biosynthesis, primary bile acid biosynthesis, cholesterol metabolism, bile secretion and phenylalanine metabolism.
Conclusion: By LC-MS approach, this study identified the potential differential metabolites between T2DM and T3cDM. T3cDM is characterized by increased carnitine, bile acid and most of lipids, providing novel biomarkers for clinical diagnosis and future direction in pathophysiological mechanisms.



Clinical Trial Registration Number: ChiCTR1800018247
Disclosure: **L. Qi:** None.

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Regional adipose tissue differences of the proteome reveals an enhanced antioxidative and chaperone activity as a feature of lower body fat in humans

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Background and aims: The special functions of gluteofemoral adipose tissue appear to convey protection against type 2 diabetes. We searched for specific features in the proteome of gluteofemoral fat tissue to further understand the specialised function.
Materials and methods: Adipose tissue biopsies were taken from abdominal subcutaneous (ASAT) and from gluteal adipose tissue (GSAT) of six healthy women. The proteome was analysed by two-dimensional gel (2-DG) electrophoresis followed by tandem mass spectrometry. Top candidates were followed up by transcriptomic profiles, scRNASeq and immuno-histochemistry.
Results: A total of 131 proteins showed ≥2-fold between ASAT and GSAT samples. The top candidate was Haptoglobin (*HP*) which was 18-fold higher expressed (p=0.01) in GSAT compared to ASAT. Single cell RNASeq of the human mature adipose and stromovascular fractions (SVF) revealed *HP* expression in both adipocytes and macrophages, and most highly in a macrophage M2 subpopulation. The pattern of expression was the same in ASAT and GSAT but significantly lower in ASAT (p=0.01). In vitro differentiation of ASAT and GSAT preadipocytes showed maintained higher *HP* expression in differentiating GSAT cells (p=0.01). Of note, the macrophage expression of *HP* had a cellular co-expression with its receptor, *CD163*. Further analysis of GSAT proteome revealed a number of additional enriched proteins protecting against redox and oxidative attack (eg, superoxide dismutase, hemopexin, catalase, peroxiredoxin-1, peroxiredoxin-2, ferritin light chain) and certain chaperones (heat-shock protein beta-5, heat-shock protein 27).
Conclusion: The present study reveals a co-ordinated defence against oxidative stress in GSAT. We propose that local production of haptoglobin provides a first line defence for preadipocytes in the adipose tissue to support the safe long-term storage of fat in the tissue.
Disclosure: **M. Ahmed:** None.

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Distinct associations of plasma methionine and cysteine with regional fat distribution: the CODAM and the Maastricht studies

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Background and aims: Sulfur amino acids (SAAs), including the essential amino acid methionine and its main derivative cysteine, have been associated with obesity and related metabolic diseases, including type 2 diabetes (T2D). Yet, it is not known if SAAs are related to specific patterns of regional fat distribution.
Materials and methods: We examined the relationships of fasting methionine and total cysteine, measured in EDTA plasma using LC-MS/MS, with measures of obesity, body fat distribution and liver fat in two cross-sectional studies enriched with (pre)diabetic individuals: the CODAM cohort (n=478, 61.5% men, 67±7 yrs) and The Maastricht Study (DMS; n=424, 54.7% men, 61.6±8.7 yrs). Main outcomes included BMI, waist circumference, trunk subcutaneous or visceral fat (CODAM and DMS), body composition on dual-energy X-ray absorptiometry (DMS), a liver enzyme score calculated from the standardized plasma levels of the three liver enzymes AST, ALT and GGT (CODAM), fatty liver on ultrasound (CODAM) and liver fat % on MRI (DMS). Associations were examined with linear or logistic regressions, as appropriate, with z-standardized primary exposures and outcomes. Adjustment was made for relevant confounders related to cohort structure, demographics and lifestyle (including protein and alcohol intake, and physical activity), and in DMS also for lean mass (not available in CODAM).
Results: In CODAM, methionine was associated with liver enzyme score (β=0.24; 95% CI: 0.14, 0.36) and fatty liver (OR=1.49; 1.19, 1.88), but not with any measure of obesity. Cysteine was associated with BMI (β=0.19; 0.09, 0.28), waist (β=0.17; 0.08, 0.25) and subcutaneous fat (β=0.15; 0.05, 0.24), but not with visceral fat (β=0.05; -0.04, 0.14) or fatty liver (OR=1.15; 0. 29, 1.43). In DMS, methionine, but not cysteine, was associated with liver fat % (β=0.13; 0.01, 0.26). Cysteine was associated with BMI (β=0.11; 0.02, 0.19) and gynoid fat distribution (β=0.14; 0.06, 0.22), but not with waist (β=0.04; -0.04, 0.11), subcutaneous fat (β=0.07; -0.03, 0.17), visceral fat (β=0.04; -0.05, 0.12) or android fat distribution (β=0.07; -0.02, 0.15).
Conclusion: Methionine and cysteine showed distinct associations with different fat depots, which were similar between studies.

Methionine was associated with liver fat, implicated in the development of insulin resistance and T2D. By contrast, cysteine was associated with BMI and peripheral fat depot. These results call for further research on the role of SAAs in regional fat distribution, and their relationship with T2D.
Supported by: Zon-MW and JPI-HDHL
Disclosure: **E.C. Tore:** Grants; Zon-MW, JPI-HDHL.

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Thrifty energy phenotype predicts weight regain: results of a randomised controlled trial

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Background and aims: Weight loss is associated with an improvement of insulin sensitivity. Both, a negative energy balance and changes of body composition are integrative components of weight loss interventions. However, the individual impact of these two components on insulin sensitivity and energy metabolism is unclear.
Materials and methods: We performed a randomized controlled trial including 80 overweight or obesepost-menopausal women. Participants randomly assigned to the intervention group underwent an 800 kcal/d liquid diet for 2 months followed by four weeks in which the formula diet was substituted by a calorie reduced healthy diet to facilitate further weight loss. This weight loss phase was followed by a 4-week weight maintenance phase, where weight stability was achieved by individualized daily caloric intake without negative energy balance. Volunteers of the control group were instructed to keep their weight stable during the entire period of 4 months. Metabolic phenotyping was performed in both groups at baseline (M0), after weight loss (M3) and after the maintenance period (M4). Additional phenotyping was performed during follow-up at 12 (M12) and 24 months (M24). Primary outcomes were changes of lean body mass (LBM) and changes of insulin sensitivity (ISI_{Clamp}) between baseline and M3 and M4. Estimates of energy metabolism were secondary endpoints.
Results: No significant changes of body weight or LBM were found in the control group between any time points. A significant reduction of body weight, fat mass (FM) and LBM was found in the intervention group between M0 and M3, while no further change was seen between M3 and M4. Only subjects of the intervention group were characterized by an improvement of the second primary outcome ISI_{Clamp} at M3, which was preserved until M4. Notably, a lower resting energy expenditure per LBM (REE_{LBM}) at M3 as well as the individual difference of REE_{LBM} between M3 and M4 significantly predicted a stronger regain of fat mass during follow-up.
Conclusion: In summary, our data demonstrate that modulation of LBM and insulinsensitivity during weight loss is predominantly driven by changes in body weight and body composition, rather than an individual effect of negative energy balance. However, the variance in energy expenditure during negative and steady energy balance indicates a thrifty phenotype, which is highly susceptible to future regain of fat mass.
Clinical Trial Registration Number: NCT01105143
Supported by: BMBF
Disclosure: **K. Mai:** None.

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Cardiovascular risk of former obesity in healthy-weight Americans

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Background and aims: Little research investigates whether effects of obesity persist in those who subsequently achieve and maintain healthy weight. Here we profile formerly-obese American adults and compare their cardiovascular risk factors to those who are currently obese, and to those who were always healthy weight.
Materials and methods: Using data from NHANES 1999-2013 (n=20,271), we define three groups: currently obese; always healthy-weight; and formerly obese. These groups were compared in their prevalence of hypertension, dyslipidemia, and diabetes, in models first crude and then corrected for age, gender, smoking and ethnicity.
Results: Formerly-obese subjects (n=326) were older than those who were never (n=6235) or currently-obese (n=13,710), and likelier to smoke cigarettes (36%, vs. 24 and 19%.) However, after correction their risks of hypertension and dyslipidemia were comparable to those who were never obese (ORs 1.08 and 1.13, both $p>0.10$.) Their risk of diabetes was intermediate between those who were never- and currently-obese (OR 2.93 for former obesity, OR 7.53 for current) Currently-obese subjects were also at elevated risk of hypertension (OR 3.14) and dyslipidemia (OR 3.11.) All p-values were <0.01 unless stated.
Conclusion: Major weight loss appeared to reverse most of the cardiovascular risks associated with obesity, even in those who continued to smoke and especially in those who quit. Risk of diabetes, but not hypertension or dyslipidemia, remained elevated over those who were never obese: however, even diabetes risk dropped with weight loss.
Disclosure: **M.P. Smith:** None.

OP 15 Fat in the liver: where it comes from and how it can be prevented

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Long-term effect of a diet high in unsaturated fat and dietary protein on intrahepatic lipids and circulating FGF21 levels: results of a randomised controlled trial

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Background and aims: Short-term trials indicate reduction of intrahepatic lipids (IHL) by either high protein or increased unsaturated fat intake beyond energy restriction. Human Fibroblast Growth Factor 21 (FGF21), a hepatokine closely linked to hepatic lipid accumulation, was shown to be strongly affected by dietary protein content. However, human data on dietary induced changes of FGF21 levels are scarce. We investigated the long-term effect of a diet focusing on high protein and increased unsaturated fat intake (NutriAct) on IHL as well as circulating FGF21 levels to analyze potential underlying mechanisms.
Materials and methods: Within a 36-months multi-center trial, 502 subjects (50-80 years) were randomized to either NutriAct intervention group (IG) high in mono-/polyunsaturated fat (15-20%E/ 10-15%E), plant protein (15-25%E) and fibre (≥30g/day) or control group (CG, usual care) based on recommendations of the German Nutrition Society. The intervention included nutritional sessions and supplementation of newly designed foods mirroring NutriAct pattern. IHL were analyzed by proton magnetic resonance spectroscopy in 346 subjects before and in 258 subjects after 12 months. Subjects with significant alcohol consumption were excluded from the present analysis. Plasma FGF21 levels were measured before and after 12 months using ELISA.
Results: A stronger IHL reduction was seen in the IG after 12 months by trend (-33.3% [95%-confidence interval (CI) -24.5,-41.1] vs. -21.8% [95%-CI -11.0,-31.3] in CG, p=0.071). Analysis of IG subjects adherent to NutriAct intervention revealed significantly higher IHL reduction compared to CG subjects with lower protein and unsaturated fat intake (-40.7% [95%-CI -31.9,-48.4] vs. -15.7% [95%-CI -1.1,-28.2], p=0.001), which persists after adjustment for weight loss, sex and age (p=0.014). At baseline, plasma FGF21 levels correlated with IHL (r=0.326, p<0.001, n = 345) and inversely with dietary protein content (r= - 0.191, p<0.001, n = 429). FGF21 levels decreased in both groups with comparable magnitude (IG (n = 187): - 27.1 pg/ml (SD 136.0), p = 0.007 vs. CG (n = 187): - 12.4 pg/ml (SD 133.9), p = 0.208; p for difference between groups = 0.291). Reduction of FGF21 was associated with increase of dietary protein intake (r = -0.128, p = 0.015, n = 358) and with IHL decrease (r = 0.279, p < 0.001, n= 258) among all participants.
Conclusion: The NutriAct pattern induced a more pronounced long-term improvement of liver fat. This was associated with FGF21 reduction, which could be a potential mediator in the context of diet-induced decline in liver fat. The NutriAct pattern might constitute a beneficial strategy in middle-aged and elderly people in Germany.
Clinical Trial Registration Number: DRKS00010049
Supported by: Federal Ministry of Education and Research (BMBF funding code 01EA1806)
Disclosure: **C. Wernicke:** None.

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Fructose intake from fruit juice and sugar sweetened beverages, but not from fruit, is associated with higher intrahepatic lipid content: The Maastricht Study

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[1]Internal Medicine, Maastricht University, Maastricht, [2]Epidemiology, Maastricht University, Maastricht, [3]Radiology and Nuclear Medicine, Maastricht University Medical Center+, Maastricht, [4]Internal Medicine, Maastricht University Medical Center+, Maastricht, [5]Complex Genetics and Epidemiology, Maastricht University, Maastricht, Netherlands.

Background and aims: There has been an ongoing debate on whether dietary fructose is a modifiable risk factor of non-alcoholic fatty liver disease. The aim of the present study was, therefore, to assess the relationship between different sources of dietary fructose and intrahepatic lipid content (IHL) at the population level.
Materials and methods: We used cross-sectional data from The Maastricht Study, a population-based cohort (n=3,981; 60±9 years; 50% women). We assessed the relationship between habitual fructose intake (assessed by a FFQ) - total and derived from fruit, fruit juice and sugar sweetened beverages (SSB) - and log-transformed IHL (quantified by 3T Dixon MRI) with adjustment for age, sex, type 2 diabetes (T2D), educational level, smoking status, physical activity, and intakes of total energy, alcohol, saturated fat, protein, vitamin E, and dietary fiber.
Results: Energy-adjusted total fructose intake was not associated with IHL in the fully adjusted model (p=0.647). Energy-adjusted intake of fructose from fruit was associated with lower IHL after adjustment for dietary factors (p=0.044), but this association was no longer statistically significant after additional adjustment for dietary fiber (p=0.767). In contrast, energy-adjusted intake of fructose from fruit juice and SSB was associated with higher IHL in the fully adjusted model (p=0.019 and p=0.006, respectively). Individuals in the highest quartile of energy-adjusted intake of fructose from fruit juice and SSB had a 1.05-fold (95% CI: 0.98; 1.12) and 1.08-fold (95% CI: 1.00; 1.16) higher IHL, respectively, when compared to the lowest quartile in the fully adjusted model. Finally, the association for fructose from fruit juice were stronger in individuals with T2D (p for interaction=0.071).
Conclusion: Fructose from fruit juice and SSB, but not from fruit, is independently associated with higher IHL. These findings support the recommendation to reduce intake of fructose-containing beverages as a means to prevent hepatic steatosis and cardiometabolic disease at the population level.
Supported by: The Maastricht Study was supported by the European Regional Development Fund via OP-Zuid, the Province of Limburg, the Dutch Ministry of Economic Affairs (grant 31O.041), Stichting De Weijerhorst (Maastricht, the Netherlands), the Pearl String Initiative Diabetes (Amsterdam, the Netherlands), School for Cardiovascular Diseases (CARIM, Maastricht, the Netherlands), School for Public Health and Primary Care (CAPHRI, Maastricht, the Netherlands), School for Nutrition and Translational Research in Metabolism (NUTRIM, Maastricht, the Netherlands), Stichting Annadal (Maastricht, the Netherlands), Health Foundation Limburg (Maastricht, the Netherlands), and by unrestricted grants from Janssen-Cilag B.V. (Tilburg, the Netherlands), Novo Nordisk Farma B.V. (Alphen aan den Rijn, the Netherlands), Sanofi-Aventis Netherlands B.V. (Gouda, the Netherlands), and Medtronic (Tolochenaz, Switzerland).
Disclosure: **A.M. Buziau:** None.

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Hepatic glycogen and whole-body fat oxidation are not modulated by one night of prolonged fasting in people with non-alcoholic fatty liver

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Background and aims: Time-restricted eating has been shown to improve metabolic health, even in energy balance. These beneficial effects may at least partially be due to creating a more pronounced fasting state, leading to larger fluctuations in hepatic glycogen. Here, we investigated in individuals with non-alcoholic fatty liver (NAFL) whether acutely prolonging an overnight fast from 9.5 hours to 16 hours reduces overnight hepatic glycogen and improves substrate metabolism and whether intrahepatic lipid content and lipid composition is improved after 5 days of such a regime.
Materials and methods: Ten overweight/obese volunteers (BMI 30.1 ±2.2 kg/m^2; age 62±8.7 year; 8 men) with NAFL (intrahepatic lipid content: 13.3±7.7 %weight/weight) participated in a randomized cross-over trial, in which hepatic glycogen was measured by ^{13}C-MRS after a standardized lunch at 2 pm and in the morning at 6.30 am after a 9.5h or 16h fast. Substrate oxidation was determined overnight as well as in the morning upon a meal using whole-body respirometry, and hepatic lipid content and composition were measured by ^{1}H-MRS before and after continuing the prolonged overnight fasting protocol for 5 days.
Results: Remarkably, hepatic glycogen did not decline between afternoon and morning after a 9.5h fast (+4.1±3.3%) and prolonging the fast to 16h did not significantly improve this decline in glycogen (-2.4±4.7%) (figure 1A). In addition, prolonging the overnight fast did not have acute effects on whole-body fat oxidation during the night (2.2±0.2 vs. 2.1±0.1 kJ/min, figure 1B) or upon a breakfast meal. Continuing the prolonged overnight fasting protocol for 5 days did not significantly influence hepatic lipid saturation (SFA +0.8 vs. +2.7%) nor hepatic lipid content (-0.6 vs. -0.6% weight/weight).
Conclusion: These results suggest that hepatic substrate metabolism is rather inert in individuals with NAFL and not sensitive to extending acute fasting periods.

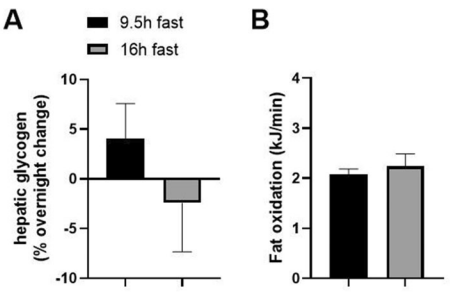

Figure 1: Relative change in hepatic glycogen from 2 pm in the afternoon to 6.30 am in the morning (A) and whole-body fat oxidation during the night (B) when fasting for 9.5h and 16h. * p<0.05, paired sampled t-test, n=10.

Clinical Trial Registration Number: NCT03593343
Supported by: This research was in part financed by the Ministry of Economic Affairs and Climate Policy by means of the PPP Allowance made available by the Top Sector Life Sciences & Health to stimulate public-private partnerships and by Unilever R&D Wageningen. HP was an employee of Unilever at the time this research was designed and conducted and has since changed his professional affiliation to Superfoods, Landsmeer.
Disclosure: **K.H.M. Roumans:** None.

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Visceral adipose tissue mitochondrial function is reduced in humans with non-alcoholic fatty liver disease and correlates with insulin resistance

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Background and aims: Adipose tissue mitochondrial dysfunction may drive the development of type 2 diabetes (T2D) and non-alcoholic (NA) fatty liver disease (NAFLD), comprising NA fatty liver and steatohepatitis (NAFL and NASH). Potential underlying mechanism comprise impaired adipose tissue mitochondrial mass and respiration favoring increased fatty acid flux to ectopic tissues. Thus, we examined subcutaneous (SAT) and visceral adipose tissue (VAT) mitochondrial oxidation in humans with different degrees of insulin resistance and varying liver histology.
Materials and methods: Obese people without NAFL (OBE-CON, n=20, 38±8 years, body mass index 53±6 kg/m^2, 10% T2D), with NAFL (OBE-NAFL, n=20, 40±8 years, 51±5 kg/m^2, 25% T2D) or NASH (OBE-NASH, n=20, 42±10 years, 51±6 kg/m^2, 40% T2D) underwent intensive baseline metabolic characterisation and tissue biopsies. O_2 flux rates from different substrates were measured with high resolution respirometry in SAT and VAT.
Results: In VAT, maximal uncoupled respiration was lower in OBE-NAFL (least square means (LSM): 0.46 pmol*mg wet weight^{-1}*s^{-1} [95% confidence interval 0.21;0.71], p<0.05) and OBE-NASH (LSM: 0.51 [0.26;0.76], p<0.001) compared to OBE-CON. Similar differences were seen for maximal ADP-stimulated respiration in VAT (following the administration of succinate). In Pearson correlation analysis, maximal ADP stimulated O_2 flux in VAT positively correlated with whole-body insulin sensitivity (M value; r= 0.29, p<0.05). Mitochondrial content in VAT, as assessed from mitochondrial DNA copy number, was not different between the groups. Protein expression of electron transport chain complexes was similar in VAT and SAT of all groups, except for complex IV that tended to be lower in VAT in OBE-NASH than in OBE-CON (p=0.065). Of note, a comparable oxidative capacity was revealed in all three groups in the SAT, despite a higher mitochondrial content in OBE-NASH than in OBE-CON (p=0.045).
Conclusion: VAT mitochondrial respiration was reduced in obese humans with NAFLD compared to those without and correlated positively with whole-body insulin sensitivity, demonstrating an adipose tissue-specific effect of mitochondrial function on insulin resistance and hepatic lipid accumulation in the context of obesity.

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Multi-organ multiparametric magnetic resonance imaging reveals distinct ectopic fat distribution in type 2 diabetics with and without co-existing obesity

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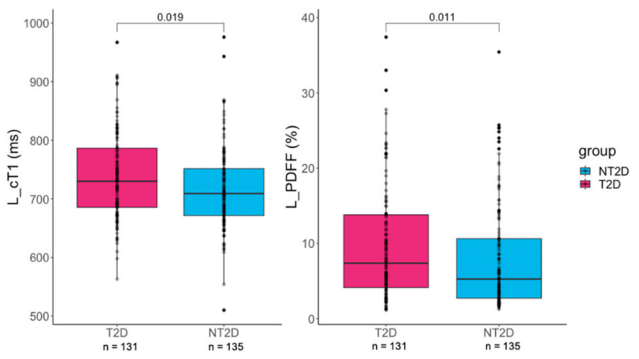
Background and aims: Type 2 diabetes (T2D) is associated with multi-organ dysfunction and ectopic fat accumulation within the liver, pancreas and as visceral adipose tissue (VAT). Multiparametric magnetic resonance (mpMR) can provide a non-invasive assessment of multi-organ health, including measures of steatosis (PDFF) and fibro inflammation (cT1). Here we collect mpMR markers of the liver, pancreas, VAT, subcutaneous adipose tissue (SAT) and skeletal-muscle index (SMI), comparing T2D and non-T2D (NT2D) participants matched for age, gender and BMI.
Materials and methods: MR images and patient demographics were collected from the UK Biobank online resource. Liver and pancreas images were analysed by placing three regions of interest (ROIs) within the organ parenchyma only on PDFF and T1 maps. Images extracted from the 3rd lumbar vertebrae were manually segmented for SAT, VAT and SMI. Measures of high density lipoprotein (HDL), glycated haemoglobin (HbA1c), aspartate aminotransferase (AST) and alanine transaminase (ALT) were collected. Data from 297 participants (131 T2D, 136 NT2D) were included for analysis. Wilcoxon tests were performed to compare differences in biomarkers between the two groups. Data is median [IQR].
Results: Participants with T2D had significantly higher liver fat (7.4% [4.1-13.8] vs 5.3% [2.7-10.6]; p=0.011) and cT1 (730ms [685-786] vs 709ms [671-753]; p=0.019), despite no differences in AST (p=0.35) or ALT (p=0.11). Significantly lower measures of SMI (45.2cm^2/m^2 [38.1-52.9] vs 50.6cm^2/m^2 [42.6-55.9]; p=0.003) and HDL (1.1mmol/L [1-1.3] vs 1.3mmol/L [1.1-1.5]; p<0.0001) were observed within the T2D group. We found no significant differences in VAT (p=0.35), SAT (p=0.43) or pancreas PDFF (p=0.22). However, all mpMR metrics were significantly greater (p<0.001) in obese vs normal weight participants, irrespective of T2D status.
Conclusion: In BMI, age and gender matched participants from the UK Biobank, mpMR reveals significantly greater liver fat and fibroinflammation in those with T2D, despite no differences in AST or ALT. Pancreatic fat and VAT were significantly greater in obese participants but were similar in both T2D and NT2D groups.



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Disclosure: **T. Waddell:** Employment/Consultancy; Employee at Perspectum Ltd.

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Inferring causal pathways between metabolic processes and liver fat accumulations: an IMI DIRECT study

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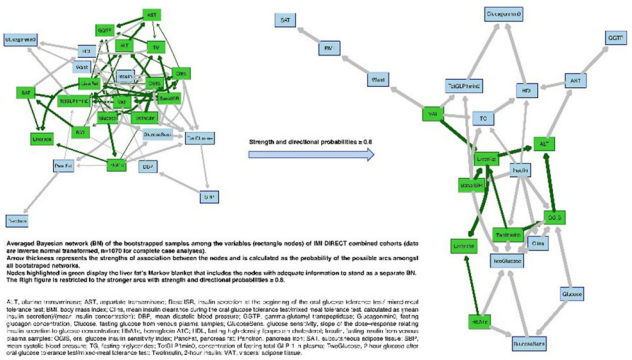
Background and aims: Many observational studies show that type 2 diabetes (T2D) and non-alcoholic fatty liver disease (NAFLD) coincide. However, the causal nature of these relationships is unclear. Here, we aimed to assess the strength and magnitude of the putative causal pathways linking dysglycemia and fatty liver using a combination of causal inference methods.
Materials and methods: Measures of glycemia, insulin dynamics, MRI abdominal and liver fat content, serological biomarkers, and anthropometry were obtained in participants from the IMI DIRECT cohorts (n=795 with new-onset T2D and n=2234 without T2D). UK Biobank was also used for modeling and replication purposes (n=4617 who had liver fat MRI measurement). Bayesian networks (BNs) were employed to build graphical models from joint probability distributions of variables to infer causal pathways. To attain a stable structure, model averaging was performed on the bootstrapped BNs. To validate these causal pathways, a series of bidirectional two-sample Mendelian randomization (MR) analyses was undertaken among all possible combinations of the selected variables.
Results: BNs in the IMI DIRECT combined cohorts identified higher basal insulin secretion rate (BasalISR) and excess visceral fat (VAT) accumulation most likely to cause liver fat accumulation (shown in the figure). Conditioning on high levels of BasalISR and VAT significantly increased the unconditional probability of high liver fat level on the random observations generated by the BNs; 23% increase after conditioning on VAT, 32% increase after conditioning on BasalISR, and 40% after conditioning on both. Similarly, VAT was the primary causal variable for liver fat accumulation in the UK Biobank BN analysis (BasalISR was not measured in the UK Biobank). Through the MR analyses, several nominal directional associations between hepatic biomarkers, glycemic, and adiposity measures were suggested after Bonferroni correction and pleiotropy sensitivity analysis. In most cases, the effects predicted by the BNs were confirmed in the MR analyses.
Conclusion: Through the conducted analyses BasalISR had the highest upstream causal effect on the liver fat content, highlighting the strong link between NAFLD and T2D. Understanding more about the BasalISR mechanism of action on liver fat accumulation and considering it as a biomarker measurement in clinical practice may help in the diagnosis, prevention, and treatment of these highly prevalent and linked conditions.



Supported by: 'IMI DIRECT was supported by the Innovative Medicines Initiative Joint Undertaking under grant agreement n°115317 (DIRECT), resources of which are composed of financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013) and EFPIA companies' in kind contribution.'
Disclosure: **N. Atabaki Pasdar:** None.

OP 16 CKD in diabetes - a costly complication

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Inside CKD: modelling the clinical and economic impact of routine screening for albuminuria in people with type 2 diabetes

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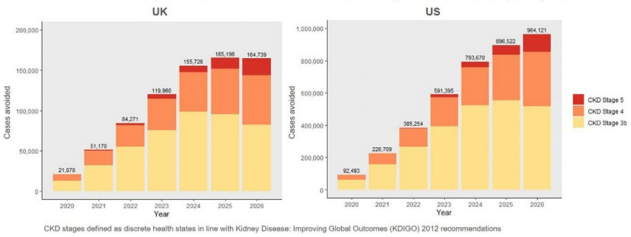
Background and aims: Early diagnosis of chronic kidney disease (CKD) in patients with type 2 diabetes (T2D) followed by guideline-recommended interventions is key to slowing CKD progression, but adherence to screening recommendations is suboptimal. *Inside CKD* models the global burden of CKD using country-specific, patient-level microsimulation models. We modelled the effects of targeted implementation of urine albumin:creatinine ratio (UACR) measurement and intervention in patients with T2D.
Materials and methods: We used the *Inside CKD* microsimulation to model the impact of measuring UACR during routine primary care visits with subsequent intervention in patients with T2D aged ≥ 45 years with a range of kidney functions, versus current practice. Virtual populations were constructed using published country-specific data on demographics, CKD (albuminuria and estimated glomerular filtration rate status), T2D, comorbidities and complications.
Results: Preliminary data for three countries show that from 2020 to 2026, the measurement of UACR with subsequent intervention in patients with T2D would prevent CKD progression to stages 3b to 5 in 164 739 patients in the UK, 964 121 in the US (**Figure**) and 156 482 in Canada. Associated cost savings would be £0.14B, US$13.81B and C$2.34B. Additional countries will be analysed.
Conclusion: Routine UACR measurement with subsequent intervention could potentially reduce the global burden of CKD and healthcare costs in patients with T2D and improve patient outcomes.

Figure: Cumulative numbers of CKD cases prevented from progressing to CKD stages 3b–5 from 2020 to 2026 by routine measurement of UACR and subsequent intervention in primary care



CKD stages defined as discrete health states in line with Kidney Disease: Improving Global Outcomes (KDIGO) 2012 recommendations

Disclosure: **S. Nolan:** Employment/Consultancy; Stephen Nolan is an employee of AstraZeneca. Stock/Shareholding; Stephen Nolan holds stock options at AstraZeneca.

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Hyper-filtration most strongly predicts decline in estimated glomerular filtration rate: results from analysis of 73,583 person-years

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Background and aims: We examined predictors of change in estimated glomerular filtration rate (ΔeGFR) per year calculated using serum creatinine.

Materials and methods: This retrospective study comprised from Periods I from April 2005 to September 2006, Period II from April 2009 to September 2010, Period III from April 2013 to September 2014, Period IV from April 2017 to September 2018. Participants were 10,698 people (7137 men, 3561 women; mean age 48 years) who underwent annual medical checkups during the above periods, and we detected the longest observation period for each person in order to evaluate ΔeGFR (ml/min/1.73 m^2/year) during the observation period. We performed stepwise regression analysis with ΔeGFR as the dependent variable to examine its associations with baseline variables and use of lipid-lowering drugs.

Results: There were 73,583 person-years of observation, and the mean observation period was 7 ± 3 years. Stepwise regression (SR) in people with diabetes (n = 1037) revealed that baseline eGFR (standardized β [Sβ] = -0.512, $p < 0.001$) was the strongest independent determinant of ΔeGFR (-1.1 ± 2.6 ml/min/1.73 m^2/year), and age (Sβ = -0.259, $p < 0.001$), fasting plasma glucose (FPG) (mg/dL) (Sβ = -0.107, $p < 0.001$), uric acid (UA) (mg/dL) (Sβ = -0.096, $p = 0.004$), female sex (Sβ = -0.094, $p = 0.006$), exercise habit (EH) (min/week) (Sβ = 0.124, $p < 0.001$), aspartate aminotransferase (AST) (U/L) (Sβ = 0.100, $p = 0.001$), and ethanol intake (EI) (g/week) (Sβ = 0.064, $p = 0.038$) were also significantly associated with ΔeGFR, but BMI (kg/m^2), waist circumference (WC) (cm), systolic BP (mmHg), diastolic BP (mmHg), total cholesterol (mg/dL), HDL-cholesterol (mg/dL), triglycerides (mg/dL), LDL-cholesterol (mg/dL), alanine aminotransferase (U/L), gamma-glutamyl transpeptidase (GGTP) (U/L), cholinesterase (U/L), hepatitis B antigen or hepatitis C antibody positivity, C-reactive protein (CRP) (mg/dL), smoking index (SI) (cigarettes per day × years of smoking), vegetable intake (VI) (at least one plate/every day), and use of lipid-lowering drugs were not significantly associated. SR in people without diabetes (n = 9661) revealed that baseline eGFR (Sβ = -0.625, $p < 0.001$) was the strongest independent determinant of ΔeGFR (-1.6 ± 2.9 ml/min/1.73 m^2/year), and age (Sβ = -0.189, $p < 0.001$), female sex (Sβ = -0.128, $p < 0.001$), BMI (Sβ = -0.092, $p < 0.001$), UA (Sβ = -0.067, $p < 0.001$), diastolic BP (Sβ = -0.023, $p = 0.025$), WC (Sβ = 0.112, $p < 0.001$), EH (Sβ = 0.093, $p < 0.001$), HDL-cholesterol (Sβ = 0.061, $p < 0.001$), EI (Sβ = 0.044, $p < 0.001$), FPG (Sβ = 0.034, $p = 0.001$), GGTP (Sβ = 0.026, $p = 0.011$), VI (Sβ = 0.024, $p = 0.010$), and SI (Sβ = 0.020, $p = 0.033$) were also significantly associated with ΔeGFR, but other variables were not significantly associated. ΔeGFR was significantly higher in people with diabetes than in those without diabetes ($p < 0.001$).

Conclusion: Among the variables investigated, baseline eGFR indicating hyper-filtration was significantly and most strongly associated with decline in eGFR during the observation period in people with diabetes as well as those without diabetes.

Clinical Trial Registration Number: 20-130 5420

Disclosure: **S. Katoh:** None.

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Metabolic syndrome, and not obesity, is associated with chronic kidney disease in the general US population

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Background and aims: Obese patients are at increased risk of chronic kidney disease, but it is still unclear whether this can be attributed to obesity per se or to the associated metabolic derangements. The aim of this study is to evaluate the relative impact of obesity and metabolic syndrome (MS) on kidney disease.

Materials and methods: This is a cross-sectional study based on data obtained in the 2005-2016 cycles of the National Health and Nutritional Examination Survey (NHANES). Adult participants were considered eligible if data were available on body mass index (BMI), estimated glomerular filtration rate (eGFR), urine-albumin to creatinine ratio (UACR) and each of the MS components. Based on the presence of obesity and MS, participants were categorized in four groups: metabolically healthy non-obese (MHNO), metabolically unhealthy non-obese (MUNO), metabolically healthy obese (MHO) and metabolically unhealthy obese (MUO). Primary outcomes were eGFR<60 ml/min and UACR≥30 mg/g. Logistic regression analysis was performed to evaluate the association between obesity, MS and kidney outcomes after adjustment for age, sex and race/ethnicity.

Results: The studied population comprised 12174 participants (MHNO: 5937; MUNO: 1844; MHO: 1574; MUO: 2819). MHO patients were younger and more commonly female. Compared with MHNO participants, an increased prevalence of albuminuria and reduced eGFR were present in both the MUNO group (OR 1.57, 95% CI 1.28-1.93, p<0.001 and OR 1.56, 95% CI 1.17-2.07, p=0.003, respectively) and the MUO group (OR 2.25, 95% CI 1.88-2.70, p<0.001 and OR 1.76, 95% CI 1.37-2.26, p<0.001, respectively), but not in the MHO group (OR 0.95, 95% CI 0.73-1.27, p=0.774 and OR 0.94, 95% CI 0.61-1.46, p=0.789, respectively). When each of the MS components was evaluated separately, elevated blood pressure and HDL cholesterol were associated with both albuminuria and reduced eGFR, while elevated blood glucose and triglycerides were only associated with albuminuria.

Conclusion: This large cross-sectional study suggests that MS and not obesity per se is associated with kidney damage. In particular, the MHO patients does not seem to carry an increased risk of kidney disease.

Disclosure: **E. Muraca:** None.

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Non-dipping of nocturnal blood pressure is associated with increased risk of mortality and kidney disease in type 1 diabetes

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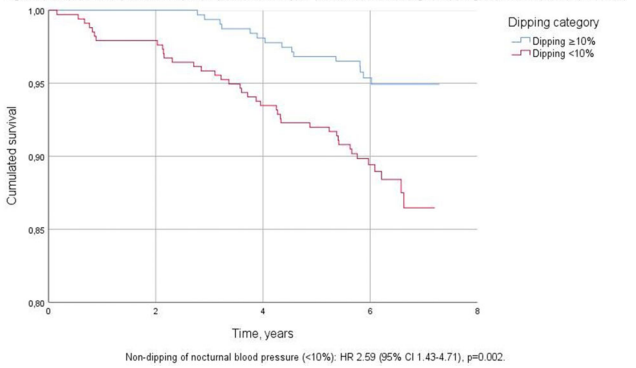
Background and aims: Persons with type 1 diabetes (T1D) have increased risk of cardiovascular disease (CVD) and mortality. A 24-hour ambulatory BP measurement (ABPM) evaluates diurnal variations in BP and has previously been shown to be superiorly correlated to future CVD compared to office and home BP measurements; however, its role in T1D remains to be determined. This study aims to determine the prognostic significance of 24-hour ABPM and non-dipping of nocturnal BP for CVD, mortality and kidney disease in persons with T1D.

Materials and methods: A cohort of 654 participants with T1D was examined from 2009 through 2011 at Steno Diabetes Center Copenhagen, including measurements of 24-hour ABPM using a tonometric wrist-watch device (BPro, HealthStats, Singapore). In 2016, outcomes (CV events, all-cause mortality, decline in eGFR ≥30%, end-stage kidney disease (ESKD) and a composite kidney event of decline in eGFR ≥30%, ESKD and mortality) were registered and hazard ratios (HR) were calculated using Cox regression analyses. Non-dipping was defined as a nocturnal decline in systolic BP of less than 10%.
Results: Participants were mean ± SD 55 ± 12 years old, had median (IQR) 35 (24-44) years duration of diabetes, 55.4% were men, BMI 25.5 ± 5.8 kg/m^2, eGFR 88 (65-110) ml/min/1.73m^2, HbA_{1c} 64.4 ± 12.6 mmol/mol and office systolic and diastolic BP of 132 ± 17 mmHg and 74 ± 9 mmHg. After a median of 6.2 years for all-cause mortality and 5.2 years for the remaining outcomes, we registered 90 new CV events, 54 mortality events, 92 events of decline in eGFR ≥30%, 21 events of ESKD and 120 composite kidney events. The participants had a mean daytime systolic BP of 133 ± 16 mmHg and nighttime systolic BP of 121 ± 16 mmHg with 337 (51.5%) participants being classified as having non-dipping of nocturnal BP. In unadjusted analyses, non-dipping was associated with all-cause mortality (HR 2.59 (95% CI 1.43-4.71), p=0.002, see Figure 1), decline in eGFR ≥30% (HR 1.85 (1.21-2.83), p=0.004), ESKD (HR 4.24 (1.43-12.60), p=0.009) and the composite kidney event (HR 2.45 (1.66-3.62), p<0.001), but not with new CV event (HR 1.25 (0.82-1.89), p=0.30). After adjustments for age, sex, diabetes duration, HbA_{1c}, BMI, HDL, smoking, previous CVD, use of antihypertensive medication/statins/diuretics, office systolic BP, urine AER and eGFR, non-dipping was associated with all-cause mortality (HR 2.01 (1.08-3.91), p=0.03) and the composite kidney event (HR 1.85 (1.21-2.84), p=0.005).
Conclusion: We find that non-dipping of nocturnal BP in persons with T1D is associated with an increased risk of mortality and kidney events. Although the BP measurements were from a wrist-watch device, this is the first time such results are demonstrated in persons with T1D.

Figure 1. Kaplan-Meier survival curve for persons with type 1 diabetes according to dipping of nocturnal blood pressure.



Disclosure: **H. Hjortkjær:** None.

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High plasma concentrations of folic acid are associated with increased risk of graft failure in renal transplant recipients with type 2 diabetes

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Background and aims: The role of folic acid in the progression of kidney disease has gained attention in recent years. Furthermore, alterations in one carbon metabolism, and deleterious effects of circulating folate in T2D patients have been described. Nevertheless, the association of circulating folic acid and graft failure remains undetermined. Hence, we aimed to investigate the prospective association of folic acid with graft failure in renal transplant recipients (RTRs) with and without Type 2 Diabetes.
Materials and methods: We included 340 RTRs from the of the TransplantLines prospective cohort, conducted in the Groningen region, in the north of the Netherlands. Out of the 340 RTRs, 170 RTRs with T2D were matched with RTRs without diabetes using a Propensity Score, with an Optimal Matching method. Cox proportional-hazards regression analyses were performed to study the association of plasma folic acid with graft failure. The main outcome was graft failure, which was defined as re-transplantation or return to dialysis and was censored for death.
Results: Among RTRs (age 57.2 ± 10.3 years; 55% males), baseline median folic acid was 11.0 (6.0, 20.0) μmol/L in RTRs with T2D and 10.0 (5.3, 18.0) μmol/L in RTRs without T2D ($p = 0.68$). After a median follow-up of 6.1 (5.6-6.7) years, graft failure was observed in 24 RTRs with T2D and in 18 RTRs without T2D. In RTRs with T2D folic acid was associated with increased risk of graft failure, independent of age, sex, systolic blood pressure, HDL-cholesterol, triglycerides, immunosuppressive therapy, albuminuria and eGFR ($_{adj}$HR per 1-SD increase, 1.69 (95% confidence interval (CI): 1.10; 2.61, $p = 0.01$). Importantly, folic acid was not associated with increased risk of graft failure, neither in the crude model, nor in the full adjusted model in RTRs without T2D ($_{adj}$HR per 1-SD increase, 0.75 (95% confidence interval (CI): 0.37; 1.38, $p = 0.32$).
Conclusion: This study suggests that circulating concentrations of folic acid are associated with an increased risk of graft failure only in RTRs with T2D, but not in RTRs without T2D. Further investigation, in relation to folic acid activating mechanisms in T2D patients with kidney disease is needed.
Disclosure: **J.L. Flores-Guerrero:** None.

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Fully automated closed-loop versus standard insulin therapy in adults with type 2 diabetes requiring dialysis: a randomised controlled trial

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Background and aims: Diabetes management in patients with end-stage renal disease on dialysis is challenging due to complex interactions with glucose metabolism. We evaluated safety and efficacy of fully closed-loop compared with standard insulin therapy in adults with type 2 diabetes (T2D) requiring maintenance dialysis.
Materials and methods: In an open-label, multinational, two centre, randomised, crossover trial, 26 adults with T2D requiring dialysis (17 males, mean±SD: age 68±11 years, diabetes duration 20±10 years) underwent two 20-day periods of unrestricted living, comparing the CamAPS HX fully closed-loop system using faster insulin aspart, with standard insulin therapy (control) in random order.
Results: The proportion of time in the target glucose range (5.6 to 10.0mmol/L; primary endpoint; Table1) was 52.8±12.5% with closed-loop vs. 37.7±20.5% with control; mean-adjusted difference 15.1 percentage points (95% CI 8.1 to 22.1; P<0.001). Mean glucose was lower with closed-loop than during the control (10.1±1.3 vs. 11.6±2.8mmol/L; P=0.002). Time in hypoglycaemia (<3.9mmol/L) was reduced with closed-loop vs. control (median [IQR] 0.1 [0.0-0.4%] vs. 0.2 [0.0-0.9%]; P=0.040). Total daily insulin requirements were similar between

interventions. No episodes of severe hypoglycaemia occurred during control period and one occurred during closed-loop period but not during closed-loop operation.

Conclusion: Fully closed-loop versus standard insulin therapy in adults with T2D requiring dialysis improved glucose control whilst reducing hypoglycaemia, demonstrating safety and efficacy in this vulnerable population.

Table 1. Comparison of primary and secondary outcomes between closed-loop and control.

	Closed-loop (n=26)	Control (n=26)	P-value
Proportion of time spent at glucose level (%):			
5.6 to 10.0 mmol/L*	**52.8 (12.5)**	**37.7 (20.5)**	**<0.001**
3.9 to 10.0 mmol/L	57.1 (14.3)	42.5 (24.7)	0.001
> 10.0 mmol/L	42.6 (14.3)	56.6 (25.1)	0.002
> 20.0 mmol/ L	1.8 (2.4)	6.7 (10.7)	0.012
< 5.6 mmol/ L	3.2 (2.0, 7.0)	4.0 (0.9, 9.5)	0.869
< 3.9 mmol/ L	0.12 (0.02, 0.44)	0.17 (0.00, 1.11)	0.040
< 3.0 mmol/ L	0.00 (0.00, 0.03)	0.00 (0.00, 0.22)	0.047
Mean glucose (mmol/ L)	10.1 (1.3)	11.6 (2.8)	0.002
Standard deviation of glucose (mmol/L)	3.2 (0.7)	3.6 (0.9)	0.019
CV of glucose (%)	31.7 (4.8)	31.5 (5.4)	0.873
Between days CV of glucose (%)	30.8 (3.4)	31.2 (5.8)	0.723
Total daily insulin dose (U/kg)	0.34 (0.15, 0.54)	0.36 (0.19, 0.58)	0.368
Total daily insulin dose (U)	20.4 (9.2, 50.3)	32.2 (12.1, 54.4)	0.383
Time using sensor glucose (%)	95 (94, 96)	94 (90, 95)	0.062
Time using closed-loop (%)	93 (89, 94)	-	-

*Primary endpoint
Data presented as mean (SD), or median (interquartile range)
CV - coefficient of variation

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Disclosure: **L. Bally:** None.

OP 17 Don't stop moving: beneficial effects of exercise on diabetes and beyond

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Regular exercise training improves skeletal muscle glucose uptake in monozygotic twin pairs discordant for body weight

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Background and aims: Skeletal muscle plays an important role for whole-body insulin sensitivity. Body adiposity is associated with decreased skeletal muscle insulin sensitivity, while exercise counteracts this effect. However, it is unclear whether the response to exercise is affected by body adiposity *per se,* because training-induced adaptations may be confounded by genetic factors. Thus, we investigated the effects of body adiposity and regular exercise training on insulin-stimulated skeletal muscle glucose uptake (GU) in various muscles while controlling genetic variability by studying monozygotic (MZ) twin pairs discordant for body weight.

Materials and methods: We recruited MZ twin pairs discordant for BMI (difference at least 2 $kg \cdot m^{-2}$) from three twin cohorts (FinnTwin12, FinnTwin16 and the Finnish twin Cohort study). Of the contacted 47 MZ twin pairs, 12 MZ pairs participated in the study (8 female, 4 male pairs; mean age 40.4 (SD) 4.5 years; leaner twins mean BMI 29.1 (SD) 6.3, heavier twins mean BMI 36.7 (SD) 7.0, $p<0.001$). Of these participants 8 pairs have completed the intervention. Participants performed a 6-month-long exercise-intervention (two endurance, one high-intensity interval training and one resistance exercise session per week). Insulin-stimulated GU was studied by positron emission tomography (PET) using [^{18}F]FDG during euglycaemic hyperinsulinemia in erector spinae (ES), biceps brachii (BB), latissimus dorsi (LD), quadriceps femoris (QF) and psoas major (PM) muscles before and after the intervention. Data was analysed by hierarchical linear mixed model.

Results: At baseline, leaner twins had smaller fat percentage as well as better cardiorespiratory fitness (VO_{2max}) and whole-body insulin sensitivity (M-value; $p<0.01$ in all), than their heavier co-twins. Compared with heavier twins, the leaner co-twins had higher GU in ES, BB, LD and QF muscles ($p<0.05$, in all) but not in PM ($p=0.68$). Exercise intervention increased M-value and VO_{2max} (time $p<0.05$ for both) similarly in both leaner and heavier twins (time x group: $p>0.23$), but had no effect on BMI or whole-body fat percentage (time: $p>0.30$). Exercise intervention increased GU in ES, LD and PM (time: $p<0.05$) and tended to increase in QF ($p=0.068$) similarly in both leaner and heavier twins (time x group: $p>0.39$).

Conclusion: When genetic variability is controlled, body adiposity is associated with decreased insulin-stimulated skeletal muscle glucose uptake. Moreover, regular exercise training improves skeletal muscle insulin sensitivity, independent of body adiposity.

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Dynamic profiling of the metabolic response to endurance exercise

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Background and aims: Physical exercise is a potent remedy to prevent obesity-associated metabolic diseases. However, the mechanisms underlying its beneficial effects are still incompletely understood. Assessing the dynamic changes in the plasma metabolome during an acute exercise challenge could reveal novel aspects of the metabolic adaptation to a training intervention that are not captured by assessing the resting metabolome only.
Materials and methods: Sedentary obese, non-diabetic subjects (14 female, 8 male) performed 8 weeks of supervised endurance training (3x1 hour/week, 80% VO_2 peak). As previously published, this intervention improved VO_2 peak and aerobic threshold, with minor reduction of the BMI and a slight, but non-significant increase in insulin sensitivity (ISI_{Mats}). The first and last training session were designed as acute endurance exercise challenges. Here, subjects arrived in the fasting state and resting blood samples were obtained before the participants had a standardized breakfast. After another 45 minutes, subjects performed 30 minutes of bicycle ergometer exercise at the end of which another blood sample was drawn. Plasma samples were analyzed using liquid chromatography- mass spectrometry (LC-MS) and capillary electrophoresis (CE)- MS which together covered a broad range of metabolites (>300) with mixed polarity. Statistical analysis was performed using the Mixed Model platform in JMP 14.2.0 (SAS).
Results: The plasma metabolomics pattern was pronouncedly altered by the acute exercise challenges. Metabolites acutely affected by exercise ($p<0.05$, fold change >10%) were mostly increased. This increase was particularly pronounced (>100%) for hypoxanthine and lactate, both before and after the training intervention. The increase of several metabolites, including most amino acids, during the pre-intervention challenge correlated with the improvement of insulin sensitivity during the intervention. Compared to the acute exercise challenges, training only caused minor alterations in the resting metabolome. However, several metabolites exhibited a different regulation between the initial and the final challenge, and most of these, e.g. different steroid sulfates, were attenuated after training. Thus, the challenged metabolome exhibited a stronger response to the training intervention than the resting metabolome.
Conclusion: Our results indicate that metabolomic profiling of an acute exercise challenge is useful in assessing and, potentially, predicting metabolic adaptations to an endurance training intervention in sedentary, diabetes-prone subjects.

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Effects of regular exercise and carnosine on muscle energy metabolism and cognitive performance in the overweight elderly population

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Background and aims: Sedentary ageing accelerates decline of metabolic health and cognitive reserve, while regular exercise effectively supports metabolic and cognitive health in ageing population. Here we tested the hypothesis that carnosine, a dipeptide with the physiological function in skeletal muscle and brain, enhances the adaptive response to regular exercise in the elderly at the level of whole-body and muscle energy metabolism, physical fitness and cognitive performance.
Materials and methods: Glucose tolerance (oral glucose tolerance test), metabolic flexibility (ΔRQ) & insulin sensitivity (euglycemic hyperinsulinemic clamp/EHC & indirect calorimetry), resting energy expenditure & metabolic substrate preference (indirect calorimetry), body composition (bioelectrical impedance), physical fitness (Rockport 1 mile walk test, VO_2max; sit-to-stand test) and cognitive functions (ACE-R, computerized CogState) were assessed. Bergström needle biopsy of *m. vastus lateralis* was performed. Oxygen consumption rate (pmol/s/mg tissue wet weight) was evaluated in saponin-permeabilized muscle fibers by O_2k high-resolution respirometry (Oroboros). Sixty sedentary (BMI 27.1 ±3.9kg/m^2) elderly volunteers (age 66.9±1.2yrs, MMSE score 28.2±1.2) were subjected to either 3-month supervised aerobic-strength training (3x1h/week) (n=36) or stretching exercise training (n=24, active controls). Half of the individuals in each intervention group were randomized to take oral carnosine (2g/day), or placebo. RM-2-way ANOVA and paired T-test was used.
Results: Training intervention combined with carnosine improved performance in sit-to-stand test (9.2%, $p<0.05$), metabolic flexibility (ΔRQ 7.3%, $p<0.05$) and aerobic fitness (VO_2max 14.8%, $p<0.05$). Combination of exercise with carnosine has been more effective in stimulating coupled mitochondrial respiration rate in permeabilized muscle fibers, as compared to exercise alone (36.5%, $p<0.01$). Addenbrooke's cognitive examination (ACE-R) score correlated positively with lean body mass and muscle strength and the training-induced change in ACE-R memory subscore correlated with concurrent change of lean body mass. Metabolic flexibility (capacity to increase RQ during EHC) correlated positively with short term memory and executive functions (CogState score).
Conclusion: We provide the evidence pointing towards synergistic effects of regular exercise and carnosine on systemic and muscle metabolism which has a potential to improve cognitive functions in overweight sedentary elderly individuals.

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Resistance exercise training protects beta cell from apoptosis in an in vitro model of type 1 diabetes

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Background and aims: Resistance exercise training exerts beneficial effects on glycemic control in type 1 diabetic (T1D) patients, which could be mediated by exercise-induced humoral factors released in the bloodstream. Here, we aimed to verify if resistance training would be able to protect beta-cell from apoptosis in an in vitro model of T1D by using the serum from resistance-trained mice.
Materials and methods: C57BL/6 mice were randomly assigned into two groups: control (CON) and resistance exercise training (RET). RET mice performed 1 training session/day, 5 days/week for 10 weeks. Each training session consisted of 8 climbs, carrying progressively heavier loads (50, 75, 90 and 100% of the animal's maximal voluntary carrying capacity "MVCC"). We analyzed strength, body weight, perigonadal fat content and gastrocnemius weight (n=8). For in vitro experiments, we used a rat pancreatic beta-cell line (INS-1E) incubated with medium containing 10% of serum from control or trained mice for 24h; followed by exposure to 10 U/ml recombinant human Interleukin-1β (IL-1β) and 100 U/ml recombinant rat Interferon-γ (IFN-γ) (in vitro T1D), for 24h. The cells were used for Western Blotting analysis and apoptosis measurement by Hoechst-Propidium iodide (HO-PI) fluorescence quantification (n=2-4). Data were analyzed by Student´s t-test or One-Way ANOVA with an unpaired Tukey's post-hoc test. Data are mean ± SEM, and the difference between the groups were considered statistically significant if $P \leq 0.05$.
Results: As expected, after 10 weeks of training, RET mice presented higher MVCC, compared with CON mice (CON 42.83 ±1.75 g x RET 67.85±1.47 g). RET mice also presented reduced body weight when compared to CON (CON 30.88±1.19 g x RET 26.95±1.0 g), in addition to lower perigonadal fat pad and higher gastrocnemius weight (CON 890.2±95.98, 935.3±30.99 g x RET 521.7±33.53, 1028±23.55 g % body weight, respectively). The in vitro experiments demonstrated that in normal conditions there were no differences between treatment with control or trained serum in neither of the parameters analyzed. However, when cells were exposed to IL-1β plus IFN-γ, the increased iNOS and Cleaved caspase-3 protein content in INS-1E cells, pretreated with control serum, were partially blocked in INS-1E cells pretreated with trained serum (Serum CON 3.66±0.86 AU, 2.55±0.13 AU x Serum RET 1.98±0.45 AU, 1.34±0.16 AU, iNOS/α-Tubulin and Cleaved casp-3/α-Tubulin, respectively). We also measured the pro- and antiapoptotic proteins BAX and bcl-2, both related to the mitochondrial pathway of apoptosis, and there were no differences between groups regarding protein content (Serum CON 3.32 ±0.88 AU, 1.28±0.17 AU x Serum RET 2.18±0.69 AU, 1.35±0.17 AU, Bax/α-Tubulin and bcl-2/α-Tubulin, respectively) and BAX/bcl-2 ratio (BAX/bcl-2 ratio: Serum CON 1.98±0.45 AU x Serum RET 1.48±0.39 AU). In addition, we observed that the apoptosis rate in INS-1E cells, cultured in a medium containing trained serum was lower than in INS-1E cells, cultured in a medium containing control serum (% of dead cells: Serum CON 21.14 ±0.55 x Serum RET 11.18±0.02).
Conclusion: These results indicate that the serum from resistance-trained mice is able to protect beta-cell from cytokine-induced apoptosis. Suggesting that resistance exercise training could be an important strategy to protect beta-cell from apoptosis in the diabetes context.
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High intensity interval training improves insulin sensitivity and affects mitochondria dynamics in skeletal muscle of type 2 diabetes humans

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Background and aims: High intensity interval training (HIIT) improves insulin sensitivity and oxidative capacity in humans with type 2 diabetes (T2D). Furthermore exercising induces mitochondrial biogenesis in healthy humans, but its effect on mitochondrial remodeling in insulin resistant states is not known. This study aims to examine the effect of HIIT on mitochondrial fusion and fission as well as on mitochondria respiration in the skeletal muscle of type 2 diabetes (T2D), insulin resistant (IR) and insulin sensitive (IS) glucose tolerant humans in order to elucidate the role of mitochondrial turnover in muscle insulin sensitivity.
Materials and methods: Twenty T2D, 11 glucose-tolerant IR and 12 IS humans, age- and BMI-matched, performed HIIT 3 days per week on a cycle ergometer (4 x 4 min intervals at 90% and 3 min of recovery at 70% of maximal heart rate for a total of 35 min) for 12 weeks. Before the intervention (baseline) and 72 h after the last exercise bout, whole-body insulin sensitivity was measured by hyperinsulinemic-euglycemic clamps, mitochondrial respiration and mitochondrial dynamics were assessed in skeletal muscle biopsies by high resolution respirometry and western blot, respectively. Finally skeletal muscle citrate synthase activity was assayed spectrophotometrically by a commercial kit.
Results: After 12 weeks of HIIT, all participants uniformly improved their cardiorespiratory fitness ($p<0.001$ *vs* baseline), whereas insulin sensitivity increased only in T2D and IR individuals ($p<0.01$ *vs* baseline). Moreover, muscle maximal oxygen uptake ($p<0.001$ *vs* baseline) as well as muscle citrate synthase activity ($p<0.01$ *vs* baseline) rose in all groups after HIIT, while biomarkers of mitochondrial fission ($p<0.01$), fusion ($p<0.001$) and mitophagy ($p<0.001$ for phospho-Parkin(Ser65) and $p<0.05$ for phospho-Pink(Thr257)) increased only in T2D.
Conclusion: In conclusion mitochondrial fusion and fission as well as mitophagy are critical to maintain a functional mitochondrial pool and likely contribute to the exercise training response of insulin sensitivity in overt T2D. These findings help to better understand the metabolic adaptation induced by lifestyle intervention and to identify novel targets for the tailored prevention and treatment of insulin resistance.
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Disclosure: **L. Mastrototaro:** None.

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TGFβ/mir143/145 associated mis-differentiation affects exercise response

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Background and aims: Physical training is a promising strategy to prevent type 2 diabetes (T2D), improving insulin sensitivity. However, a substantial number of individuals lack a beneficial outcome in glycemic control. TGFβ was identified as a possible upstream regulator involved in this low-response. TGFβ is known to be a potent regulator of microRNA (miRs) expression. Aim of this study was therefore to elucidate the role of TGFβ-driven miRs on skeletal muscle differentiation and individual exercise response.
Materials and methods: By small and long RNA sequencing, we identified TGFβ-regulated transcripts in primary human myoblasts, afterwards fused to myotubes, utilizing TGFβ and SB431542. Identified miRs were investigated in human skeletal muscle biopsies obtained before and after a supervised 8 weeks-endurance training intervention, in relation to the change in insulin sensitivity (ISIMats) (n=40 consenting donors). Reported results were significant ($p \leq 0.05$) according to appropriate statistical tests e.g. ANOVA Tukey.
Results: Enrichment analyses of lcRNAseq data corroborated the negative impact of TGFβ on myotube differentiation. Non-targeted miRNAseq analyses identified several miRs including miR143/708/181/31/145 upregulated and miR499/208/146/139/502 downregulated by TGFβ in myotubes (top 5). miR expression pattern during myotube differentiation revealed that TGFβ effects on miR499/208/206/146/139 could not be distinguished from its negative effect on differentiation. Specific regulation of miR143/145/181/31 by TGFβ was validated in myoblasts and differentiated myotubes. Human skeletal muscle biopsy donors were categorized as responder (RE) or low-responder (LRE) based on their fold change in ISIMats (±1.2). TGFβ signaling as well as miR143/145 expression were stronger induced by training in skeletal muscle of LRE donors compared to RE. Over all donors, changes in miR143/145 cluster expression correlated inversely with changes in ISIMats and clustered together with changes in *MYH11*, *ACTA 2* and *MYOCD*. Global transcriptome, correlation and pathway enrichment analyses hinted towards a vascular smooth muscle cell association. Changes in miR143/145 correlated positively with changes in transcripts like *MYH11*, *ACTA2* and *PPP1R14A*. Target mining over all samples revealed several possible miR143/145 targets regulated in our system like *HDACs* and *INSR*.
Conclusion: Our results clearly associate TGFβ signaling and its regulation of miR143/145 with a lack of beneficial outcome in glycemic control as a response to training. Correlation analyses revealed a connection to smooth muscle or differentiating muscle cell markers suggesting incomplete or misguided muscle cell differentiation as a response to elevated TGFβ/miR143/145 in low-responders. Identification of the TGFβ/miR143/145 axis in skeletal muscle, and elucidation of involved targets of these miRs, has the potential to identify novel strategies to improve glycemic control in patients with risk for T2D.
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Disclosure: **S.I. Dreher:** None.

OP 18 Stressed out beta cell organelles

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Boosting the pancreatic beta cell function: the influence of nanotopographical cues on cell clustering and organelles crosstalk

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Background and aims: increasing efforts are focusing on the development of engineering microenvironments mirroring the biophysical properties of the islet niche to improve the β-cell differentiation and function *in vitro*. We have previously demonstrated that mouse and human β-cells sense and respond to the extracellular nanotopography by activating a mechanostransductive pathway, which mainly involved a reorganization of the actin cytoskeleton. Given that the cytoskeleton acts as a hard-wired structure that regulates the intercellular connectivity and the distribution of the organelles within the cytoplasm, aim of the proposed research was to evaluate whether the nanotopography could impact on the β-cell clustering, polarity and organelles organization, thus driving the β-cell fate.
Materials and methods: mouse β-cells and isolated human islets were cultured on metal oxide scaffolds with controlled roughness that mimic the extracellular nanotopography. Changes in the cellular proteome were evaluated by a shot-gun label-free proteomic approach and morphological studies were performed by means of super-resolution fluorescence microscopy (STED, TIRFM).
Results: immunofluorescence staining showed a reorganization of the cell-cell and the cell-substrate adhesion sites on the nanostructure, resulting in an increased intercellular connectivity that promotes the cell clustering. Interestingly, we also found a modification of cilia distribution and dimension in the cells grown on the nanostructure. Quantitative immunofluorescence studies revealed a more elaborated and complex mitochondrial network in the cells grown on the nanostructure, which supports the increased mitochondrial membrane potential and potentiates the glucose-stimulated insulin secretion. Furthermore, the proteomic analysis suggested that the nanotopography modulates the expression of proteins shared by the endoplasmic reticulum (ER) and mitochondria (CARL, VDAC1, HSPs) and by mitochondria and lysosomes (RAB7A and XSPA8). Accordingly, morphological studies confirmed a profound reorganization of the mitochondrial-ER and mithocondrial-lysosomes contact sites.
Conclusion: our results indicate that the extracellular nanotopography promotes the β-cell clustering and regulates the interplay of organelles within the cytoplasm, which are crucial for ensuring a rapid and coordinated cellular response to the extracellular stimuli. Understanding how to target mechanotransductive processes is of particular importance to successfully design scaffolds that can improve β-cell function and viability *in vitro*.
Disclosure: **A. Galli:** None.

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Bcl-xl limits transcriptional and functional decompensation of beta cell mitochondria during chronic exposure to high glucose

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Background and aims: In the development of type 2 diabetes, β-cells respond to increasing metabolic demand with an early phase of functional compensation, followed by pathogenic decompensation and failure.

Evidence suggests mitochondrial metabolism is enhanced in the adaptive response, while later failure involves changes to β-cell identity and mitochondrial dysfunction. It is not clear what mechanisms drive, and control, the progression from β-cell compensation to decompensation. We previously reported that anti-apoptotic Bcl-x_L dampens β-cell mitochondrial metabolism. Here, we aimed to determine if the non-apoptotic functions of Bcl-x_L limit β-cell decompensation under chronic glucose excess.

Materials and methods: Islets from Bcl-$x^{flox/flox}$:Pdx1-CreER^{TM} mice with β-cell knock-out of Bcl-x_L (BclxβKO), and Bclx$^{flox/flox}$ wild-type controls (BclxβWT), were cultured 6-days in 11 mmol/l glucose (NG) or 25 mmol/l glucose (HG). Transcriptional profiling was done by RNA-Seq and mRNA expression was quantified by qPCR. We imaged cytosolic Ca^{2+} using Fura-2, and insulin secretion was assayed in static incubation. Oxygen consumption rate (OCR) was measured in an XF^{e}24 Analyzer. Mitochondrial ROS (mitoROS) was imaged using MitoSOX and scavenged using MitoTEMPO. β-Cell mitochondria were labeled with TMRE and MitoTracker Green and imaged by 2D and 3D confocal microscopy. We quantified mitochondrial morphology and function using our "Mitochondria Analyzer" ImageJ plug-in.

Results: In both BclxβWT and BclxβKO islets, HG culture caused transcriptional re-wiring of metabolism toward glycolysis, a beginning loss of β-cell identity, and robust upregulation of adaptive ER stress responses. There was no significant activation of pro-apoptotic pathways or β-cell death. In HG, 233 genes were differentially expressed between BclxβWT and BclxβKO and these were dominated by mitochondria-related transcripts. This reflected a loss of key transcripts in BclxβKO islets, including *Tfam,* mitochondrial ribosomal genes, and numerous components of the electron transport chain such as *Ndufb8, Sdhb, Cox4l1 and Atp5e.* Total OCR, was significantly higher in HG-cultured BclxβKO islets than in BclxβWT, but ATP-coupled OCR was lower and glucose-stimulated mitochondrial hyperpolarization was impaired. Despite this, overall Ca^{2+} signaling and insulin secretion were amplified in HG-cultured BclxβKO islets. Confocal analysis indicated this was likely because of compensatory increases in mitochondrial fusion and total mitochondrial volume. HG culture increased mitoROS significantly more in BclxβKO cells than BclxβWT. Remarkably, normalizing mitoROS levels with MitoTempo rescued the glucose-induced defects in BclxβKO mitochondrial gene expression and the transcriptional changes to β-cell identity/function (*Ins2*, *Gck*, *Mafa*, *Ldha*, *Pdk1*) in islets of both genotypes.

Conclusion: We show that mitoROS is a primary driver of transcriptional re-wiring in β-cells exposed to excess glucose, and identify Bcl-x_L as a safeguard of the transcriptional and physiological integrity of β-cell mitochondria. Our findings suggest Bcl-x_L is essential to prevent early β-cell decompensation during developing hyperglycemia.

Supported by: CIHR

Disclosure: **D.J. Pasula:** None.

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The fate of intracellular sphingosine-1 phosphate regulates beta cell response to fatty acids

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Background and aims: During type 2 diabetes (T2DM) development pancreatic beta-cells are exposed to lipotoxic stress, which results in beta-cell failure. The bioactive sphingolipid S1P (sphingosine-1 phosphate) has been linked to the development of T2DM. S1P is generated by two isoforms of sphingosine kinases with specific intracellular localizations. S1P can be catabolized either by S1P phosphatase (SPP) in the dephosphorylation reaction yielding sphingosine, or can undergo an irreversible degradation to hexadecenal and phosphoethanolamine by the action of S1P lyase (SPL). The intracellular site of S1P generation was shown to differentially regulate the mode of lipotoxicity in beta-cells. Little is known, however, about the role of S1P catabolism in beta-cell sensitivity to fatty acids. Therefore the aim of this study was to compare the role of SPP and SPL in beta-cell sensitivity to lipotoxicity.

Materials and methods: Insulin-secreting INS1E ;cells were stably transfected either with an empty vector (INS1E-ctr), with human SPL (INS1E-SPL) or human SPP1 (INS1E-SPP) vectors. Cells were treated with 500 μM palmitate (PA), 500 μM oleate (OA) or a combination of both fatty acids (FFA) for 24 h. Thereafter cell viability was estimated by a MTT assay, oxidative stress by DCFDA oxidation, protein expression by Western blot and gene expression analyses were performed by qRT-PCR.

Results: The overexpression resulted in a 20-fold increase of the expression of SPL or SPP1. Exposure of INS1E-ctr cells to PA led to a significant decrease of cell viability, an effect that was potentiated by SPL overexpression (~25% stronger vs.INS1E-ctr, $p<0.05$). In contrast, SPP overexpression significantly protected against PA-mediated viability loss (only 20 % cell viability loss). OA was not toxic to INS1E-ctr cells and protected against PA-mediated cell viability loss (OA 91%, PA+OA 90% viability). Interestingly, in INS1E-SPL and in INS1E-SPP cells the incubation with OA led to a significant decrease of cell viability, and failed to prevent PA toxicity when co-incubated. These effects on cell viability correlated with the observed changes in the FFA-mediated oxidative stress induction and the expression of ER and mitochondrial stress markers.

Conclusion: Our results showed that the fate of intracellular S1P significantly participates in the regulation of beta-cell sensitivity to various FFA. SPL overexpression sensitized INS1E cells towards PA toxicity, while an increased capacity for S1P dephosphorylation by SPP overexpression provided protection against PA-mediated viability loss. An increased rate of intracellular S1P turnover induced toxicity of unsaturated fatty acid OA, which is the major toxic FFA in human beta-cells. Therefore the dynamic re-arrangements of beta-cell sphingolipid composition may impact beta-cell sensitivity to various FFA.

Disclosure: **Y. Tang:** None.

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Manf overexpression in pancreatic beta cells protects from streptozotocin-induced beta cell death and diabetes in mice

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Background and aims: Type 1 Diabetes (T1D) is characterized by a progressive autoimmune destruction of pancreatic beta cells. Unresolved endoplasmic reticulum (ER) stress induced by local pro-inflammatory cytokines and chemokines contribute to the pancreatic beta cell death and insulin deficiency in T1D. Mesencephalic astrocyte-derived neurotrophic factor (MANF) is a small ER stress-regulating factor with cell protective and regenerative roles in various rodent disease models. We previously showed that conventional and pancreas-specific MANF knockout mice develop insulin-deficient diabetes due to postnatal loss of beta cell mass caused by reduced beta-cell proliferation and increased beta cell death preceded by sustained ER stress. Conditional removal of MANF specifically from adult beta cells revealed that endogenous MANF expression is mandatory for postnatal beta cell expansion and maintenance in mice. In human, loss of MANF results in insulin-deficient diabetes due to increased ER stress and defect in proinsulin processing and secretion. Exogenous MANF protein induces proliferation of human and mouse beta cells and protects them from ER stress-induced cell death triggered by chemical ER stressor and cytokines mimicking T1D *in vitro*. *In vivo*, AAV-MANF induced overexpression in pancreas protected beta cells from streptozotocin (STZ)-induced beta cell death. Thus, we wanted to test the therapeutic effect of induced transgenic MANF overexpression in two diabetes mouse models induced by STZ.

Materials and methods: We generated doxycycline inducible bi-transgenic mice that overexpressed MANF under the insulin promoter (INS-MANF). To study the effect of MANF overexpression in beta cells, mice were given doxycycline at different ages and different time periods and islet expression of beta cell markers, proliferation and beta cell mass were analyzed. We induced diabetes in the INS-MANF mice by injection of a high single dose of STZ and by injections of multiple low doses of STZ (MLDS). Beta cell mass, proliferation, apoptosis and insulitis scores were analyzed from the pancreases. Primary beta cells isolated from INS-MANF mice were challenged with STZ, and ER stressors to elucidate the mechanism of MANF protective action in beta cells *in vitro*.
Results: Increased MANF expression in beta cells conferred protection against beta cell apoptosis and hyperglycemia in both models of STZ-induced diabetes (at the end point $p<0.01$, one-way ANOVA followed by post hoc test). In addition, MANF-overexpression efficiently protected against islet lymphocyte infiltration and insulitis in the MLDS model of T1D. Furthermore, prolonged MANF overexpression for 3 months in pancreatic beta cells showed no change in pancreatic beta cell mass, serum insulin level and ER stress in beta cells.
Conclusion: We conclude that increased endogenous levels of MANF in beta cells is protective against diabetogenic insult and inflammation and induces beta cell proliferation in adult mice, thus constituting a promising therapeutic candidate for T1D.
Supported by: JDRF; Tekes; Finnish Diabetes Research Foundation; Academy of Finland
Disclosure: **H. Li:** None.

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Loss of autophagy, Transcription Factor EB (TFEB) and lysosomal homeostasis limit beta cell function and survival under ER stress in vitro and in vivo

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Background and aims: Autophagy can help protect stressed β-cells by delivering damaged organelles and protein aggregates to lysosomes for degradation. However, autophagic flux is impaired by severe lipotoxicity and inflammation. To examine if this may be a common feature in β-cells under ER stress, we investigated lysosomal homeostasis and the importance of autophagy in β-cells under various ER stress-inducing conditions, including hypoxia and islet transplantation.
Materials and methods: β-Cell autophagy-deficiency was induced by deletion of Autophagy Related 5 (Atg5), in vitro by adenovirus-Cre transduction of $Atg5^{flox/flox}$ islet cells, and in vivo by pancreatic duct injection of $Atg5^{flox/flox}$ with dsAAV6-RIP-Cre virus. Control mice were injected with dsAAV6-Empty vector. A marginal mass of 300 wild-type or Atg5 knock-out islets were transplanted under the kidney capsule of $Atg5^{flox/flox}$ mice that were made diabetic by 175 mg/kg streptozotocin (STZ). Glucose tolerance was assessed by i.p. glucose tolerance tests (IPGTTs). Autophagic flux was assessed in cultured islet cells from CAG-RFP-EGFP-LC3 reporter mice by confocal microscopy. Cell death was quantified by propidium iodide staining. Lysosomal function was determined using the Magic Red Cathepsin B activity assay. Tfeb protein levels were determined by immunofluorescence and western blot. Islet mRNA expression was quantified by qPCR. Human islets were obtained from the University of Alberta IsletCore.
Results: Atg5 knockout reduced β-cell survival under hypoxic stress in vitro and impaired the ability of syngeneic marginal mass islet grafts to maintain glucose homeostasis in STZ-diabetic mice, >80% of recipients of Atg5-deficient islets returned to diabetes within 10 days post-transplant (vs 30% of controls, $p=0.05$). Mouse islets cultured in 1% O_2 activated pro-apoptotic ER stress pathways, and quantification of autophagosomes and autolysosomes in CAG-RFP-EGFP-LC3 islet cells revealed that hypoxia significantly disrupted autophagic flux. The impairment of autophagy was associated with reduced lysosomal cathepsin B activity and a significant loss of transcription factor EB (Tfeb), a master regulator of lysosomal physiology and biogenesis. A similar loss was seen in response to $CoCl_2$-induced hypoxic stress. Notably, hypoxia and $CoCl_2$ also reduced Tfeb in human islet cells. Treating hypoxic mouse islet cells with the mTOR-inhibitor Torin 1 restored Tfeb protein and improved lysosomal function. Lipotoxicity and thapsigargin-induced ER stress reduced Tfeb protein and viability in MIN6 cells, suggesting ER stress-specific mechanisms may drive dysregulation of Tfeb and lysosomes. Conversely, overexpression of Tfeb:GFP partially protected MIN6 cells from thapsigargin-induced death.
Conclusion: Autophagy protects β-cells under hypoxia and following islet transplantation but lysosomal clearance is impaired under prolonged hypoxic stress, possibly through Tfeb down-regulation. Our data further suggest that loss of Tfeb is a common feature of severe ER stress that may exacerbate β-cell failure and death.
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Disclosure: **Y. Zou:** None.

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Cell biology of stress granules in pancreatic beta cells

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Background and aims: Pancreatic islet β-cells maintain glucose homeostasis by secreting insulin. β-cells quickly enhance the translation of *Insulin* mRNA and related transcripts upon glucose stimulation (hyperglycemia). As the levels of these transcripts do not immediately increase after glucose stimulation, their rapid translational increment is mainly regulated by shared post-transcriptional mechanisms. Having a half-life of > 24 hours, the *insulin* mRNA is likely stored within the cytoplasm. We found that in mouse MIN6-K8 insulinoma cells kept at rest with 2.8 mM of glucose, *Insulin1* mRNA is colocalized with the stress granule marker G3BP1. Stress granules are membraneless compartments associated with the storage of mRNAs and part of the translation machinery to halt protein synthesis. Hence, β-cells store *Ins1* mRNAs and conceivably other related mRNAs in stress granules until glucose stimulation prompts the recruitment of these mRNAs to the ER to upregulate the biosynthesis of preproinsulin and other insulin secretory granule proteins. Accordingly, glucose stimulation of MIN6 cells correlates with the disappearance of stress granules and the redistribution of G3BP1 and *insulin* mRNA throughout the cytosol. However, how critical are these *insulin* mRNA stores, and more in general, the dynamic physiology of stress granules for β-cell function remains unclear. To address these questions, we are investigating the biology of stress granules in wild type and $G3BP1^{-/-}$ MIN6-K8 insulinoma cells.
Materials and methods: *In vitro* cultures of insulinoma MIN6-K8 cells treated with 2.8 mM glucose (resting) or 25 mM glucose (stimulated) were used as a model of glucose stimulation to β-cells. CRISPR-Cas9 editing was used to generate the $G3BP1^{-/-}$ MIN6-K8 cells. Western blots and immunostainings were performed for detecting G3BP1, phosphor-G3BP1 and insulin levels in wild type and $G3BP1^{-/-}$ MIN6-K8 cells. Transcripts levels were measured by qPCR.
Results: We found that $G3BP1^{-/-}$ MIN6-K8 cells lack stress granules, while their insulin content is increased. Moreover, in $G3BP1^{-/-}$ MIN6-K8 cells *Ins1* mRNA levels are reduced compared to control cells. This data suggest that G3BP1 stabilizes *Ins1* mRNA while suppressing its translation. In other cell types phosphorylation of G3BP1 on S149 and S232 regulates stress granule assembly and disassembly. However, the levels of phospho-G3BP1S149/S232 in resting and glucose stimulated MIN6-K8 were comparable, pointing to the possible existence of other post-translational modifications.

Importantly, we detected G3BP1^{+} granular structures also in the cytosol of human pancreatic β-cells of a non-diabetic living donor, supporting the physiological roles of these cytosolic organelles. Furthermore, RNAseq analysis revealed that in laser-captured microdissected islets of metabolically phenotyped living donors with type 2 diabetes (T2D) the levels of *G3BP1* mRNA are reduced compared to equivalent islets from normoglycemic donors. Hence, our data point to the first time to a link between G3BP1, stress granules and T2D.
Conclusion: In conclusion, this project aims to establish whether stress granules are physiological structures for mRNA storage in β-cells and whether their function is affected in T2D.
Supported by: DZD, IMI-RHAPSODY, IMI-INNODIA, DFG-Beta Stress
Disclosure: **E. Quezada:** None.

OP 19 Diet and nutrition

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Reduced carbohydrate and increased protein and fat during weight loss improve the atherogenic lipid profile in type 2 diabetes

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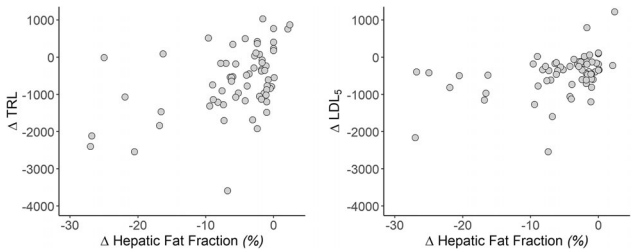
Background and aims: Elevated triglyceride-rich lipoproteins (TRL), excess small dense LDL particles (LDL_5) and decreased HDL_2/HDL_3 ratio promote atherogenesis in type 2 diabetes (T2D). Carbohydrate restriction reduced intrahepatic triglyceride (IHTG) content beyond the positive effect of weight loss in a group of T2D patients, the present study sought to determine whether parallel improvements in lipoprotein density profiles occurred in these same patients.
Materials and methods: Seventy-two adult T2D patients with a mean±SD BMI of 33±5 kg/m^2 were randomised 1:1 to 6 weeks of fully-provided hypocaloric dietary treatment aimed at ~6% weight loss, either with a carbohydrate-reduced high-protein (CRHP, C30E%/P30E%/F40E%) diet or a conventional diabetes (CD, C50E%/P17E%/F33E%) diet. Density profiles of lipoproteins were determined by ultracentrifugation of fluorescently labelled plasma. Magnetic resonance spectroscopy was used to assess IHTG. Treatment effects were evaluated using a constrained linear mixed model with inherent baseline adjustment.
Results: Body weight decreased by 5.8 kg (~6%) in both groups. Compared with the CD diet, the CRHP diet reduced TRL (mean [95% CI]) by -16 [-30;1]% (p=0.07) and LDL_5 by -13 [-22;-3]% (p=0.01), and increased HDL_2/HDL_3 by 11 [1;22]% (p=0.04). The CRHP diet reduced IHTG more than the CD diet (-26 [-45;0]%, p=0.05), and changes in IHTG including both groups correlated significantly with changes in TRL and LDL_5 (Spearman's ρ 0.39 and 0.38, p<0.01).
Conclusion: Carbohydrate restriction adds to the positive effect of weight loss in T2D patients by inducing greater improvements in atherogenic lipid profile, maybe facilitated by a reduction in intrahepatic fat.



Clinical Trial Registration Number: NCT03814694
Supported by: Arla Foods amba and the Danish Dairy Research Foundation
Disclosure: **M.N. Thomsen:** None.

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Intra-organ fat content during weight loss-induced remission of type 2 diabetes in people with normal or raised BMI

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Background and aims: The aetiology of type 2 diabetes is often assumed to be different in people who are overweight or obese compared with those with BMI in the 'normal' range. However, clinical observation suggested that losing body fat from above a 'personal fat threshold' at any BMI level can bring about remission of diabetes. We therefore compared baseline liver and pancreas fat content in people with type 2 diabetes and BMI above or below 27kg/m^2 and examined changes in intra-organ fat after weight loss in relation to remission of diabetes in each group.
Materials and methods: We examined data from two cohorts (DiRECT: BMI 35.1±4.5 kg/m^2; n=56, age 53.3±7.6 years, diabetes duration 3.0 ±1.7years; ReTUNE: BMI 24.3±2.0kg/m^2, n=17, age 58.2±7.3 years, diabetes duration 2.6±2.2 years). Age, sex and BMI-matched control groups without diabetes were also studied (BMI>27kg/m^2: n=18, age 55.4±6.0 years; BMI<27kg/m^2: n=11, age 58.8±9.5 years). Weight loss was achieved using a low calorie diet (~800 kcal/day) followed by food reintroduction and weight maintenance. In those with BMI<27kg/m^2, other types of diabetes identified by antibody and genetic tests (n=2: 1 T1DM, 1 MODY) were excluded from analysis. Intra-organ fat was quantified by 3-point Dixon magnetic resonance.
Results: In each BMI category, liver and intrapancreatic fat content were higher in diabetes compared with non-diabetic controls. For BMI>27kg/m^2: liver fat was 16±1.3 vs. 5.5±1.4% p<0.0001 for type 2 diabetes vs. control respectively, and intrapancreatic fat 8.5±0.3 vs. 6.8±0.5% p<0.010, respectively. For BMI<27kg/m^2: liver fat was 4.7±0.8 vs. 1.9 ±0.3% p=0.016 for type 2 diabetes vs. control, and intrapancreatic fat 5.0 ±0.3 vs. 3.4±1.1%, p=0.029, respectively. Both diabetic and control groups with BMI >27kg/m^2 had significantly higher liver and intrapancreatic fat than those with <27kg/m^2 (p<0.0001). After weight loss, liver fat content fell significantly in both groups (BMI>27kg/m^2: to 3.0 ±0.5%, p<0.0001; BMI<27: to 1.4±0.1%, p=0.004). Similarly, intrapancreatic fat decreased (BMI>27- to 7.6±0.3%, p<0.0001; BMI <27 to 4.5±0.6%, p=0.026). Diabetes remissions were similar between BMI groups (60% and 67%).
Conclusion: Intra-organ fat content is elevated in people with type 2 diabetes at any level of BMI. Irrespective of BMI, 10-15% weight loss decreased liver and intrapancreatic fat with notably similar rates of remission of type 2 diabetes, consistent with the twin cycle hypothesis. Lesser elevation of liver fat, below the diagnostic threshold for NAFLD, can cause metabolic problems in leaner individuals, indicating the need to develop BMI-specific normal ranges. Across the range of BMIs, early type 2 diabetes has the same reversible pathophysiology.
Clinical Trial Registration Number: ISRCTN15177113
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Disclosure: **R. Taylor:** Grants; Supported by grants from Diabetes UK. Lecture/other fees; academic lectures funded by Novo Nordisk, Lilly and Janssen. Non-financial support; RT is author of book 'Life Without Diabetes'.

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Habitual intake of dietary methylglyoxal is associated with less low-grade inflammation, but also with impaired retinal microvascular function: The Maastricht Study

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Background and aims: Dicarbonyls are highly reactive compounds and major precursors of advanced glycation endproducts (AGEs). Dicarbonyls are formed endogenously but also during food processing. Circulating dicarbonyls and AGEs are associated with inflammation and microvascular complications of diabetes, but the associations are currently unknown for dietary dicarbonyls. This study examined the associations of dietary dicarbonyl intake with low-grade inflammation and microvascular function.
Materials and methods: In 2793 participants of the Maastricht Study (60 ±8 yrs, 50% men, 26% T2DM), we estimated habitual intake of the dicarbonyls methylglyoxal (MGO), glyoxal (GO) and 3-deoxyglucosone (3-DG). Food Frequency Questionnaires were linked to our food composition database, including MGO, GO and 3-DG concentrations in >200 foods. Low-grade inflammation was determined as a z-score of plasma inflammation biomarkers (CRP, SAA, sICAM-1, IL-6, IL-8 and TNF-α). Microvascular function was determined in plasma as a z-score of endothelial function biomarkers (sVCAM, sICAM, eSelectin and vWF), in arterioles and venules of the retina as flicker light-induced dilation and diameters, and in skin as heat-induced skin hyperemic response. Cross-sectional associations of dietary dicarbonyls with markers of low-grade inflammation and microvascular function were investigated using linear regression adjusting for age, sex, glucose metabolism status (GMS), BMI, energy intake, smoking, alcohol, physical activity, education, triglycerides, systolic blood pressure, total cholesterol/HDL ratio and medication.
Results: Higher intake of MGO was associated with a lower z-score for inflammation, which was significant in the fully adjusted model (age, sex and GMS adjusted: -0.03 [-0.06;0.01]. Fully adjusted: -0.05 [-0.10;-0.01]). Intake of MGO was inversely associated with each individual inflammation biomarker, with strongest and significant associations for CRP and TNF-α. On the other hand, higher MGO intake was associated with impaired retinal venular dilation in the age, sex, and GMS adjusted model (-0.05 [-0.10;-0.004]), and remained so after full adjustment (-0.08 [-0.14;-0.02]). MGO intake was also associated with impaired retinal arteriolar dilation and skin hyperemia, although not significantly. We observed no association of dietary MGO with retinal diameters and plasma biomarkers of endothelial function. GO and 3-DG intake were not associated with any of the outcomes. The associations did not differ between individuals with NGM, prediabetes or T2DM (P int.>0.10).
Conclusion: Higher habitual intake of MGO was associated with less low-grade inflammation, but also with impaired retinal venular dilation. This suggests that food-derived MGO may induce anti-inflammatory effects, but also contributes to impaired retinal microvascular function, possibly via inflammation independent pathways. These associations in opposite directions require further investigation.
Supported by: NVWA, JPI HDHL, ERA-NET, ERDP, PoL, DMoEA, SDW, PSID, CVC, CARIM, CAPHRI, NUTRIM, SA, HFL, P, IS, JCBV, NNF, SAN
Disclosure: **K. Maasen:** None.

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Very-low dose pre-meal whey protein microgels reduce postprandial glucose in type 2 diabetes: a randomised, placebo-controlled cross-over study

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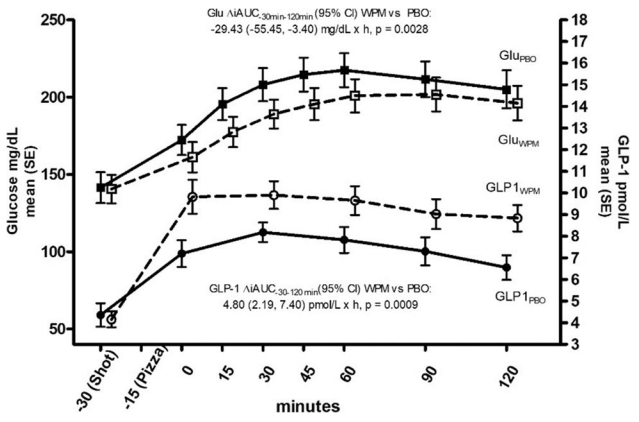
Background and aims: Whey protein (WP) is found in dairy products, and are rich in branched chain amino acids and bioactive peptides that stimulate secretion of incretin peptides and insulin. Its applicability in routine nutraceutical clinical use has however been limited by 1) requiring a relatively high dose with a high caloric content, and 2) the need to take it well in advance of a meal. New technologies allowing for a more rapid absorption could enable use of a lower WP-dose, as well as allow to take the WP closer to the meal.
Materials and methods: In this single-intervention crossover study in individuals with drug-naïve or metformin-treated type 2 diabetes, we studied the effects of 10g WP (40 kcal) prepared with novel technology to enhance absorption (micelle-technology [WPM]), or placebo (0 kcal), provided as a 125 mL shot 15 min ahead of a 250 g pizza meal (622 kcal [29.0 g protein, 22.6 g fat, 72.6 g carbohydrates]). Postprandial (PP) glucose response over 4 hours, and incretin response (intact glucagon-like peptide [GLP]-1, peptide-YY [PYY], glucose-dependent insulinotropic polypeptide [GIP]) over 2 hours were assessed in blood, and the difference between WPM and placebo were assessed by comparing incremental areas under the curve (iAUC) between the two interventions.
Results: In total 26 individuals (14 females, mean [standard deviation] age 62.0 [8.3] years, baseline HbA1c 58 [12] mmol/mol /7.5% [1.1], eGFR 96.6 [25.7] ml/min/1.73m^2, BMI 29.2 [4.8] kg/m^2) completed both sequences. The pre-meal WPM shot significantly altered the early PP glucose trajectory, reducing the 2h iAUC by 22% (mean [95% CI] difference $iAUC_{-30min-120min}$ WPM vs placebo -29.43 [-55.45, -3.40] mg/dLxh, p=0.0283). The $iAUC_{-30min-180min}$ was similar (-31.58 [-68.43, 5.26], p=0.0896). A 66% increase in GLP-1 $iAUC_{-30min-120min}$ was observed (4.80 [2.19, 7.40] pmol/Lxh, p=0.0009), while responses for both PYY and GIP were similar between WPM and placebo (respectively, 5.21 [-1.14, 11.56] pmol/Lxh, p=0.1035, and 8.52 [-15.27, 32.31] pmol/Lxh, p=0.4668).
Conclusion: In sum, 10g WPM significantly reduces the early glycaemic response and significantly augments the GLP-1 response to a mixed meal in subjects with type 2 diabetes. These results support its use as a convenient pre-meal shot to improve PP metabolic profile.



Clinical Trial Registration Number: NCT04639726
Supported by: Nestlé Health Science
Disclosure: **O. Johansen:** Employment/Consultancy; Nestlé Health Science.

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Investigation of sex-dependent effects of a one-year low-carb- vs low-fat intervention in patients with high-risk prediabetes - a randomised controlled trial

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Background and aims: Current guidelines point out, that diets of different macronutrient composition (low-carb vs. low-fat) are equally recommendable for prevention and therapy of T2DM, when achieving sufficient nutrient quality. Low-fat diets were the gold standard for more than 50 years, today a Mediterranean-style low-carb diet - low in meat, rich in fish and plant oils - is considered at least comparable, if not superior in reducing cardiovascular risks. Underlying risk factors are differentially affecting women and men; thus, RCTs need to assess their diet-induced metabolic outcomes under consideration of sex as well. Our DiNA-P study (Diabetes Nutrition Algorithms in Prediabetes) compares individualized two-phase low-carb- and low-fat diets. We present here data of the one-year intervention period, split up by sex.
Materials and methods: 267 subjects with high-risk prediabetes (age 59 ±10 years, BMI 32,5 ± 6,2 kg/m²; 38 % male) were identified by screening oGTT, characterized by insulin secretion deficit and/or insulin-resistant fatty liver disease, and 1:1 randomized to either low-carb or low-fat diet. They underwent an initial hypocaloric (1200-1500 kcal/d) dietary intervention for three weeks (< 40 g of carbs vs. < 30 kcal% of fat), followed by a moderate maintenance phase (1500-1800 kcal; < 40 kcal% vs. < 30 kcal% of fat) of 49 weeks. Clinical follow-up tests included oGTTs, routine lab, anthropometric assessment as well as MRI and 1H-MRS for body fat and liver fat quantification. Statistical analysis was conducted stratified by sex, assessed interaction effects between diet and sex, and was adjusted for differences in weight change.
Results: In the first diet phase, women showed a superior outcome in the low-carb group with respect to body weight, fasting glucose, blood pressure, triglycerides and LDL/HDL ratio, while in men, low-carb was inferior regarding levels of uric acid and 2-hours glucose (trend-wise). Effects on fasting glucose were characterized by a significant sex-by-diet interaction.
In the second diet phase, a significantly stronger reduction of body fat content was seen in men, only, but no other differences between the diets or sex and no significant interaction were determined. Compliance to the diets was considerably lower during maintenance phase, with the low-carb group showing clearly better long-term adherence compared to low-fat.
Conclusion: Only short-term benefits of the investigated diets seemed to be affected by a relevant sex-specific superiority for low-carb (women) and low-fat (men). After one year, both diets achieved comparable outcomes despite higher compliance to the low-carb diet. A modulation of dietary adherence or molecular metabolic mechanisms by sex needs to be evaluated by further analyses and upcoming trials.
Clinical Trial Registration Number: NCT02609243
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Disclosure: **S. Kabisch:** Grants; German Center of Diabetes Research (DZD).

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The effect of dietary carbohydrate restriction beyond weight loss on health-related quality of life and cognition

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Background and aims: Carbohydrate restriction is emerging as a viable treatment strategy in type 2 diabetes, but its effect on health-related quality of life and cognitive function during a weight loss remains largely unknown. We aimed to evaluate the effect of weight loss induced by a carbohydrate-reduced high-protein (CRHP) diet on self-reported physical and mental health and cognition in patients with type 2 diabetes.
Materials and methods: In this randomized parallel trial, 72 adults with type 2 diabetes and overweight or obesity (mean±SD, HbA_{1c}: 7.4±0.7% and BMI: 33±5 kg/m^2) were randomly assigned 1:1 to CRHP diet (C30E%/P30E%/F40E%) or conventional diabetes (CD: C50E%/P17E%/F33E%) diet for 6 weeks. The two diets were intended to induce similar weight losses (~6%). Physical and mental component summary (PCS and MCS) scores were assessed from the short-form 36 questionnaire (SF-36), and global cognition, verbal memory, psychomotor speed and executive function from a neuropsychological test battery. Treatment differences were estimated from constrained linear mixed models using baseline adjustment.
Results: Both groups achieved a 5.8 kg (~6%) weight loss and improved PCS (median (IQR), CD: 2.7 (1.1;4.2)% and CRHP: 2.1 (0.7;3.7)%; both p<0.001). In addition, CRHP diet improved MCS (1.8 (-0.7;5.7)%, p<0.01) and 6 out of 8 domains of the SF-36 compared with 2 out of 8 after the CD diet. Global and specific domains of cognition did not change within or between groups, but CD scored better on the symbol digit modality test (SDMT) of psychomotor speed compared to CRHP (p<0.01; table 1).
Conclusion: Weight loss improves self-reported physical health independently of diet composition, and carbohydrate restriction may further benefit mental health, without adversely affecting overall cognition.

Table 1. Health-related quality of life and neuropsychological assessment before and after matched weight loss by a conventional diabetes (CD) or a carbohydrate-reduced high-protein (CRHP) diet in patients with overweight or obesity and type 2 diabetes.

	CD diet			CRHP diet			Between diets	
	Baseline	Treatment effect	n	Baseline	Treatment effect	n	Difference	*p*-value
Health-related quality of life								
PCS	48.9 (37.0;51.6)	2.7 (1.1;4.2) ‡	31	47.2 (42.5;52.3)	2.1 (0.7;3.7) §	33	0.5 [-1.6;2.5]	0.65
MCS	56.7 (51.0;59.7)	0.4 (-0.9;2.3)	31	55.7 (52.9;58.6)	1.8 (-0.7;5.7) †	33	2.0 [-0.7;4.8]	0.15
Neuropsychological assessment								
Global cognition	0.0 (±0.8)	0.1 (±0.4)	26	0.0 (±0.6)	-0.1(±0.4)	25	-0.2 [-0.4;0.0]	0.10
Verbal memory (RAVLT)	0.1 (±0.8)	-0.1 (±0.8)	26	0.1 (±0.8)	-0.2 (±0.7)	26	-0.0 [-0.4;0.4]	0.92
Psychomotor speed	0.2 (±0.9)	0.1 (±0.5)	28	-0.1 (±0.9)	-0.1 (±0.5)	27	-0.2 [-0.5;0.0]	0.09
- SDMT	53.1 (±13.4)	1.7 (±6.1)	28	49.5 (±13.4)	-1.8 (±6.2)	27	-4.1 [-7.2;-1.1]	0.01
- TMT A ¤	33.5 (29.0;46.3)	1.0 (-6.0;3.0)	28	37.0 (33.5;47.5)	-1.0 (-6.0;6.5)	27	3.6 [-7.6;16.2]%	0.54
Executive function (TMT B) ¤	81.5 (63.8;114.5)	-7.5 (-26.3;5.3) *	28	87.0 (72.3;114.5)	-3.0 (-12.0;6.5)	26	10.8 [-2.5;25.9]%	0.11

Data at baseline and changes from baseline are presented as means (±SD) or medians (25th–75th percentiles). Between diet differences are estimated marginal means [95% CI], presented as absolute or relative differences (CRHP vs CD) for normally distributed or log-transformed data, respectively, and derived from constrained linear mixed models with inherent baseline adjustment using all available data. ¤ Higher test score reflects worse performance. * p<0.05; † p<0.01; ‡ p<0.001; § p<0.0001 vs baseline. CD, conventional diabetes; CRHP, carbohydrate-reduced high-protein; PCS, physical component summary; MCS, mental component summary; RAVLT, Rey Auditory Verbal Learning Test; SDMT, Symbol Digit Modalities Test; TMT, Trail Making Test.

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Disclosure: **N.J. Jensen:** None.

OP 20 Keeping the balance in islet secretion

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Re-internalised Phogrin/IA-2beta is targeted to multigranular bodies devoted to degradation of young insulin secretory granules

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Background and aims: Phogrin is a transmembrane protein of insulin secretory granules (SGs) and a target of autoimmunity in type 1 diabetes. Although it belongs to the receptor protein-tyrosine phosphatase family, it recognizes inositol phospholipids PI3P and PI(4,5)P2 as substrates. Its role and life-cycle in beta cells remains unclear. Previous studies indicated that upon SG exocytosis phogrin is incorporated into the plasma membrane, then endocytosed and recycled either to the Golgi complex or immature SGs. Here we revisited its lifecycle by using a time- and space- dependent CLIP/SNAP labelling approaches and high-resolution 3D microscopy.
Materials and methods: For age- and space-dependent conditional labelling of phogrin in rat insulinoma INS-1 cells a CLIP tag was inserted in its extracellular region after second convertase cleavage site. Imaging of living INS-1 cells expressing CLIP-phogrin was performed with a Nikon Eclipse Ti microscope equipped with a Andor iXON 897 EMCCD camera, a Yokogawa CSU-X spinning disk and a Plan Apochromat Oil objective (100x/NA1.45).
Results: INS-1 cells were incubated for 72h after transient transfection with insulin-SNAP and CLIP-Phogrin, and then with blocking non-fluorescent CLIP and SNAP permeable substrates, before being stimulated with 25mM glucose while being labelled with permeable fluorescent CLIP^{TMR} and SNAP^{SiR} substrates. After a 2h-long chase, cells were fixed, stained with various antibodies and imaged in 3D. At this time point newly-synthesized CLIP^{TMR}-phogrin$^+$ and insulin-SNAP^{SiR+} had left the ER and Golgi and colocalized by 75% within compact vesicular objects. <2% of CLIP^{TMR}-phogrin$^+$ objects were TGN38$^+$ (Golgi marker), while ~15%, ~6% and ~2% of them were either ICA69$^+$ (immature SGs), or EEA1$^+$ or APPL1$^+$ respectively, with the two latter being early endosome markers. Hence, CLIP-phogrin is properly sorted to the SGs. A 19°C block-and-release chase for SG biogenesis revealed that CLIP^{TMR}-phogrin leaves the ER/Golgi ~30 min before insulin-SNAP^{SiR+}, possibly explaining their apparent non-complete colocalization. Surprisingly, after 2h-chase ~15% of CLIP^{TMR}-phogrin$^+$ and 8% of insulin-SNAP^{SiR+} objects were also LAMP2$^+$ (late endosome/lysosomal marker). We used the impermeable substrate CLIP$^{Surface-647}$ to selectively label CLIP-phogrin at the cell surface after 25mM glucose stimulation. Quantitative analysis of deconvoluted images revealed that re-internalized CLIP$^{Surface-647}$-phogrin first colocalized with EEA1, while later ~80% of it was found in multigranular bodies (MGB) which included multiple newly-synthesized insulin-SNAP^{SiR+} cores. Concomitantly, the colocalization of CLIP$^{Surface-647}$-phogrin with LAMP2 and cathepsin D increased ~2 fold with a peak after 6h-chase.
Conclusion: After SG secretion, phogrin is endocytosed, but it is neither directly targeted to degradation, nor recycled to Golgi or immature SG for re-usage. Instead, we hypothesise that phogrin is part of a mechanism coupling insulin secretion with degradation of a pool of young granules (crynophagy).
Supported by: BMBF-DZD, IMI-RHAPSODY, IMI-INNODIA
Disclosure: **I. Kalaidzidis:** Grants; BMBF-DZD, IMI-RHAPSODY, IMI-INNODIA.

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The importance of islet δ-cell ATP-sensitive K^+channel (K_{ATP}) function for somatostatin secretion and islet hormone balance

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Background and aims: Previous scRNAseq and RNAseq studies identified the ATP-sensitive K^+ (K_{ATP}) channels within the islet δ-cells to be the same (Kcnj11 for Kir6.2 and Abcc8 for SUR1) and expressed at similar levels to those found in the β- and α-cells. However, the specific nature of the δ-cell K_{ATP} channels in driving glucose-induced somatostatin secretion (GISS) is unclear. We investigated the role of the K_{ATP} channels on δ-cell function and somatostatin (Sst) secretion by introducing a conditional gain-of-function Kir6.2-V59M point mutation into the MgATP-interacting pore-forming Kir6.2 subunits. This equates to a mutation associated with human neonatal diabetes.
Materials and methods: Insertion of the Kir6.2-V59M gain-of-function mutation specifically within δ-cells was achieved by crossing hemizygous Sst-iCre$^{+/-}$ mice with ROSA26-STOP*loxP*-Kir6.2$^{+/-}$ animals (δV59M mice). Cre/loxP-mediated excision of the STOP codon and Kir6.2-V59M expression was confirmed by the expression of an eGFP reporter situated behind the Kir6.2V59M sequence using qPCR. Intraperitoneal glucose (2 g/kg) and insulin (0.75 IU/kg) tolerance tests were performed on adult (12-14 wks) male/female δV59M mice. Sst-iCre$^{+/-}$, ROSA26-STOP*loxP*-Kir6.2$^{+/-}$, and WT littermates served as controls. The electrophysiology of δV59M islet cells was investigated *via* whole cell patch clamp. Sst, insulin, and glucagon secretion from δV59M and control animals exposed to various glucose concentrations was assessed by in situ pancreas perfusion (ISPP) and in static islet incubations.
Results: eGFP mRNA expression was detected within δV59M but not littermate control islets (δV59M: 0.23 ± 0.08; controls: n.d, relative to β-actin, n = 3), showing successful expression of the Kir6.2-V59M mutation. Adult mice displayed no adverse phenotype, difference in weight gain or *ad libitum*-fed blood glucose at 4-14 wks of age compared to controls. Adult male δV59M mice demonstrated significantly impaired glucose tolerance (AUC glucose excursion δV59M vs controls; 1800 ± 79 vs 1546 ± 67, $p < 0.01$, n = 10) and minor insulin insensitivity, whereas adult female mice revealed no change in glucose tolerance or insulin sensitivity compared to controls. Both male and female δV59M mice showed elevated plasma insulin in response to i.p. glucose. Male and female δV59M δ-cells were hyperpolarised in the presence of elevated glucose consequently causing a reduction in action potential firing compared to control cells. δV59M islets exhibited impaired GISS (ISPP % increase from 1 mM to 20 mM G δV59M vs controls: 95.5 ± 19.4 vs 150.5 ± 12.6, n = 3, $p < 0.05$) causing a loss of glucose-induced glucagon suppression and amplified 1st and 2nd phase glucose-stimulated insulin secretion, when compared to control islets (ISPP AUC δV59M vs controls 1 mM G to 20 mM G; 1st phase, 519 ± 81 vs 304 ± 46, $p < 0.01$; 2nd phase, 341 ± 60 vs 138 ± 23, $p < 0.01$, n = 4).
Conclusion: These data indicate that constitutive opening of the K_{ATP} channels specifically within Sst-expressing cells causes an *in vivo* and islet phenotype comparable to the reported global Sst$^{-/-}$ mouse, supporting an influential role for K_{ATP} channels in driving normal islet δ-cell function and Sst secretion. As with the β- and α-cells, these findings highlight the likely translational loss of normal islet δ-cell function in neonatal diabetic patients carrying the Kir6.2-V59M mutation.
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Disclosure: **T.G. Hill:** None.

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Islet beta cell synchrony and second phase activity are governed by α cells upon physiological nutrient mixtures

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Background and aims: Pancreatic β cells are central in nutrient homeostasis and diabetes. Dynamic influences of non-β cells on β cell activity between and during meals and their putative deregulations in type 2 diabetes remain poorly understood. It is also crucial to decipher these functional interactions for cell therapies based on surrogate islets. α cells represent 20-35% of islet cells and have been mainly considered as β cell counter-regulators. We addressed their influence on β cell function in vivo and ex vivo in pre- and post-prandial situations with glucose and amino-acid mixtures.
Materials and methods: A model of induced α cell ablation in mice, termed GluDTR, was used (males, 13-26 weeks). In vivo i.p. glucose tolerance tests (IPGTT) were performed with and without a physiological mix of 19 amino acids (AAM). Islets were subsequently isolated and cultured on multi-electrode arrays (MEAs) for extracellular electrophysiological recordings of slow potentials (SPs), the β cell-specific coupling signals. Levels of β cell activity and synchrony were both monitored as published through SP frequencies and amplitudes, respectively.
Results: GluDTR islets contained insulin levels similar to WT (14.2 ±1.3 and 12.9±1.8 ng/islet respectively; N=3-4 mice), but were depleted in glucagon (4±1 vs 234±4 pg/islet). Fasting glycaemia were similar in WT and GluDTR mice (7.3±0.6 vs 7.8±0.5 mM respectively, N=11-15) as well as IP glucose tolerance. Co-administrations of glucose and AAM significantly improved glucose tolerance in WT (AUC glycaemia: $p<0.001$ for glucose with vs without AAM) but not in GluDTR mice, and glycaemia during the second phase were higher in GluDTR than in WT ($p<0.05$). Under these conditions, plasma C-peptide levels increased more in WT than in GluDTR mice (2.2±0.2 vs 1.5±0.2 fold respectively, $p<0.05$, N=3-6). In isolated islets analysed on MEAs, stimulations with glucose only from 3 (G3) to 6 (G6) or 8.2 mM (G8.2) triggered similar biphasic SP patterns between WT and GluDTR islets (n=80-106 islets; N=3-4 mice). In the additional presence of AAM, differences appeared between WT and GluDTR. AAM increased basal β cell activity and synchrony at G3 in WT but not in GluDTR islets. At G6, AAM increased similarly β cell activity and synchrony in WT and GluDTR islets in the first phase ($p<0.0001$, n=98-106, N=3), whereas the second phase was strongly increased in WT ($p<0.0001$) but not in GluDTR islets. At G8.2, AAM did not change the first phase in WT and GluDTR islets. During the second phase, β cell activity was increased by AAM in both WT and GluDTR islets ($p<0.0001$ and $p<0.001$ respectively), whereas β cell synchrony was stable in WT but decreased in GluDTR islets ($p<0.05$, n=80-102, N=3-4). Finally, inhibiting the action of glucagon-related peptides on GLP-1 receptors with exendin(9-39) in WT islets produced alterations of SP patterns similar to those observed in GluDTR islets.
Conclusion: These in vivo and ex vivo data provide a new model of β cell activity and synchronization upon physiological nutrient mixtures in which α cells are pivotal. Amino acids act directly on β in the first phase but mainly through α cells at low glucose and during the second phase with a strong influence on β cell synchrony. This regulation of intra-islet β cell networks by α cells will be further explored with increased spatial resolution by high-density MEAs.
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Disclosure: **M. Raoux:** None.

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Pancreatic alpha and beta cells are globally phase-locked

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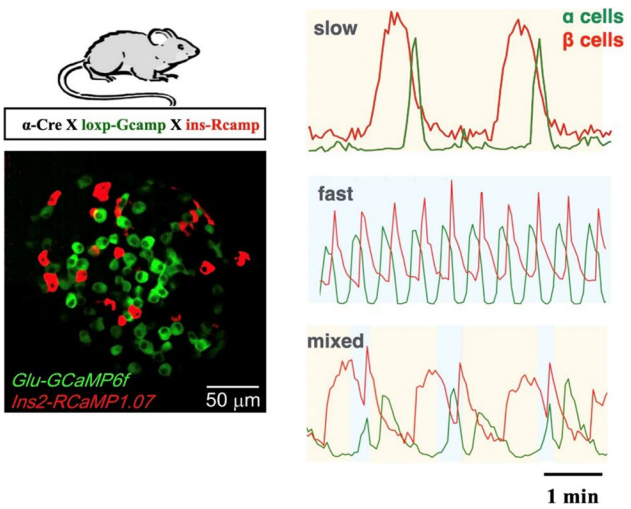
Background and aims: The Ca^{2+} modulated pulsatile secretion of glucagon and insulin by pancreatic α and β cells plays a key role in glucose metabolism and homeostasis. However, how different types of islet cells couple and coordinate via autocrine and paracrine interactions to produce various Ca^{2+} oscillation patterns are still elusive.
Materials and methods: We generated transgenic mice to label α and β cells and designed a microfluidic device to facilitate long-term recording of islet Ca2+ activity at the single-cell level and simultaneously identifying different cell types in live islet imaging. We used T-distributed stochastic neighbor embedding and negative matrix factorization for spatial-temporal image analysis. Mathemitical modeling is used to link paracrine interaction and oscillation modes.
Results: we developed a microfluidic chip realized the quantitive observation of a long-term Ca^{2+} signal and showed heterogeneous but intrinsic Ca^{2+} oscillation patterns of islets upon glucose stimulation. Spatial-temporal analysis using T-distributed stochastic neighbor embedding and negative matrix factorization independently revealed that an oscillatory islet was composed of two well-separated out-of-phase cells groups. Combining with the α-Gcamp-β-Rcamp transgenetic mice, it was found that the α and β cells were globally phase-locked to various phase delays, causing fast, slow or mixed oscillations. For each oscillation cycle, the waiting time of α cells was fixed to 20s. And the waiting time of β cells determined the oscillation period. Since isolated single β cells only show slow oscillation with a period of 5 min, we have not followed the mathematical model of simplifying islet into single β cell in the past. The αβ-coupling oscillator islet model proposed in this work recaptured the oscillation modes and the quantitative relationship between phase and period. It was found the islet oscillation mode was tuned by the coupling strength of α to β cell. And the the waiting time of β cells was determined by glucagon concentration.
Conclusion: Our study highlights the importance of cell-cell interaction to generate stable but tunable islet oscillation patterns. And islet serve as the minimal unit to determine different oscillation modes.



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Disclosure: **H. Ren:** None.

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Investigating the presence of proglucagon-derived peptides in human pancreas

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Background and aims: Emerging evidence suggest production of GLP-1 in pancreatic islets. Prohormone convertase 1/3 (PC1/3), the enzyme responsible for GLP-1 cleavage from the proglucagon precursor, has been detected in rodent glucagon-producing cells, especially under β-cell stress conditions. However, only few α-cells are thought to produce GLP-1 in non-stressed conditions. Here, we evaluate whether fully processed active GLP-1 and N-terminally extended GLP-1 can be detected in biopsies obtained at partial pancreatectomy. The patients were carefully characterized regarding glucose tolerance: normal (NGT), impaired glucose tolerance (IGT), diabetes (DM)).
Materials and methods: We enrolled n=33 individuals with no known history of type 2 diabetes (18F/15M, age 66.2±9.29 yrs., BMI 25.1±4.74 kg/m2) scheduled for partial pancreatectomy periampullary tumors. To detect differences in the glucose tolerance and insulin secretion among subjects enrolled we performed a preoperative 75 gr OGTT and subjects were classified into n=9 NGT, n=14 IGT and n=10 T2D. Pancreas biopsies were collected during surgery. In extracts of frozen specimens of pancreatic tissue we measured chromogranin A (CgA), GLP-1 1-36, GLP-1 7-36, GIP, glucagon, insulin, c-peptide. Tissue proglucagon-derived peptides levels were also adjusted for CgA and expressed as percentage of CgA levels. Tissue measurements were correlated with patients' clinical parameters.
Results: In the entire cohort, extractable levels of intact GLP-1 was 10 times lower compared to GLP-1 1-36 levels (mean levels of pancreatic GLP-1 1-36: 8.14 ± 1.41 pmol/g vs. mean levels of intact GLP-1: 0.81 ± 0.13 pmol/g). Further, CgA levels correlated to levels of GLP-1 1-36 (r=0.47, p=0.02) and intact GLP-1 (r=0.41, p=0.02), indicating that the expression of proglucagon-derived peptides is directly linked to the amount of endocrine tissue available in the biopsies. When subjects were classified according glucose tolerance and proglucagon-derived peptides levels adjusted for CgA, we observed similar levels of glucagon, while GLP-1 1-36 and intact GLP1 ($p<0.01$) levels were increased in T2D subjects compared to IGT and NGT. Moreover, we observed that intact GLP-1 tissue levels were positively correlated to in-vivo 2h glucose levels during OGTT (r=0.5, p=0.01)
Conclusion: Our data revealed that GLP-1 detected in human pancreas primarily consist of biological inactive GLP-1 1-36, while expression of intact GLP-1 is very low. We furthermore demonstrated that levels of intact GLP-1 were significantly increased in subjects with increased 2-h glucose levels. Our findings suggest that poor glucose metabolism is linked to increased islet levels of GLP-1, but the functional implication of this is still uncertain.
Supported by: EFSD Future Leaders Mentorship Programme for Clinical Diabetologists 2017 supported by an unrestricted educational grant from AstraZeneca
Disclosure: **T. Mezza:** None.

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Modulation of cholesterol homeostasis via pancreatic LDL receptor alteration: impact on beta cell secretory activity

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Background and aims: the accumulation of cholesterol in pancreatic islets has been associated with reduced glucose-induced insulin secretion and cellular toxicity pointing to the critical role of cholesterol metabolism

in pancreas. Cholesterol influx in β-cells mainly results from LDL uptake through the low-density lipoprotein receptor (LDL-R), abundantly expressed in these cells. The proprotein convertase subtilisin/kexin Type 9 (PCSK9) has been identified as the main target of LDL-R in liver. Interestingly, increased LDL-R expression and cholesterol content were observed in the pancreas of *Pcsk9*-deficient mice, suggesting a potential role for the protein in this tissue. Aim of this work was to understand the contribution of selective pancreatic PCSK9 production on β-cell function and to investigate the mechanisms involved.
Materials and methods: *Pdx1Cre+/Pcsk9*$^{LoxP/LoxP}$ (endocrine pancreas-selective *Pcsk9* knock-out mice) and *Pdx1Cre-/Pcsk9*$^{LoxP/LoxP}$ mice were generated and fed to a standard or chow diet for 20 weeks, and the metabolic phenotype was characterized. The expression of LDL-R, the cholesterol accumulation and the insulin secretion were evaluated in islets from *Pcsk9* deficient and control mice and in βTC3-cell lines treated with different levels of recombinant PCSK9 protein.
Results: pancreas specific *Pcsk9*-KO mice, as expected, lack detectable PCSK9 expression in pancreatic islets and showed decreased glucose clearance. The phenotype associated with impaired insulin secretion as insulin levels following fast and refeeding experiments were significantly lower than in littermates. In line with these findings, the glucose-stimulated insulin secretion (GSIS) test performed *ex vivo* resulted significantly decreased only in isolated islets from *Pdx1Cre+/Pcsk9*$^{LoxP/LoxP}$ mice. The analysis of pancreatic sections from littermates showed that LDL-R is prevalently present in β cells and its expression is increased in pancreas derived from specific *Pcsk9*-KO mice. Western blot analysis on βTC3-cell lines incubated with the PCSK9 recombinant protein showed a downregulation of the LDL-R, decreased BODYPY uptake and changes in the lipidomic profile. Studies are in progress to understand the role of PCSK9/LDL-R/cholesterol axis on β-cell function.
Conclusion: pancreatic *Pcsk9* deficiency results in increased expression of LDL-R in β cells, thus leading to increased accumulation of cholesterol which impacts glucose-stimulated insulin secretion, resulting in hyperglycaemia, and impaired glucose tolerance. Our findings identify a new player in regulating pancreatic lipid homeostasis and insulin secretion.
Disclosure: **A. Marku:** None.

OP 21 The adipocentric angle

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Yes-associated protein 1 in adipocytes plays an important role in glucose homeostasis

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Background and aims: Adipose tissue hypertrophy in obesity is associated with glucose intolerance and insulin resistance underlying type 2 diabetes. Furthermore, diabetes and obesity are associated with increased fibrosis formation in adipose tissue and metabolic dysregulation. Yes-associated protein 1 (YAP) is a transcription cofactor that promotes fibrosis in the lung, liver, and kidney. However, the role of YAP in adipocytes and glucose homeostasis is unknown. Therefore, the aim of this study was to elucidate the role of adipocyte YAP in glucose metabolism *in vivo*.
Materials and methods: To assess the impact of metabolic stress conditions on adipocyte YAP levels, we placed 6-week-old C57BL/6 mice on high-fat diet for 12 weeks. To further investigate the role of adipocyte YAP in glucose homeostasis, we generated novel adipocyte-specific YAP knockout mice (*AdipoqYap*$^{-/-}$) using an adiponectin-Cre loxP recombination system. Littermate mice (*AdipoqYap*$^{+/+}$) were used as controls and mice were fed chow or high-fat diet for 12 weeks at 6 weeks of age. Body weight, fasting blood glucose, and GTT were measured prior to sacrificing the mice. Mice were randomly assigned to groups by the experimenter; no formal blinding was used.
Results: YAP protein was increased in inguinal and perigonadal white adipose tissue from mice fed a high-fat diet for 12 weeks. This suggests that YAP is upregulated in adipose tissue with metabolic dysfunction and could potentially play an important role in the setting of obesity and glucose intolerance. When fed a high-fat diet, *AdipoqYap*$^{-/-}$ mice had significantly lower fasting blood glucose and improved glucose tolerance on glucose tolerance testing compared to littermate controls (n=8-10 mice/group, p-value<0.05, Student's t-test). *AdipoqYap*$^{-/-}$ mice on high-fat diet also showed significantly lower levels of genes associated with fibrosis in inguinal and perigonadal white adipose tissue compared to littermate controls (n=5-6 mice/group, p-value<0.001, Student's t-test). Overall, this suggests that disruption of YAP in adipocytes improves glucose tolerance and protects mice from high-fat diet-induced adipose tissue fibrosis.
Conclusion: Together, these data indicate that YAP increases in adipose tissue with weight gain and disruption of YAP in adipocytes improves glucose tolerance; suggesting that adipocyte YAP plays an important role in modulating glucose homeostasis under metabolic stress conditions. Past studies have shown that YAP is an important regulator of fibrosis development in various tissues. Our data show that knocking out YAP in adipocytes decreases the expression of key genes involved in the development of fibrosis. This suggests that adipocyte YAP also regulates fibrosis signalling in adipose tissue under metabolic stress conditions. Overall, our study identifies a potential novel therapeutic target for the treatment of adipose tissue fibrosis and obesity-associated glucose intolerance.
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Aberrant overexpression of HOTAIR inhibits abdominal adipogenesis through the epigenetic remodelling of genome-wide DNA methylation and transcription

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Background and aims: Abdominal adiposity is strongly associated with diabetic and cardiovascular comorbidities. The long non-coding RNA *HOTAIR* (HOX Transcript Antisense Intergenic RNA) is an important epigenetic regulator, that is lowly expressed in abdominal subcutaneous adipose tissue (SAT) compared to gluteal SAT. *HOTAIR* also locates under the genome-wide association *HOXC13* locus for human fat distribution. Hence, we aim to examine the phenotypic effects of *HOTAIR* overexpression on abdominal adipogenesis and link *HOTAIR*-mediated DNA methylation and transcriptome changes to identify its downstream regulated genes and functional pathways.
Materials and methods: The expression level of *HOTAIR* was compared among different fat-depots collected from six healthy, five morbidly obese, and five uremic subjects, correlated with dual-energy x-ray absorptiometry (DXA) defined regional adiposity. The human immortalized preadipocyte was used to assess the phenotypic effects of *HOTAIR* overexpression on abdominal adipogenesis. The integrative analysis of reduced representation bisulfite sequencing (RRBS) and RNA-sequencing was performed to identify putative *HOTAIR*-regulated genes and the associated signaling pathways. *HOTAIR*-repressed genes were further validated using RNA/chromatin immunoprecipitation with real-time qPCR and correlated with human body fat distribution.
Results: We found that the expression of *HOTAIR* was high in gluteal SAT, and low in arm/abdominal SAT and visceral (omental) adipose tissue. It could be aberrantly increased in uremic arm SAT. Notably, the lower expression of *HOTAIR* was correlated with higher abdominal adiposity in morbidly obese subjects, whereas a higher expression of *HOTAIR* was found to correlate with lower arm adiposity in uremic patients. *HOTAIR* overexpression in human immortalized abdominal preadipocyte remarkably suppresses the *in vitro* adipogenesis. We further identified 10 *HOTAIR*-mediated genes showing strong changes of DNA methylation associated with gene expression during abdominal adipogenesis, suggesting potential epigenetic regulation. Two *HOTAIR*-repressed genes, particularly *SLITRK4* and *PITPNC1,* were further highlighted and validated with real-time qPCR and RNA/chromatin immunoprecipitation. Both presented an obesity-driven fat-depot specific expression pattern positively correlated with the central body fat distribution.
Conclusion: Our study indicates that *HOTAIR* is an important regulator for abdominal adipogenesis via intricate DNA methylation likely to associate with transcriptional regulation of specific genes, such as *SLITRK4* and *PITPNC1*.
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Disclosure: **F. Kuo:** None.

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The type 2 diabetes gene RREB1 plays a role in high-fat diet induced adipogenesis in mice

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Background and aims: Genome wide association studies have identified multiple independent signals for type 2 diabetes risk, glycaemic traits and ectopic fat distribution at the zinc finger transcription factor Ras-responsive element binding protein 1 (RREB1) locus. RREB1 is expressed in multiple diabetes relevant tissues, including adipose, but the impact of RREB1 loss *in vivo* and the mechanisms underlying these metabolic phenotypes remain unknown. The overall aim of this study was to determine the role of RREB1 in adipose tissue and its contribution in ectopic fat distribution.
Materials and methods: Global heterozygous *Rreb1*$^{tm1b(EUCOM)Wtsi}$ knockout C57BL/6N mice (homozygotes are embryonic lethal) were generated and characterised from 4 to 26 weeks of age on a high fat (HFD) and a matched low-fat (LFD) diet. Body weight (g), fat and lean mass (g) were measured, the latter using Echo-MRI. Tissue isolated from the inguinal adipose tissue depot of *Rreb1*$^{+/-}$ mice were subjected to histological analysis of cell size. Lipid formation using Oil Red O staining was measured in precursor adipocytes isolated from inguinal adipose tissues and grown and differentiated *in vitro*. Intraperitoneal insulin tolerance tests (IPITT) were performed to determine insulin sensitivity in *Rreb1*$^{+/-}$ mice.
Results: *Rreb1*$^{+/-}$ male mice were significantly protected from weight (p<0.0001) and fat mass (p<0.0001, Figure 1) gain compared to wild-type (WT) litter mates. Fat mass remained significantly lower in males (8-14 weeks) when adjusted for body weight (p<0.0001). Whilst the weights of multiple visceral adipose depots were reduced on a HFD in females (gonadal adipose tissues, p<0.0001; mesenteric adipose tissue, p=0.003; n=46). Adipocytes isolated from the inguinal adipose tissue depot were smaller in size compared to wild-type mice (p=0.0002), indicating that hypertrophic and hyperplastic processes may be altered in these mice. Precursor adipocytes isolated from the inguinal adipose tissue were less capable of differentiating into mature adipocyte as measured by lipid accumulation (p=0.03, n=12). Finally, IPITT showed reduced plasma glucose levels within the first 15 minutes following insulin injection in *Rreb1*$^{+/-}$ male mice compared to WT mice (p< 0.0001; n=30).
Conclusion: Our results demonstrate that RREB1 is important in fat deposition and glucose homeostasis exhibiting sex, diet and age effects and provide evidence that RREB1 is critical in adipocyte differentiation and lipid storage.

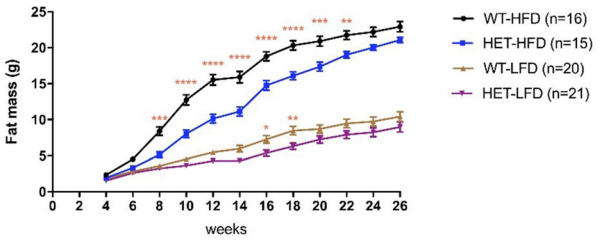

Figure 1 – Global *Rreb1*$^{+/-}$ male mice show reduced fat mass gain on a high fat diet (HFD) compared to wide-type male mice. Error bars presented as mean values ± SEM. Statistical analysis was performed using 2-way ANOVA. **p<0.01; ***p<0.001; ****p<0.0001.

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Deletion of CD44 promotes adipogenesis and insulin signalling in adipocytes

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Background and aims: Growing evidence suggest a close link between adipose fibrosis, inflammation, and insulin resistance in obesity. Hyaluronan, one of the main components of the extracellular matrix is increased in adipose tissue of obese and diabetic mice. CD44, the main hyaluronan receptor is associated with Type 2 diabetes from expression-based genome-wide association studies and its expression level in adipose tissue is positively correlated with adipose inflammation and insulin resistance. This study is to determine the role of CD44 in adipose function and insulin resistance.

Materials and methods: Stable, CD44-deficient 3T3-L1 cells were generated by *Crispr Cas 9* technology using guide RNAs targeting the exon 3 of cd44 gene. The CD44 knockout (KO) cells were confirmed by Western blot and site mutations were determined by biallelic sequencing. Cells that were transfected but maintained normal level of CD44 protein were used as Crispr wildtype (WT) controls. Differentiation of 3T3-L1 cells to adipocytes was included by a cocktail of isobutylmethylxanthine, insulin, and dexamethosone. Mouse primary adipocytes were derived from stromal cells of the subcutaneous adipose tissue in 10 week old C57BL/6 WT and global CD44 KO mice. Adipogenesis was measured by Oil Red O staining and insulin sensitivity was measured by phosphorylation of Akt. Insulin resistance was induced by treating the cells with 250μM palmitic acid for 24 hours.
Results: CD44 gene expression decreased by 81% after differentiation in WT 3T3-L1 cells (P<0.05). Deletion of CD44 in 3T3-L1 cells increased adipogenesis as assessed by Oil Red O staining (3.24±0.86 arbitrary units vs 2.52±0.67 in 3T3-L1 naïve cells and 1.98±0.86 in Crispr WT cells) (P<0.05). Gene expression of the adipogenic markers PPARγ and CEBPα were also consistently increased in the CD44 KO cells when compared with the control cells. Upon insulin stimulation, knocking out CD44 enhanced phosphorylation of AKT at S473 in differentiated 3T3-L1 adipocytes (2.1±0.8-fold increase, P<0.05). Palmitate acid induced a blunted response of Akt phosphorylation and P38 dephosphorylation in 3T3-L1 WT adipocytes, which was reversed in CD44 KO 3T3-L1 adipocytes (P<0.01). Consistent with the results in 3T3-L1 cells, primary adipose stromal cells isolated from CD44 KO mice displayed an enhanced adipogenic capacity and increased phosphorylation of Akt after insulin stimulation compared to those from the WT mice.
Conclusion: Deletion of CD44 promoted adipogenesis and improved insulin signalling in vitro in 3T3-L1 cells and mouse primary adipocytes. This study extends our knowledge of the role of CD44 in regulating adipocyte function, representing a potential target for mitigating adipose dysfunction in metabolic disorders.
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Disclosure: **X. Weng:** None.

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The role of CDKN2C in the regulation of human adipocyte metabolism

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Background and aims: CDKN2C (Cyclin-Dependent Kinase Inhibitor 2C)/p18 is a member of the INK4 family of cyclin-dependent kinase inhibitors that controls cell cycle progression and has been shown to be involved in murine adipocyte cell differentiation. In a genome-wide association study *CDKN2C* gene polymorphisms have been associated with insulin resistance phenotypes and impaired peripheral adipose tissue (AT) storage capacity. This study aims to explore the role of CDKN2C in human adipocyte metabolism.
Materials and methods: Abdominal subcutaneous AT (SAT) needle biopsies were collected from 20 metformin-treated subjects with T2D and 20 sex, age- and BMI- matched healthy controls (20F/20M, age 58 ± 9 vs 58 ± 11 years, BMI 30.8 ± 4.6 vs 30.7 ± 4.9 kg/m^2). Glucose control (HbA1c) was 6.6 ± 0.8 vs 5.6 ± 0.3 % (mean ± SD). Expression levels of *CDKN2C* were measured and correlated with expression of genes involved in lipid storage and differentiation and clinical markers of insulin resistance and obesity. Body fat volumes were measured by magnetic resonance imaging. Furthermore, *CDKN2C* expression was measured in paired samples of SAT and visceral AT (VAT) (15F/8M). CDKN2C was knocked out in human primary preadipocytes using CRISPR/Cas9 gene editing to study the effect on adipocyte proliferation and differentiation rates. Glucose uptake and lipolysis were done in differentiated adipocytes.
Results: *CDKN2C* mRNA expression levels in SAT were 30% lower in the T2D group compared to the control (P<0.05). In the T2D group, *CDKN2C* expression in SAT was negatively correlated with FFA AUC during OGTT (P<0.01), markers of dysglycemia (HbA1c, glucose AUC during OGTT; all P<0.05), insulin resistance (HOMA-IR, P<0.05), and visceral obesity (waist-hip ratio (WHR), VAT volume; all P<0.01), whereas it was positively correlated with SAT/VAT ratio (P<0.01) and the expression of genes promoting lipid storage (e.g. *FASN*, *PPARG*, *FABP4,* all P<0.01). In the control group, *CDKN2C* expression was negatively correlated with WHR and VAT volume and positively with the SAT/VAT ratio (all P<0.05). The *CDKN2C* expression was also down-regulated by 30% in VAT compared to SAT when measured in paired samples (P<0.05), and it correlated negatively with BMI and HOMA-IR in both SAT and VAT. In CRISPR/Cas9 experiments, the loss of CDKN2C did not affect human preadipocyte proliferation or differentiation rates when compared to wild type cells. Interestingly, basal and insulin-stimulated glucose uptake rates were decreased up to 30% in the knockout cells compared to wild type but no effect was seen on lipolysis rate (basal, isoproterenol-stimulated, or insulin-inhibited).
Conclusion: T2D patients have lower *CDKN2C* expression in SAT compared to healthy controls, and this expression is associated with markers of insulin resistance and visceral adiposity. Furthermore, our knockout results support a role of CDKN2C in human adipocyte glucose metabolism but not in the regulation of human adipocyte differentiation. Our findings suggest that downregulation of *CDKN2C* in T2D might contribute to insulin resistance in adipose tissue.
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Disclosure: **M. Vranic:** None.

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Zmat3 hypomethylation contributes to early senescence of adipose precursor cells from healthy individuals with a family history of type 2 diabetes

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Background and aims: Senescence of adipose precursor cells (APC) impairs *de novo* adipogenesis and contributes to the age-related subcutaneous adipose tissue (SAT) dysfunction increasing risk of type 2 diabetes (T2D). First-degree relatives (FDR) of T2D individuals feature restricted adipogenesis in SAT, reflecting detrimental effects of APC senescence earlier in life and rendering FDR more vulnerable to T2D. Recent evidence indicates that epigenetics may contribute to these abnormalities. Our previous methylome analysis in APC from FDR and subjects with no family history of diabetes (CTRL) identified *ZMAT3* as one of the top-ranked senescence-related genes which feature hypomethylation in the FDR subjects and associates with T2D risk. In this study, we investigated whether and how DNA methylation changes at *ZMAT3* promote early APC senescence.
Materials and methods: APC were obtained from healthy and non-obese FDR (n = 12) and CTRL (n = 12) subjects (Table 1). A panel of senescence markers were assayed. Gene expression was assessed by both qPCR and western blot analyses. DNA methylation was assessed by bisulfite sequencing and its impact on transcriptional activity was further

verified by luciferase assay. Gain-of-function experiments were performed to establish the role for *ZMAT3* in inducing APC senescence.
Results: Senescence-related marks, including the acquisition of a senescence-associated secretory phenotype, were observed in APC from the FDR subjects. In FDR APC, reduced DNA methylation at the *ZMAT3* gene caused *ZMAT3* upregulation and accompanied development of the senescent phenotype. Interestingly, demethylation of *ZMAT3* in the APC from CTRL donors led to both increased *ZMAT3* expression and premature senescence. In addition, APC overexpressing *ZMAT3* exhibited senescence and activation of the p53/p21 pathway similar to that of the APC from FDR individuals. Adipogenic differentiation was also inhibited in APC overexpressing *ZMAT3*. Indeed, *ZMAT3* expression was enhanced in the poorly differentiated FDR APC while remaining unchanged in normally differentiated CTRL APC. Finally, in the FDR subjects, senolytic clearance of senescent APC was accompanied by increased DNA methylation and decreased expression of *ZMAT3* as well as by improved adipocyte differentiation.
Conclusion: Our findings indicate that DNA methylation induces *ZMAT3* upregulation in the FDR APC accompanied by acquisition of a senescent phenotype and impaired adipogenic differentiation, likely contributing to FDR insulin resistance and propensity to develop T2D.

Variables	FDR subjects	CTRL subjects
N (female/male)	12 (6/6)	12 (6/6)
Age, years	42.9 ± 2.4	38.3 ± 2.2
BMI, Kg/m^2	25.1 ± 0.6	24.6 ± 0.4
Fat percent, %	26.9 ± 2.4	24.0 ± 1.9
Waist to Hip Ratio (WHR)	0.90 ± 0.02**	0.81 ± 0.02
Cell size, µm	100.3 ± 1.7***	89.5 ± 1.3
f-insulin, pmol/L	60.1 ± 8.0**	35.4 ± 3.9
fb-glucose, mmol/L	4.8 ± 0.1**	4.4 ± 0.1
OGTT p-glucose 2h, mmol/L	6.6 ± 0.6*	4.8 ± 0.4

Table 1. Characteristics of FDR and CTRL subjects. All data shown are the mean ± SEM. Statistical analysis was tested by two-tailed Mann Whitney test. *p<0.05, **p<0.01, ***p<0.001 *vs* CTRL subjects. FDR, first-degree relatives of T2D patients; CTRL, control individuals; BMI, body mass index; f-insulin, fasting insulin; fb-glucose, fasting blood glucose; OGTT, oral glucose tolerance test.

Disclosure: **R. Spinelli:** None.

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Meta-analysis of whole exome and whole genome sequencing data for diabetic nephropathy in individuals with type 1 diabetes

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Background and aims: Diabetic nephropathy (DN) is a major risk factor for severe kidney failure, end-stage renal disease (ESRD) and cardiovascular diseases in individuals with diabetes. The genetic landscape behind this complication is still relatively unexplored, although recent studies have discovered a few causal common variants. To find novel rare variants for DN, we performed meta-analysis of whole exome (WES) and whole genome sequencing (WGS) studies in individuals with type 1 diabetes (T1D) and extreme phenotypes of diabetic nephropathy.
Materials and methods: A total of 1100 individuals with T1D were recruited from the Finnish Diabetic Nephropathy Study (FinnDiane) to undergo WES and WGS. Post-QC, 493 individuals in WES and 583 in WGS were included in the analyses. In both cohorts, the cases had developed either macroalbuminuria or ESRD, whereas the controls had retained normal albumin excretion rate for at least 26 years on WES cohort and 35 years on WGS cohort. Samples were sequenced using Illumina platforms (WES: HiSeq2000 with >80% exome coverage, >20x capture, WGS: HiSeqX >30x capture). Variants were called jointly using Broad Institute's best practices guidelines for Genome Analysis Toolkit, and they were filtered with 98% call rate and 10×10^{-50} HWE. Single variant meta-analysis was performed using rvtest and METAL, and we consider P-values < 1×10^{-6} as exome-wide significant. The gene-based SKAT-O meta-analysis was run with MetaSKAT. The SKAT-O analyses were performed for protein altering variants (PAV) and protein truncating variants (PTV) separately, and we consider P-value of 2.5×10^{-6} as gene-based test significance threshold.
Results: No variant or gene reached genome-wide significance after adjustments for sex, age at T1D onset and two first genetic principal components. The most significant SNP in single variant analysis was a synonymous variant rs10979729 in *EPB41L4B* p=6.13×10^{-6}. However, it is in high LD (r^2=0.94 in Finnish) with a missense variant rs2230794 (Ile830Met) in *ELP1* with the 9th most significant P-value of 5.680×10^{-5}. *ELP1* has been associated with kidney abnormality and renal insufficiency, offering a more plausible explanation for the region's association with DN. The top gene in SKAT-O meta-analysis yielded a P-value 3.55×10^{-5} for *NOM1*, which includes 10 PAVs with MAF<0.10 across the two datasets. *NOM1* was also the top gene associated to diabetic kidney disease in a common variant gene burden analysis from the type 2 diabetes knowledge portal.
Conclusion: Both the WES-WGS single variant and SKAT-O meta-analyses offer novel risk loci and plausible genes for DN, such as *NOM1*. However, due to the loss of statistical significance after Bonferroni correction, the results need further validation.
Disclosure: **J. Haukka:** None.

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Long non-coding RNA from cells derived from urine in biopsy confirmed kidney disease with diabetes to differentiate diabetic kidney disease from non-diabetic kidney disease

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Background and aims: Renal involvement in Type 2 diabetes can be those due to diabetes per se (Diabetic Kidney Disease) or causes other than diabetes (Non-diabetic Kidney Disease NDKD). Currently available clinical, biochemical and radiological markers fail to differentiate the two

accurately and renal biopsy remains gold standard for correct diagnosis. In animal model in those with diabetes and kidney disease it is observed that there is deregulation of several lncRNA. However, this has not be explored in humans with biopsy proven kidney disease in those with Type 2 diabetes. Aims: To determine expression of lncRNA in cells derived from urine (which mainly originate from the kidney) and to evaluate if this could be used as non-invasive markers to differentiateDKD from NDKD.

Materials and methods: We recruited consecutive patient with renal involvement (eGFR >30ml/min/m^2 to <60ml/min/m^2 and/or ACR>300mg/mcg or proteinuria >500 mg/24 hr. Patients without any contradiction for biopsy were subjected to biopsy.Histopathological classification was done as perInternational Society of Nephrology and the Renal Pathology Society (ISN/RPS) Classification Second morning urine sample in fasting state was collected for analysis.From 30 ml of freshly collected urine samples, RNA was isolated from urinary cell debris by commercially available kit. Concentration and purity of RNA was determined in spectrophotometer. Differential expression of 10 Long-Non coding RNAs (chosen from list of lncRNA found to be deregulated in previous animal model studies) were determined by Quantitative Real time PCR with respect to internal control. The fold changes were calculated using SDS software.Mann-Whitney U andReceiver operating characteristic (ROC) curves) were performed.

Results: 94 patients were included of whom 60(63.8%) were DKD, 19 (20.2%) were NDKD and 15 (15.9%) had both DKD &NDKD.We included only those with pure DKD or NDKD for comparison of expression of long non-coding RNA. Amongst the panel we found difference in level of expression of MALAT1($p<0.005$), NEAT($p<0.05$) and PVT1 ($p<0.05$).Amongst them MALAT1 had the best utility in discriminating DKD from NDKD(AUCs of 0.73 (95% CI: 0.5-0.88), $p<0.005$)).

Conclusion: Long-noncoding RNA expression in cells derived from urine may help distinguish DKD from NDKD. The result need validation in larger cohort.

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Novel cross-species transcriptional networks, effective genes and signalling pathways of diabetic nephropathy in human and mouse kidney

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Background and aims: Diabetic nephropathy (DN) or diabetic kidney disease is a serious kidney-related complication of chronic type 1 and type 2 diabetes. Mouse models are effective approach in describing the pathophysiology of (DN), however, it is incompletely summarizing disease presentations of human (DN). To describe the molecular similarities and differences between human and mouse (DN) and to maximize the potential of utilizing these models, we performed a cross-species comparison of kidney tissue transcriptional networks, enriched genes and biological pathways.

Materials and methods: Gene expression profiles for (DN) were downloaded from Gene Expression Omnibus (GEO) data repository for human and mouse and filtered only for diabetic and normal condition samples, normalized and batch corrected. Differentially expressed genes were obtained using bioconductor package "limma". We performed modular repertoire analysis for each dataset separately, where differentially expressed genes are represented in fixed repertoire of transcriptional modules. To gain insight into the biological processes and pathways that could mediate the DN, KEGG enrichment analyses were performed on common DEGs between both datasets using the KEGG KAAS server. To identify the possible protein-protein interaction (PPI) between DEGs, STRINGDB was utilized. To identify critical TFs mediating genes involved in DN *Encode*, *JASPAR* and *ChEA*databases were utilized.

Results: Based on differential expression analysis, 1283 and 2677 genes were significantly dysregulated (p-value < 0.05) in mouse and human datasets respectively. Of the DEGs, a total of 908 and 2202 genes were significantly up-regulated in (DN) samples in both mouse and human datasets when compared with control samples. Interestingly, we found signalling pathways like *PI3K-Akt, MAPK,* and *PPAR* among top enriched pathways. Key genes mediating these signalling pathways are *COL4A1, TLR4, FGFR2/4, ACE, PIK3CD, SPP1, CD36, FABP4, LCN2*, and *LPL*. The PPI network of common DEGs between 2 contrast groups consisted of 22 genes and 45 interactions. Two topological features Maximal Clique Centrality (MCC) and degree were calculated to identify key nodes. Higher the two quantitative values of a gene, the more important it is in the PPI network. The top nodes ranked by degree and MCC were identified, which included *COL4A1, SP1, NFKB1, TP53, STAT3, VIM*, *LCN2, DNMT1* and *HDAC1*. The TF-gene interaction network consisted of 27 nodes (12 TFs and 15 genes). The top-ranked TFs were SMAD4, *SMARCA5, SIN3A, POLR2A, E2F5, ZFX*, and *MAZ*.

Conclusion: The discovered human-mouse shared transcriptional networks enriched genes and biological pathways are most likely contributing to disease progression in type 2 diabetic patients with nephropathy and in commonly used mouse models of DN.

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Disclosure: **B.A. Bhat:** None.

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Soluble Nogo-B upregulates Tie1 receptor: implication for vegfa/vegfr2 signalling in diabetic nephropathy

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Background and aims: The angiopoietin (angpt)/Tie2 system has been implicated in the permselective properties of the glomerular filtration barrier with angpt1 mediating Tie2 phosphorylation (activation) promoting capillary stability and angpt2 (competing for angpt1/Tie2 binding) preventing the action of angpt1. Recent reports have supported the notion that Tie1, an orphan receptor of the Tie receptor family, is required for angpt1 mediated activation of Tie2 phosphorylation and that could also facilitate the action of angpt2 as a Tie2 activator. We recently explored the role of the endoplasmic reticulum protein NogoB and its soluble (sNogoB) circulating isoform (~200 aa N-terminus of NogoB) in experimental animal models of diabetic kidney disease. NogoB and sNogoB bind, with their N-terminus, to their receptor NgBR, expressed in endothelial cells. NogoB is downregulated in diabetic glomeruli and overexpression of sNogoB improves diabetes-mediated albuminuria and prevents diabetes-mediated upregulation of VEGFA/VEGFR2 system, recognised pathway involved in diabetic glomerulopathy. Recent reports have suggested that Tie1 upregulation favours angpt1/2 Tie2 phosphorylation and inhibition VEGFA/VEGFR2 receptor system favouring vascular stability. We hypothesise that Tie1 is implicated in the renal protective response mediated by the sNogoB/NgBR system.

Materials and methods: VEGFR2 phosphorylation and Tie1 protein expression was studied with western immunoblotting in kidney cortex cell lysate of control and diabetic (streptozotocin induced) DBA2J mice overexpressing sNogoB in the circulation (adenoviral vector). In glomerular endothelial cells (GECs)(gift of Dr Satchell), with or without sNogoB overexpression, VEGFA-mediated VEGFR2 phosphorylation was studied by incubating starved GECs with VEGFA (50 ng/ml) for 15 min and ratio VEGFR2 phosphorylation/total VEGFR2 expression studied with western immunoblotting on total cell lysate. NgBR deletion in GECs was performed with siRNA technology and Tie1 expression studied with western immunoblotting.

Results: *In-vivo,* non-diabetic (ND) and diabetic (D) mice administered with AAV-sNogo-B were characterized by a 12-fold increase in circulating

sNogoB when compared to controls (AAV-GFP) (ND-GFP vs ND-sNogoB and D-GFP vs D-sNogoB, p<0.001). sNogo-B overexpression in the circulation was paralleled by 4-5 times upregulation of Tie1 expression in both non-diabetic and diabetic mice (ND-GFP vs ND-sNogoB and D-GFP vs D-sNogoB, p<0.001, n=3-6 per group). Similarly, sNogoB overexpression in GECs upregulated the sNogo-B protein level in the supernatant by 10-fold and partially inhibited VEGFA-mediated VEGFR2 phosphorylation (p<0.05, n=4). Deletion of NgBR in GECs was paralleled by a near total downregulation of Tie1 (p<0.001, n=3-4).
Conclusion: sNogoB overexpression prevents the VEGFA-mediated VEGFR2 phosphorylation possibly by upregulating Tie1 receptor. Downregulation of Tie1 in NgBR deficient cells implicates the sNogo/NogoB-NgBR system in the regulation of the angpt Tie2/Tie1 receptor system. The sNogoB-mediated Tie1 upregulation represents a novel concept and opens new investigations looking at the potential protective role of sNogoB diabetic chronic vascular complications.
Supported by: KRUK
Disclosure: **C. Ricciardi:** None.

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Novel compounds found to regulate VEGF-A splicing in diabetic podocytes

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Background and aims: Alternative splicing (AS) gives rise to multiple proteins from the same gene, by skipping or including exons or parts of them, or by keeping introns as coding sequences. The resulting AS isoforms may function differently from each other. Vascular endothelial growth factor A (VEGF-A) is an angiogenic protein. The alternatively spliced variant, VEGF-A_{165}b, is formed when a distal 3' splice site in exon 8 is selected. The anti-angiogenic and anti-permeability VEGF-A_{165}b has reno-protective properties and has been shown to rescue kidney function in mouse models of diabetic nephropathy. The aim of this study was to investigate three novel compounds that regulate VEGF-A AS in podocytes exposed to a diabetic environment.
Materials and methods: Two of the VEGF-A AS regulatory compounds, trovafloxacin (10μM) and 10058-F4 (5-[(4-Ethylphenyl) methylene]-2-thioxo-4-thiazolidinone) (10μM), were identified from nine synthetic compounds to switch VEGF-A splicing. A third compound, delphinidin (10μg/ml), was found to be key compound regulating VEGF-A AS in a natural blueberry and seabuckthorn extract (DIAVIT). Human podocytes were exposed to a diabetic environment (glucose soup [GS]: 25 mM glucose, 1 ng/ml TNF-α, 1 ng/ml IL-6, and 100 nM insulin), in comparison to a normal glucose (5.5 mM glucose) and an osmotic (5.5 mM glucose + 19.5 mM mannitol) control, for 48 hours. RNA and protein were extracted for RT-PCR and Western blotting analysis of splice isoforms.
Results: Trovafloxacin (mean=2.48, ±0.45 SEM, *p=0.0212, N=17), 10058-F4 (mean= 4.20, ± 0.97 SEM, ****p<0.0001, N=18) and delphinidin (mean= 2.96, ± 1.01 SEM, *p<0.0446, N=9) significantly increased the anti-angiogenic VEGF-A_{165}b relative to pro-angiogenic VEGF-A_{165}a in podocytes exposed to a normal glucose environment at either the mRNA or protein level. Furthermore, 10058-F4 (mean= 5.75, ± 0.53 SEM, ***p=0.0008, N=9) and delphinidin (mean= 2.67, ± 0.64 SEM, *p<0.0269, N=11) were also found to increase the VEGF-A_{165}b/VEGF-A_{165}a ratio in podocytes exposed to a diabetic environment. Statistical analysis were done by using a one way ANOVA followed by a Tukey's post-hoc analysis with p values <0.05 considered as significant. Regarding 10058-F4 and delphinidin, pilot data suggests that these compounds may influence the expression of Clk-1, a kinase known to regulate VEGF-A splice site selection, in diabetic podocytes. Additionally, delphinidin was found to significantly increase the phosphorylation of SRSF6, a splice factor known to promote VEGF-A_{165}b splice site selection, in diabetic podocytes. 10058-F4 was also found to downregulate SRSF1 may be through c-myc inhibition.
Conclusion: We have identified three novel compounds that regulate VEGF-A AS to promote the expression of the reno-protective VEGF-A_{165}b isoform in podocytes. This study will further investigate the mechanism of action of these three compounds regarding VEGF-A splicing regulation in diabetic podocytes. The final goal of this project is to identify whether these novel AS regulatory compounds can be used to develop new therapeutic strategies in diabetic nephropathy.
Supported by: Diabetes UK
Disclosure: **M.L. Ayine:** Grants; Diabetes UK. Lecture/other fees; Richard Bright VEGF research Trust.

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Circulating tenascin-C levels predict renal progression in type 2 diabetes

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Background and aims: Tenascin-C (TN-C) is an extracellular matrix glycoprotein highly expressed in inflammatory states. In individuals with type 2 diabetes, a condition associated with chronic low-grade inflammation, serum TN-C levels have been reported to correlate with baseline eGFR and albuminuria status, and predict major adverse cardiovascular events and mortality. Whether serum TN-C level is prospectively associated with renal progression in type 2 diabetes remains to be investigated. Therefore, we carried out this cohort study to address this issue.
Materials and methods: We conducted a nested case-control study involving participants recruited from the Hong Kong West Diabetes Registry (HKWDR), who had type 2 diabetes followed up regularly at the medical specialist clinics of the Hong Kong West Cluster since 2008. In this study, only those with baseline estimated glomerular filtration rate (eGFR) ≥60 mL/min and followed up for at least 1 year were included. Renal progression was defined by doubling of serum creatinine, which was equivalent to a 57% decline in eGFR. We compared TN-C level between the participants with and without renal progression, while controlling for underlying baseline demographic and clinical differences (glycemic control, eGFR and albuminuria status) and follow-up interval between two groups using propensity score matching. Each case of renal progression was matched to one control. Multivariable conditional logistic regression was employed to identify the independent factors associated with renal progression.
Results: A total of 760 individuals (380 cases and 380 controls) were included after propensity score matching. Their baseline characteristics were as follows: 57.1% men, mean age 63.0±12.0 years, BMI 26.5±5.0 kg/m^2, glycated hemoglobin (HbA1c) 7.9±1.6%, duration of diabetes 12.2±8.7 years. The mean baseline eGFR was 82.7±14.4 mL/min with 42.2% of the cohort having albuminuria of <30mg/g (A1), 37.8% having albuminuria of 30-300mg/g (A2), and 20.0% having albuminuria of >300mg/g (A3). The mean follow-up period was 5.5±2.1 years. The baseline characteristics between individuals with and without renal progression were well-balanced in terms of age, sex, HbA1c, duration of diabetes, lipid profile, eGFR and albuminuria status, use of renin-angiotensin-aldosterone-system blockade, statin and aspirin. However, there were more prevalent cardiovascular diseases (p=0.049) and insulin users (p=0.038) in those with renal progression. Notably, despite comparable high-sensitivity C-reactive protein levels at baseline, we observed higher serum TN-C levels in those with renal progression compared to those without (44.2 ng/mL [95% CI 33.0-59.6] vs 41.7 ng/mL [95% CI 29.6-55.5], p=0.016). Multivariable conditional logistic regression analysis revealed that serum TN-C levels remained independently associated with renal progression (adjusted OR 1.39 [95% CI 1.03-1.89], p=0.034) after adjustments for prevalent cardiovascular diseases and insulin usage.
Conclusion: Our data would suggest serum TN-C level to be an independent predictor of renal progression among individuals with type 2 diabetes and relatively preserved kidney function.
Disclosure: **D.T.W. Lui:** None.

OP 23 Advances in insulin therapy

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Ado09, a co-formulation of pramlintide and insulin A21G improves post-prandial glucose and body weight versus insulin aspart in type 1 diabetes

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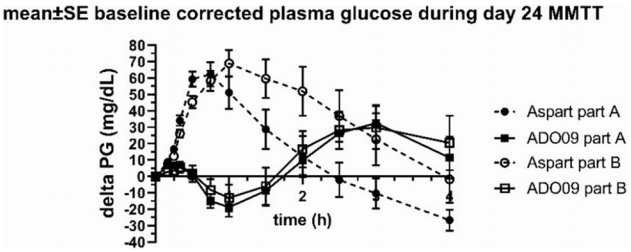
Background and aims: Pramlintide improves post-prandial glucose through delaying gastric emptying, reducing glucagon secretion, and promoting satiety. ADO09 is a co-formulation of pramlintide and insulin A21G. This study was a two-part, double-blind, randomised, 2-period cross-over trial comparing pre-meal ADO09 versus insulin aspart over 24 days in subjects with type 1 diabetes. Part A was conducted in participants using less than 40 U prandial insulin per day, part B in subjects using 40 to 75 U prandial insulin per day.
Materials and methods: During a 28 days run-in period, basal insulin was switched to insulin degludec and titrated. Each treatment period consisted of 3 inpatient days [baseline assessments including a mixed-meal-tolerance test (MMTT) on Day 1], followed by 3 outpatient weeks and a final inpatient MMTT on day 24. Blood glucose, glucagon, and kinetics of gastric emptying were analyzed, as were CGM-metrics. The two treatment periods were separated by a 5 to 7 days washout.
Results: 28 subjects were enrolled in part A and 16 in part B. BMI was higher in those using more prandial insulin, with a mean of 24.54±2.03 kg/m² in part A compared to 30.54±3.08 kg/m² in part B. Incremental plasma glucose AUCs during day 24 MMTT with ADO09 were reduced by >100% in the first 2hrs ($p<0.001$) in both parts vs insulin aspart and, after 4hrs by 39% (p=NS) in part A and 69% (p=0.027) in part B (figure). Gastric emptying was slower with ADO09 (Tmax part A: ADO09 2.47 h vs aspart 0.50h; part B: ADO09 1.89 h vs aspart 0.69 h) and glucagon levels lower with ADO09 over 0-2h ($\Delta AUC_{Glucagon\_0\text{-}2h}$ part A: ADO09 3.29 pmol*h/L vs aspart 11.32 pmol*h/L; part B: ADO09 3.45 pmol*h/L vs aspart 11.66 pmol*h/L). Looking at CGM, BG improved significantly with ADO09 treatment (mean BG over 24h part A: -8.2 mg/dL with ADO09 vs aspart ($p<0.001$); part B: -7.0 mg/mL with ADO09 vs aspart (p=0.013)), and a higher Time-In-Range was observed (part A: +51 min (p=0.013); part B: +58 min (p=0.043) vs aspart). Time <70 mg/dL was slightly higher with ADO09 (part A: +10 min (p=0.046); part B: +13 min (p=NS)), as were hypoglycemic events (part A: ADO09 142 vs aspart 115; part B: ADO09 96 vs aspart 79). ADO09 reduced body weight in both parts, but the reduction was more pronounced in part B (part A -0.7 kg p=0.012 and part B - 1.6 kg (p=0.007) vs baseline), while insulin aspart had not such an effect (part A +0.0 kg (p=NS) and part B +0.4 kg (p=NS) vs baseline). Both treatments were well tolerated with more, but transient, gastrointestinal adverse events with ADO09 in both parts (24 vs 6 in part A and 11 vs 3 in part B), consistent with the known side effect profile of pramlintide. Overall treatment satisfaction measured with a questionnaire was improved with ADO09.
Conclusion: ADO09 was well tolerated and significantly improved post-prandial blood glucose control, hyperglucagonemia, CGM-metrics and body weight versus insulin aspart over 24 days across a wide range of prandial insulin doses.



Clinical Trial Registration Number: NCT03981627
Disclosure: **G. Meiffren:** Employment/Consultancy; Adocia. Stock/Shareholding; Adocia.

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Once weekly basal insulin Fc is safe and efficacious in patients with type 2 diabetes previously treated with basal insulin

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Background and aims: Basal insulin Fc (BIF; LY3209590) is a novel, once-weekly, long-acting IgG Fc-fusion protein that is being assessed for the treatment of diabetes mellitus. The presented study evaluated the safety and efficacy of BIF compared to insulin degludec over 32 weeks in patients with type 2 diabetes mellitus previously treated with oral antidiabetic drugs and a basal insulin.
Materials and methods: The study design included 2 different dosing algorithms for BIF (BIF-A1 and BIF-A2) with two different fasting glucose (FG) targets of ≤7.8 mmol/L (BIF-A1) and ≤6.7 mmol/L (BIF-A2). Insulin degludec was titrated to a FG target of ≤5.6 mmol/L using a modified Riddle treat-to-target algorithm.
Results: Study participants (N=399) were randomized in a 1:1:1 ratio to 1 of 3 parallel treatment groups. The average age of participants was 60.2 years, baseline HbA_{1c} was 65.2 mmol/mol (8.1%) and duration of diabetes 14.7 years. There were no statistically significant differences in demographics or baseline characteristics across the 3 treatment groups. Both BIF groups achieved non-inferiority (non-inferiority margin = 0.4%) for the primary endpoint of HbA_{1c} change from baseline to Week 32 with a mean±SE reduction for BIF-A1, BIF-A2 and insulin degludec of 0.6±0.1%, 0.6±0.1% and 0.7±0.1%, respectively. In line with the different fasting serum glucose (FSG) targets, insulin degludec achieved greater FSG lowering from baseline as compared to the BIF arms. Similarly, both BIF dosing groups showed significantly fewer hypoglycaemic events compared to insulin degludec (all documented events as well as nocturnal events) when assessing events ≤3.9 mmol/L. Hypoglycaemic events <3.0 mmol/L (all documented events as well as nocturnal events) were not significantly different between the three dosing groups. Two severe hypoglycaemic events were reported in BIF-A2. The reported treatment-emergent adverse events and serious adverse events (SAEs) were balanced across the 3 treatment groups. Both BIF groups had a statistically significantly smaller increase in body weight compared to insulin degludec from baseline to Week 32.
Conclusion: In summary, BIF, when administered weekly according to either dosing algorithm, was noninferior to insulin degludec for glycaemic control as measured by change in HbA_{1c} after 32 weeks with a lower rate of documented and nocturnal hypoglycaemia ≤3.9 mmol/L and less weight gain. Additionally, no safety signals were detected. While higher FG targets were chosen in this first Phase 2 study with BIF, the safety and tolerability results allow assessment of lower target glucose ranges in future trials. The results from this study support continued development of BIF as a once-weekly insulin treatment of diabetes mellitus.
Clinical Trial Registration Number: NCT03736785
Supported by: Eli Lilly and Company
Disclosure: **J. Frias:** Employment/Consultancy; Altimmune, Axcella, Boehringer Ingelheim, Coherus Therapeutics, Echosens, 89bio, Eli Lilly, Gilead, Intercept, Merck, Novo Nordisk, Sanofi. Grants; Allergan, AstraZeneca, Boehringer Ingelheim, BMS, Eli Lilly, Intercept, Janssen, Madrigal, Metacrine, Merck, NorthSea Therapeutics, Novartis, Novo Nordisk, Oramed, Pfizer, Poxil, Sanofi, Theracos. Other; Merck, Sanofi.

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Similar hypoglycaemia duration with once-weekly insulin icodec vs insulin glargine U100 in insulin naive or experienced patients with type 2 diabetes

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Background and aims: Insulin icodec (icodec) is a novel once-weekly basal insulin analogue in development. This post hoc analysis explored the duration of hypoglycaemia using double-blinded continuous glucose monitoring (CGM) (Dexcom G6®) data from two phase 2, randomized, open-label, treat-to-target 16-week trials.

Materials and methods: One trial compared three titration algorithms of icodec vs insulin glargine U100 (IGlar U100) in 205 insulin-naïve patients with type 2 diabetes (T2D), the other assessed 154 basal insulin-treated patients with T2D switching from any daily basal insulin to icodec with or without a 100% loading dose vs IGlar U100. In line with ATTD guidelines, a hypoglycaemic episode was defined as a CGM period of interstitial glucose (IG) <3.9 mmol/L for at least 15 min, ending when IG ≥3.9 mmol/L for at least 15 min.

Results: In the titration trial, the duration of hypoglycaemia was similar across all arms: median (interquartile range [IQR]) overall hypoglycaemic episode duration was 35.0 (20.0, 70.0) min for icodec titrations A (self-measured blood glucose target 4.4-7.2 mmol/L; adjusted ±21 U/week) and B (4.4-7.2 mmol/L ±28 U/week) and for IGlar U100 (4.4-7.2 mmol/L ±4 U/day), and 39.0 (24.0, 70.0) min for icodec titration C (3.9-6.0 mmol/L ±28 U/week). The distribution pattern of hypoglycaemic episodes by duration was similar across all treatment arms. Results were similar for nocturnal hypoglycaemic episodes. Similarly, in the switch trial, the duration of hypoglycaemia was similar between arms, irrespective of loading dose use: median overall hypoglycaemic episode duration (IQR) was 40.0 (20.0, 75.0) min for icodec with loading dose, 40.0 (25.0, 80.0) min for icodec without loading dose, and 35.0 (20.0, 60.0) min for IGlar U100. The distribution pattern of hypoglycaemic episodes by duration was similar across treatment arms. Similar results were seen for the nocturnal period.

Conclusion: In conclusion, CGM-based hypoglycaemic episode duration was similar with icodec vs IGlar U100 in insulin-naïve and insulin-experienced patients with T2D, regardless of titration algorithm or initial loading dose use.

Clinical Trial Registration Number: NCT03951805 and NCT03922750

Disclosure: **R. Silver:** None.

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A first in human, single ascending dose study to assess the safety and tolerability, pharmacokinetics, and pharmacodynamics of AB101 in subjects with type 1 diabetes

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Background and aims: AB101 is a slow release microsphere form of polyethylene glycol-human recombinant insulin (peginsulin) for potential use as an ultra long acting basal insulin in patients with diabetes mellitus. In animals, including alloxan-induced diabetic mini pigs, AB101 resulted in slow onset, sustained, and peak less increases in insulin and corresponding glucose lowering for a week or more. This first in human study was designed to assess the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of single subcutaneous (SC) administration of AB101 in subjects with T1DM who were on background continuous SC insulin infusion (CSII).

Materials and methods: This study utilized the euglycemic clamp technique, CSII, and continuous glucose monitoring (CGM). Cohorts 1 thru 3 received single doses of AB101 (0.25, 0.5, or 1.0 mg/kg peginsulin) in sequential ascending dose fashion. Serial measurements of peginsulin were obtained. Three clamps of 24-36h duration were performed at cohort-specific timepoints adapted over the course of study to capture the time-action profile of AB101, between Day 3 and Day 30 relative to dosing. A follow-up safety visit occurred after clinic discharge.

Results: The mean (SD) age and BMI were 35.2 (10.3) and 25 (3.1), respectively; 69% and 88% of participants were male and caucasian, respectively. There were no serious adverse events (AEs) or discontinuations due to treatment emergent AEs, and no clinically significant changes in laboratory evaluations, ECGs, or vital signs. The PK profile showed a delayed onset of AB101 in humans compared to animals. The concentration-time profile demonstrated a dose-dependent increase in serum peginsulin concentrations beginning at Day 15 to Day 20 relative to dosing, with sustained insulin levels for a week or more. There was no evidence of initial or delayed sudden insulin release (burst). Corresponding to the temporal PK profile and supporting a time-action relationship, there was a trend toward dose dependent increases in glucose disposal as reflected by increases in glucose infusion rate (GIR), at clamp timepoints as described in the table. Additionally, AB101 resulted in decreased glycemic variability by CGM, and Cohort 3 consistently required less insulin during clamp run-in and non-clamp days (via CSII) compared to Cohorts 1 and 2.

Conclusion: Overall, single SC doses of AB101 in patients with T1DM were safe and well tolerated, with no evidence of sudden insulin release. Administration of AB101 resulted in slow onset and sustained insulin levels and activity for more than seven days with dose-dependent improvements in glycemic variability, background insulin requirements, and glucose disposal. However, at active doses, the necessary dose volume was greater than anticipated, and delayed or variable kinetics was observed. Formulation optimization would be necessary to to achieve the target product profile for a weekly basal insulin.

	PD Parameters		
	$AUC_{GIR\ 0\text{-}24h}$ (mg/kg)	C_{GIRmax} (mg/kg/min)	T_{GIRmax} (min)
AB101 0.25 mg/kg			
Clamp 1 (Day 3)	N/A	N/A	N/A
Clamp 2 (Day 7)	N/A	N/A	N/A
Clamp 3 (Day 10)	N/A	N/A	N/A
AB101 0.50 mg/kg			
Clamp 1 (Day 7)	N/A	N/A	N/A
Clamp 2 (Day 21)	183.608 (232.587)	1.110 (0.927)	616.000 (472.249)
Clamp 3 (Day 30)	N/A	0.115 (0.230)	452.000 (904.000)
AB101 1.00 mg/kg			
Clamp 1 (Day 14)	27.775 (55.550)	0.550 (0.662)	544.750 (883.306)
Clamp 2 (Day 21)	1254.255 (1015.300)	2.125 (1.633)	793.000 (729.2910)
Clamp 3 (Day 28)	297.520 (511.2290)	0.670 (0.838)	233.000 (347.978)

AUC: Area Under the Curve
GIR: Glucose Infusion Rate

Disclosure: **B.K. Roberts:** Employment/Consultancy; Rezolute. Stock/Shareholding; Rezolute.

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Final data from a long-term observational study of continuous intraperitoneal insulin infusion in a vulnerable population with diabetes

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Background and aims: Continuous intraperitoneal insulin infusion (CIPII) is an alternative method of insulin delivery in the management of people with type 1 or type 2 diabetes (T1D/T2D) requiring insulin, for whom subcutaneous insulin administration is not achieving target glucose, including people who do not tolerate subcutaneous insulin administration. The Accu-Chek® DiaPort system is intended for CIPII and was previously shown to be safe and effective. To assess long-term safety and performance of CIPII therapy with the Accu-Chek® DiaPort we conducted an observational post-market clinical follow-up (PMCF) study to capture real life information from people using the system.

Materials and methods: People aged from 6 years onwards with diagnosed T1D/T2D treated with insulin, either requiring the implantation of the Accu-Chek® DiaPort system, or with the system already implanted, were included. Participants routinely visited the clinic at least every 6 months for 24 months. Additional visits occurred if clinically required. There were no additional appointments or treatments for study purposes. After 24 months, additional safety data were captured at routine visits if the patient agreed to this. All recorded and derived variables are presented, stratified by treatment group and visits, using descriptive summary tables (continuous and ranked data: e.g. sample size, mean, SD).

Results: In 2019 we presented the first data from this study including 88.4 patient years. The most frequent indications for CIPII were unacceptable glucose fluctuations, hypoglycemia unawareness, and severe hypoglycemia, as well as subcutaneous insulin resistance, lipodystrophies and skin disorders. The first data showed that glycemic control (HbA1c and hypoglycemia) improved markedly. Furthermore, no additional unexpected risks were observed and no death occurred in this vulnerable patient population. The study finished at the end of 2020 reaching the goal of collecting data from over 100 patient years. The final data show a marked improvement of mean HbA1c between Visit 1 and last measurement from 8.2 to 7.65% without increasing the number of severe hypoglycemia. We will present further safety and efficacy as well as patient satisfaction data from this 2-5 years long observation time.

Conclusion: This will be the first presentation of safety, therapy efficacy and patient satisfaction data from 49 people with type 1 or type 2 diabetes treated with CIPII therapy using the Accu-Chek® DiaPort system.

Supported by: Roche Diabetes Care GmbH

Disclosure: **B. Gehr:** Honorarium; Participation in Roche Diabetes Care GmbH Advisory Boards.

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Comparative effectiveness of insulin glargine 300 U/mL and insulin degludec 100 U/mL in insulin naive type 2 diabetes adults: the Restore-2 naive cohort

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Background and aims: Second generation basal insulins (2BI) provide similar or improved efficacy with a better safety profile compared to first generation BI. Comparative real-world data on 2BI in European patients with type 2 diabetes (T2DM) are scant. The aim of the study was to assess comparative effectiveness and safety of 2BI [Insulin Glargine 300U/mL (Gla-300) vs. Insulin Degludec 100 U/mL(IDeg-100)] in naive T2DM patients at 6 months. Persistence of HbA1c reduction after 12 months from 2BI initiation was also assessed as long-term outcome.

Materials and methods: RESTORE-2 was a retrospective, non-inferiority, multicenter study, based on electronic medical records. All patients initiating Gla-300 or IDeg-100 in January 2017 - 2020 were 1:1 propensity score matched (PSM). Linear mixed models for repeated measures were applied to assess changes from baseline to 6 months in HbA1c and FBG levels, body weight and insulin dose, and changes in HbA1c levels after 12 months in the two groups. Incidence rates (IR) of hypoglyacemic events were compared between-group using Poisson regression models.

Results: Overall, 357 patients in each PSM cohort were identified. Participants had mean age of 69 years, diabetes duration of 14 years, and baseline HbA1c of 9.2% (77 mmol/mol). After 6 months, marked reductions in HbA1c were documented in Gla-300 and IDeg-100 group [-1.70% (95%CI -1.90;-1.50) vs. -1.69% (95%CI -1.89;-1.49)] without statistically significant between-group difference (p=0.49). The non-inferiority of Gla-300 vs. IDeg-100 was confirmed (margin of non-inferiority of 0.30%; between group mean difference at 6 months: 0.01%; 95%CI -0.29;0.27). Statistically significant within-group reductions were documented in FBG levels: -63 mg/dl (3.5 mmol/L) in Gla-300 group and -61 mg/dl (3.4 mmol/L) in IDeg-100 group (between group difference p=0.74). Minor changes in body weight were documented in both groups. No between-group differences were documented in insulin doses; after 6 months, the dose was 0.20 U/Kg in both groups. IR (episodes per patient-months) of blood glucose (BG) ≤70 mg/dl was 0.13 (95%CI 0.07;0.26) in Gla-300 group and 0.14 (95%CI 0.07;0.27) in IDeg-100 group (p=0.87) during 6 months. IR of BG <54 mg/dl was 0.02 (95%CI 0.01;0.05) in Gla-300 group and 0.02 (95%CI 0.01;0.04) in IDeg-100 group (p=0.49). No severe hypoglycaemic episodes were reported. HbA1c reduction from baseline to 12 months was greater in the Gla-300 group than in Ideg-100 group [-1.71% (95%CI (-1.94;-1.48) vs. -1.44% (95%CI (-1.67;-1.21)], although the between-group difference did not reach the statistical significance (p=0.052).

Conclusion: In adults with T2DM, initiating Gla-300 or IDeg-100 in real world practice was associated with similar improvements in glycemic control, with no weight gain, low hypoglycaemia rates, and no severe episodes. After 12 months, persistence of HbA1c reduction was documented in both groups.

Supported by: Sanofi

Disclosure: **G.P. Fadini:** Grants; AstraZeneca, Novo Nordisk, Mundipharma. Honorarium; Abbott, AstraZeneca, Boehringer, Lilly, Daichi-Sankyo, Mundipharma, Sanofi. Lecture/other fees; Abbott, AstraZeneca, Boehringer, Lilly, Mundipharma, Sanofi, Servier.

OP 24 Epidemiology of diabetes complications

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Young-onset type 2 diabetes: clinical outcomes in Norwegian general practice

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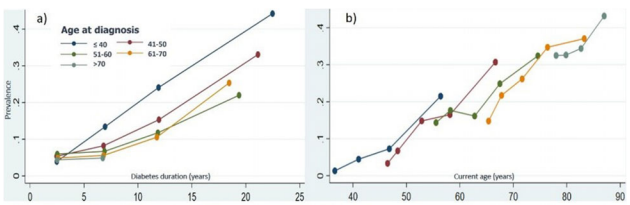
Background and aims: People diagnosed with type 2 diabetes (T2D) early in adult life incur a high lifetime risk of complications and reduced life expectancy. General practitioners are responsible for the predominance of diagnosis and management of young-onset T2D (YOD) in Norway. In this study we aim to estimate the prevalence of YOD in Norway and study the relationships between age at diagnosis, duration of T2D and rates of macro- and microvascular complications.
Materials and methods: Cross-sectional data from general practice electronic medical records of 10 242 adults with T2D were collected in 2015. YOD was defined as diagnosis by age 40. We studied clinical outcomes by age at diagnosis, taking into account diabetes duration or current age. Associations were analysed by multivariate regression, using diabetes diagnosis after age 60 as baseline comparator.
Results: In our study sample, 11.8 % of those with a recorded year of diagnosis (n=9605 non-missing values) were diagnosed with T2D by age 40. In the unadjusted analysis, mean HbA1c (n=9346) was 50.8 mmol/mol among those diagnosed after age 60. Levels were on average 7.79 (6.98, 8.61) mmol/mol higher in YOD. In YOD, HbA1c showed a greater increase with age and diabetes duration. LDL (n=8105) and total cholesterol (n=8550) were also higher in YOD. Retinopathy (n=5880) was found in 8.1% of those diagnosed after age 60. In comparison, retinopathy in YOD had an OR of 3.87 (3.06, 4.88). After adjustment for diabetes duration, sex, highest education level, HbA1c, systolic blood pressure and LDL cholesterol, logistic regression analysis gave an OR of 1.89 (1.37, 2.59). In YOD, retinopathy arose earlier after diagnosis. Macrovascular disease was less frequent in YOD overall. Coronary heart disease had an OR of 0.24 (0.20, 0.30) in YOD. However, in a time to event model with age-based baseline frailty and adjusting for the same covariates as above, cox regression analysis gave a hazard ratio for coronary heart disease in YOD of 1.96 (1.53, 2.50). Macrovascular complications showed a stronger relation to current age than to diabetes duration.
Conclusion: All diabetes complications tended to increase more with age in young onset diabetes compared to older onset. Retinopathy showed the most severe development in YOD, with much higher prevalence and onset starting earlier after diabetes diagnosis. These findings may have implications for future guidelines and individualization of diabetes care.



a) Prevalence of retinopathy by age at diabetes diagnosis and diabetes duration
b) Prevalence of coronary heart disease by age at diabetes diagnosis and current age
The lines represent age at diabetes diagnosis in five strata. The dots represent diabetes duration in years, stratified from left to right: 0, 1-4, 5-9, 10-14, 15 and longer. Strata with less than 100 individuals in total were omitted from the figure.

Supported by: Katrina Tibballs is supported by AMFF with a PhD grant.
Disclosure: **K.L. Tibballs:** Grants; Allmennmedisinsk forskningsfond.

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Diabetes complications among patients from metropolitan versus non-metropolitan cities in India: one year results of LANDMARC

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Background and aims: As type 2 diabetes (T2D) progresses, it inflicts damage to the vasculature. Micro- and macro-vascular complications are the major causes of morbidity and mortality associated with T2D. In India, data on complications in patients with diabetes from metropolitan and non-metropolitan cities are unavailable. Hence, micro- and macro-vascular complications during the first year of LANDMARC, a 3-year nationwide prospective observational study, were evaluated in patients from metropolitan versus non-metropolitan cities.
Materials and methods: LANDMARC is the first nation-wide, prospective, long-term, multicenter, observational, and longitudinal study including patients with T2D. LANDMARC study included patients with T2D who were on ≥2 antihyperglycemic medications. Each participant is intended to be evaluated over the 3-year period (March 2018 to March 2021), comprising 7 visits at 6-months interval.
Results: Of the total 6236 enrolled patients, 2378 and 3858 were from metropolitan and non-metropolitan cities, respectively. Age, duration of T2D, and baseline A1C were similar across groups (Table). At 1-year, microvascular complications were significantly higher in those from non-metropolitan than metropolitan cities (19.08% vs. 10.89%; P<0.0001) (Table). Neuropathy was the most common microvascular complication reported in both the groups. Among macrovascular complications reported, acute coronary syndrome and heart failure were the most common and were significantly higher in participants from non-metropolitan cities (Table).
Conclusion: The present, first-of-its-kind data from India demonstrates that patients from non-metropolitan cities may have higher complications, particularly microvascular. Results from this ongoing study present patterns of disease progression among patients with T2D.

Table. Prevalence of complications

	Metropolitan cities (n=2378)		Non-metropolitan cities (n=3858)	
Age (years), mean (SD)	52.4 (9.33)		52.0 (9.03)	
Duration of type 2 diabetes (years), mean (SD)	8.62 (5.73)		8.57 (5.57)	
HbA1c (mmol/mol), mean (SD)	64.6 (17.36)		64.4 (17.41)	
Complications	**Baseline**	**1 Year**	**Baseline**	**1 Year**
No. of microvascular complications, n	244	272	788	878
No. of patients with microvascular complications	236 (9.9)	259 (10.9)	666 (17.3)	736 (19.1)
Neuropathy	188 (79.7)	205 (79.2)	549 (82.4)	610 (82.9)
Nephropathy	29 (12.3)	36 (13.9)	125 (18.8)	144 (19.6)
Retinopathy	27 (11.4)	31 (12.0)	114 (17.1)	121 (16.4)
No. of macrovascular complications, n	55	65	94	116
No. of patients with macrovascular complications	54 (2.3)	64 (2.7)	91 (2.4)	109 (2.8)
Non-fatal MI	27 (50.0)	30 (46.9)	47 (51.6)	48 (44.0)
Non-fatal stroke	11 (20.4)	12 (18.8)	19 (20.9)	20 (18.3)
Cardiovascular death	0	2 (3.1)	0	13 (11.9)
PVD	17 (31.5)	21 (32.8)	28 (30.8)	34 (31.2)
Comparison of complications between metro and non-metro cities				
Microvascular complications				
Neuropathy	205 (8.62)		610 (15.81)*	
Nephropathy	36 (1.51)		144 (3.73)*	
Retinopathy	31 (1.30)		121 (3.14)*	
Macrovascular complications				
MI	30 (1.26)		48 (1.24)	
Stroke	12 (0.50)		20 (0.52)	
PVD	21 (0.88)		34 (0.88)	
ACS	25 (1.05)		70 (1.81) **	
Heart failure	3 (0.13)		20 (0.52)**	
Unstable angina	10 (0.42)		31 (0.80)	

Data presented as n (%), unless otherwise specified. *P<0.0001; **P<0.05.
Percentages are based on number of patients in Evaluable population. Evaluable population is defined as subset of the patients in the eligible population who are compliant with the protocol and do not have any critical protocol violations. P values are reported from Fishers test if the cell frequency is lesser than 5 and P values are reported using Chi Square test otherwise.
Metropolitan cities include Bengaluru, Chennai, Delhi, Hyderabad, Kolkata, and Mumbai.
Abbreviations: HbA1c, haemoglobin A1C; ACS, acute coronary syndrome; MI, myocardial infarction; PVD, peripheral vascular disease, SD, standard deviation.
Note: This is an interim analysis & possible modifications on variables & data could be performed for the subsequent interim analyses and final analysis

Clinical Trial Registration Number: CTRI/2017/05/008452
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Disclosure: **S. Kalra:** Honorarium; SK received honoraria/speaker fees from Eli Lilly, Novo Nordisk and Sanofi.

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Risk factors, incident dementia, cognitive performance and structural brain abnormalities in individuals with type 2 diabetes

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Background and aims: Type 2 diabetes is associated with increased risks of cognitive dysfunction and structural brain abnormalities. The extent to which risk factor modification can mitigate these excess risks is unclear. We investigated the associations between incident dementia, domain-specific cognitive performance and structural brain abnormalities among individuals with type 2 diabetes, according to the number of risk factors within target range, compared to controls without diabetes.
Materials and methods: Prospective data from UK Biobank of 87,856 individuals (n=10,663 diabetes; n=77,193 controls; baseline 2006-2010; dementia follow-up until February, 2018). Analysis was replicated using data from the Netherlands (the Maastricht Study; cohort with oversampling of type 2 diabetes; examination 2010-2019; cross-sectional data). Individuals with type 2 diabetes were categorized according to the number of seven selected risk factors within target range (nonsmoking; guideline-recommended levels of HbA_{1c}, blood pressure, BMI, albuminuria, physical activity, diet). Outcomes were incident dementia, domain-specific cognitive performance, white matter hyperintensity volume and total brain parenchyma volume.
Results: After a mean follow-up of 9.0 years, 147 (1.4%) individuals with type 2 diabetes and 412 (0.5%) controls had incident dementia. Compared to controls, individuals with type 2 diabetes had a higher incidence of dementia (HR: 1.88 (95% CI: 1.55;2.27)). Among individuals with type 2 diabetes, excess dementia risk decreased stepwise for a higher number of risk factors within target range. Among individuals with type 2 diabetes who had 5 to 7 risk factors within target range, compared to controls (incidence rate per 1,000 person-years 0.62 (95%CI: 0.56; 0.68)), the absolute rate difference per 1,000 person-years for dementia was 0.20 (-0.11; 0.52) and the hazard ratio for dementia was 1.32 (0.89; 1.95). Similarly, differences in processing speed, executive function, and brain volumes were progressively smaller for a higher number of risk factors within target range; these results were replicated in the Maastricht Study.
Conclusion: Among individuals with type 2 diabetes, excess risk of dementia, lower cognitive performance and brain abnormalities decreased stepwise for a higher number of risk factors within target range.
Supported by: ERDF, PoL, SDW, PSID, CVC, CARIM, CAPHRI, NUTRIM, SA, HFL, JCBV, NNF, SAN, ZonMW, NCDC, MP, DHF
Disclosure: **A.C.E. van Gennip:** None.

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Associations between chronic kidney disease, prior cardiovascular conditions and increased mortality in 36.303 type 1 diabetes patients between 2015-2017

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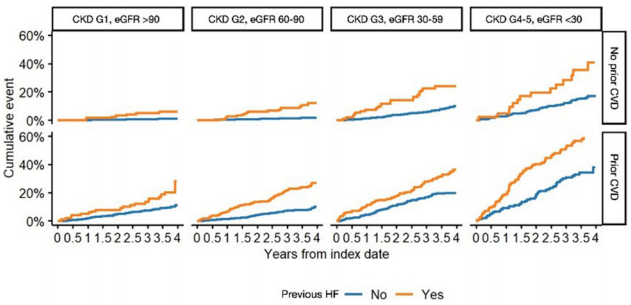
Background and aims: People with type 1 diabetes (T1D) still have an increased risk of premature death compared to the general population, usually caused by cardiovascular disease. The aim of this study was to present updated information on the significance of previous heart failure, kidney disease, and atherosclerotic vascular disease for the risk of mortality in a nationwide, registry-based cohort of T1D.
Materials and methods: All T1D patients in the Swedish National Diabetes Register (NDR) during the inclusion period between January 1, 2015 and December 31, 2017 were included (n=36 303). NDR data were linked to data from several national health registries through each patient's unique personal identification number. The cumulative mortality was described using Kaplan-Meier curves, and evaluated for association to potential risk factors using a Multiple Cox regression model including age, gender, diabetes duration, HbA1c, BMI, SBP, DBP, albuminuria, previous cardiovascular disease (CVD), coronary heart disease, acute myocardial infarction or stroke, heart failure (HF) and eGFR stage as independent variables.
Results: Mean age at index day was 40.1 years, 55% were men and mean duration of diabetes 25.8 years. Record of previous CVD, was found in 8.7%, and prior HF in 1.7%. Normal kidney function (chronic kidney disease (CKD) stage G1; eGFR ≥90) was seen in 50%, and 70% were normoalbuminuric. The mean follow-up time was 3.3 years with a minimum patient follow-up time of 1 year. In total 1127 patients died, with an observed crude total mortality rate of 0.92 deaths/personyear.In patients with prior CVD the risk in all-cause mortality was HR (hazard ratio) 1.91 (95% CI 1.66-2.20) in multivariate analysis compared with persons free of CVD at baseline.In patients with HF adjusted HR was 1.92 (1.66-2.23).Patients with impaired kidney function (moderate, high and very high CKD stages) had HR 1.47 (1.19-1.80), 2.78 (2.11-3.66), and 3.80 (2.80-5.16), respectively, in multivariate analysis.Figure 1 shows cumulative mortality for people with T1D and varying degrees of CKD, with and without previous CVD and HF.
Conclusion: In our registry-based observational study, we found that for patients with T1D, a previous history of CVD, HF, and CKD all were associated with an increased risk of death, and a combination of risk conditions with substantially elevated risk.

Figure 1. Kaplan Meier curves: All-cause mortality by CKD stage and prior CVD or HF



Supported by: Sanofi
Disclosure: **L. Lyngfelt:** None.

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Heart failure and renal complications in young- and usual-onset type 2 diabetes among white Caucasians from US and UK

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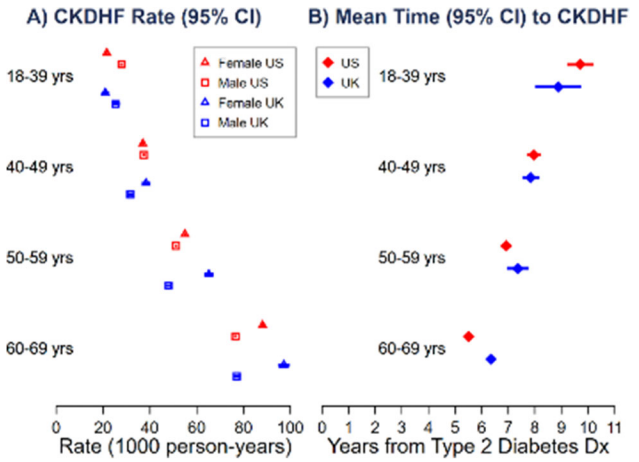
Background and aims: While people with T2DM are known to have increased risk of chronic kidney disease (CKD) and heart failure (HF), the population-level evidence on the risk of these events in young- and usual-onset T2DM in different healthcare setups is scarce. The aim of this study was to evaluate the risk of CKD and/or HF in White Caucasians with T2DM in two different healthcare setups following the same study design. We also evaluated the potential difference in risk dynamics between male and female across age groups.

Materials and methods: Using nationally representative electronic medical records of US and UK, 1491672 and 103233 White Caucasians diagnosed with T2DM between 2000 and 2018 in the age groups of 18-39 /40-49 / 50-59/ 60-69 years were identified. Date of T2DM diagnosis was considered as baseline, with first date of CKD or HF diagnosis (CKDHF) or end of follow-up from baseline considered as the time to event. The risk of CKDHF (hospitalisation or physician coded events) was evaluated in males and females in different age groups adjusting for time-varying covariates. Adjusted mean time to CKDHF was estimated using a propensity score based modelling approach, balancing and adjusting for confounders.
Results: At T2DM dx in US /UK, 50 /59% were male, 10 /13% had CKD, 2 /2% had HF, 48 /55% had hypertension, 39 /31% had dyslipidaemia, 13/15% had cardiovascular disease, and 20 /15% had microvascular disease. 24 /9% were on insulin and 34 /33% had depression prior to CKDHF or end of follow-up. With mean 4.9-5.1 /7.3-7.5 years of follow-up across all age groups in US /UK, 95% CIs for incidence rates per 1000PY for CKDHF were: (23-24) /(22-24) in 18-39 years, (37-38) /(33-35) in 40-49 years, (53-54) /(53-55) in 50-59 years and (82-83) /(83-86) in 60-69 years in US /UK. In the youngest age group in US /UK, compared to female, male had significantly higher CKDHF rate (Figure A) and 25% (HR CI: 1.20-1.29) /31% (HR CI: 1.14-1.53) higher adjusted risk. However, in the 50+ year groups, compared to female, male had significantly lower rate and 3-11 / 21-23% lower risk [range of HR CI: 0.88-0.98 /0.73-0.82] in US /UK. Adjusted years (CI) to CKDHF in 18-39 years group were 9.7 (9.2, 10.2) /8.9 (8.0, 9.7) in US/UK, only 4.2 / 2.6 years later compared to 5.5 (5.4, 5.6) /6.3 (6.2, 6.5) years in 60-69 years group (Figure B). Within age groups < 60 years, the time to event(s) was similar in US and UK.
Conclusion: While healthcare systems differ across countries, the risk paradigm for CKD and HF in White Caucasian males and females with young- and usual-onset T2DM is similar. This clearly suggests a common global approach in the proactive management of macro- and microvascular risk simultaneously in people with T2DM, particularly among young-onset T2DM who develop CKD or HF only 3-4 years later than the usual-onset.



Disclosure: **S. Paul:** Employment/Consultancy; Novartis, Sanofi Avantis, GI Dynamics, Roche, AstraZeneca, Guangzhou Zhongyi Pharmaceutical and Amylin Pharmaceuticals LLC. Grants; Merck, Novo Nordisk, AstraZeneca, Hospira, Amylin Pharmaceuticals, Sanofi-Aventis and Pfizer.

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Risk of long term HbA_{1c} variability on cancer events and cause-specific death in 15,286 patients with type 2 diabetes (The Hong Kong Diabetes Register 1995-2019)

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Background and aims: In high income areas, cancer is emerging as a leading cause of death in type 2 diabetes (T2D). The association of glycaemic variability (GV) with cancer and cause-specific death in type 2 diabetes (T2D) has not been well defined.
Materials and methods: In the Hong Kong Diabetes Register (1995-2019), we used clinic-based HbA1c values to calculate GV using 1) haemoglobin A1c variability score (HVS): percentage of HbA1c values varying by 0.5% compared with prior values, 2) standard deviation of HbA1c (SD_ HbA1c), and 3) SD independent of mean (SDIM) as GV indexes.
Results: We included 15,286 patients with ≥10 years of T2D before cancer occurred or censor date, ≥3 years of observation and ≥5 HbA1c measurements (51.7% male, age: 61.04±10.73 years, HbA1c: 7.54±1.63%, body mass index [BMI]: 25.65±3.92 kg/m^2). We excluded 3 years of HbA1c values prior to cancer to avoid reverse causality. There were nonlinear relationships between HVS and cancer events as well as cause-specific death. In patients with HVS above the median (interquartile range) value of 42.31 (27.27, 56.28), every 1 SD increment of HVS increased the fully-adjusted hazard ratio (aHR) of all-site, breast and liver cancer by 1.15 (95% confidence interval: 1.04, 1.26), 1.44 (1.07, 1.94), and 1.37 (1.08, 1.74), respectively. The respective aHRs were 1.21 (1.06, 1.39), 1.27 (1.15, 1.40), and 1.15 (1.09, 1.22) for cancer, vascular, and noncancer nonvascular death. The significant association between HVS and clinical events was observed only in patients with above-median HVS. The aHRs of SD_HbA1c and SDIM for these events were consistent with that of HVS. Using obesity (BMI ≥25 kg/m^2) and HVS median as risk stratifiers, the obese/high HVS group had aHRs of 1.42 (1.16, 1.73), 2.44 (1.24, 4.82), and 2.63 (1.45, 4.74) respectively for all-site, breast and liver cancer events versus the non-obese/low GV group. The respective aHRs were 1.45 (1.07, 1.96), 1.47 (1.12, 1.93), and 1.35 (1.16, 1.57) for cancer, vascular, and noncancer nonvascular death.
Conclusion: Obesity and GV were jointly associated with increased risk of cancer events and cause-specific death in T2D. Avoidance of glycaemic fluctuation and obesity might reduce the risk of cancer events and cause-specific death in T2D.
Supported by: This project was supported by the CUHK Direct Grant and CUHK Diabetes Research and Education Fund of the Department of Medicine and Therapeutics, Faculty of Medicine, CUHK.
Disclosure: **D. Mao:** None.

OP 25 Disparities and diversity in diabetes epidemiology

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Marked and widening and socio-economic inequalities in prevalence of type 2 diabetes in Scotland

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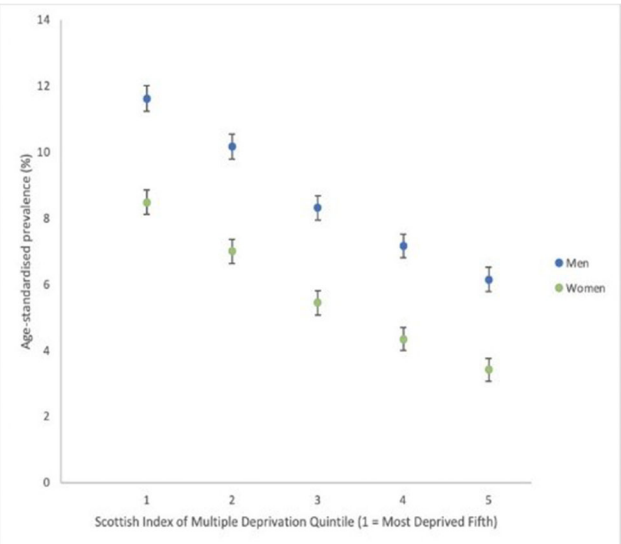
Background and aims: Socio-economic inequalities in prevalence of type 2 diabetes in Scotland were last described in 2007. Since then widening health inequalities in the United Kingdom in general have been reported. The aim of this study was to describe the association between socio-economic status and type 2 diabetes prevalence in Scotland in 2021 and to compare the findings with those reported in 2007.
Materials and methods: A February 2021 extract of the Scottish electronic population-based register of diagnosed diabetes and mid-year population estimates for 2019 (the most recent year for which data are available) were used to estimate diabetes prevalence for 35-84 year olds. The European Standard Population was used to generate age-standardised prevalence by sex and quintiles of an area-based measure of socio-economic status, the Scottish Index of Multiple Deprivation (SIMD) with Q1 and Q5 used to describe the most and least deprived fifths of the population respectively. Sex-specific relative risks for age-standardised diabetes prevalence for Q1 compared to Q5 were estimated.
Results: Data on age, sex and SIMD were available for 255,764 people (98.9%) of 35-84 years of age with diagnosed type 2 diabetes giving an overall prevalence in this age-group of 8.3% in 2021 (as compared to 7.3% in 2007). Prevalence of type 2 diabetes was lowest in women aged 35-39 years from Q5 (0.5%) and highest in men aged 75-79 years from Q2 (22.4%). The figure shows that age-standardised prevalence was higher for men than for women for all SIMD quintiles and was inversely associated with socio-economic status. Relative risks for age-standardised diabetes prevalence for Q1 compared to Q5 have increased from 2.00 (95% CI 1.52-2.62) in 2007 to 2.48 (95% CI 2.15-2.89) in 2021 for women and from 1.58 (95% CI 1.20-2.07) in 2007 to 1.89 (95% CI 1.73-2.08) in 2021 for men.
Conclusion: Prevalence of type 2 diabetes in Scotland varies by age, sex and socio-economic deprivation. Socioeconomic inequalities in prevalence of type 2 diabetes in Scotland have widened between 2007 and 2021.



Disclosure: **S.H. Wild:** Other; The Scottish Diabetes Research Network is supported by National Health Service (NHS) Research Scotland, a partnership of Scottish NHS Boards and the Chief Scientist Office of Scottish Government.

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Prevalence and characteristics associated with antidepressant and antipsychotic prescribing prior to diagnosis of type 2 diabetes in Scotland

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Background and aims: The prescribing of antidepressant and antipsychotic drugs is increasing worldwide for a range of indications including mental ill-health. People with diabetes may be prescribed these drugs more often relative to the general population, due to the increased co-morbidity of diabetes and mental illness as well as the use of some types of antidepressants in treating neuropathy. The aim of this study was to describe the prevalence and patterns of antidepressant and antipsychotic drug prescribing prior to diagnosis of diabetes in Scotland.
Materials and methods: The Scottish Care Information-Diabetes Collaboration dataset (SCI-Diabetes) is a registry which includes information gathered routinely in primary and secondary care for almost all patients diagnosed with diabetes in Scotland. We used the SCI-Diabetes dataset to describe the prevalence and patterns of antidepressant and antipsychotic drug prescribing in adults with type 2 diabetes. We classified people as being prescribed antidepressant or antipsychotic drugs if they had received at least one prescription of these drugs in the four years prior to their diabetes diagnosis. Key characteristics were compared within the cohort by prescription history with P values for ANOVA for continuous variables and for chi-squared tests for categorical variables.
Results: Our cohort included 266,186 adults with type 2 diabetes. Of these, 22.5% were prescribed antidepressants, 5.3% were prescribed antipsychotics, and 6.6% were prescribed both antidepressants and antipsychotics. Of people who were prescribed antidepressants, 32.9% were prescribed a selective serotonin reuptake inhibitor, 30.5% were prescribed a tricyclic antidepressant, 9.9% were prescribed an antidepressant of a different subtype, and 26.8% were prescribed antidepressants from multiple subtypes. Of people who were prescribed antipsychotics, 80.4% were prescribed a first-generation antipsychotic, 14.2% were prescribed a second-generation antipsychotic, and 5.5% were prescribed antipsychotics from multiple subtypes. Compared to people not prescribed antidepressant or antipsychotic medication, a greater proportion of people prescribed antidepressants or antipsychotics were women, lived in more socioeconomically deprived areas, were current smokers, were obese, had hypertension, and had high total cholesterol. People prescribed antidepressant or antipsychotic medication were also more likely to have had a hospital admission for a psychiatric disorder.
Conclusion: Antidepressant and antipsychotic prescribing is common prior to diabetes diagnosis among people with type 2 diabetes in Scotland. In general, a prior prescription for these drugs is associated with a poorer risk factor profile. Further work is needed to investigate prescription patterns post-diabetes diagnosis and to determine whether use of these drugs influences the risk of macrovascular and microvascular complications of type 2 diabetes.

Antidepressant and antipsychotic exposure status (4-year lookback period)		*Non-exposed*	*Antidepressant exposed only*	*Antipsychotic exposed only*	*Antidepressant and antipsychotic exposed*
		174,567 (65.6%)	59,926 (22.5%)	14,197 (5.3%)	17,496 (6.6%)
Men, n (%)		110,996 (63.6%)	26,782 (44.7%)	6,344 (44.7%)	5,668 (32.4%)
Mean age at diabetes diagnosis (years) (SD)		60.9 (13.3)	59.7 (12.7)	63.4 (14.1)	58.7 (13.7)
Ethnicity	White	134,862 (77.3%)	49,064 (81.9%)	11,211 (79.0%)	14,261 (81.5%)
	Other	13,746 (7.9%)	2,815 (4.7%)	970 (6.8%)	804 (4.6%)
	Missing	25,959 (14.9%)	8,047 (13.4%)	2,016 (14.2%)	2,431 (13.9%)
SIMD Quintile	5 (least deprived)	26,790 (15.3%)	6,598 (11.0%)	1,883 (13.3%)	1,490 (8.5%)
	1 (most deprived)	38,352 (22.0%)	17,004 (28.4%)	3,635 (25.6%)	5,673 (32.4%)
	Missing	1,964 (1.1%)	641 (1.1%)	125 (0.9%)	188 (1.1%)
Glycaemic control	HbA1c ≥ 75 mmol/mol)	30,370 (17.4%)	9,393 (15.7%)	2,096 (14.8%)	2,508 (14.3%)
	Missing	24,868 (14.2%)	5,758 (9.6%)	1,441 (10.2%)	1,794 (10.3%)
Blood pressure	Hypertension (systolic ≥ 140 mmHg or diastolic ≥ 90 mmHg) or medication	127,175 (72.9%)	46,534 (77.7%)	10,935 (77.0%)	13,018 (74.4%)
	Missing	13,447 (7.7%)	1,906 (3.2%)	431 (3.0%)	567 (3.2%)
Cholesterol	High total cholesterol (> 5 mmol/L) or medication	127,086 (72.8%)	47,680 (79.6%)	10,976 (77.3%)	13,803 (78.9%)
	Missing	18,543 (10.6%)	4,215 (7.0%)	1,051 (7.4%)	1,448 (8.3%)
BMI	Obese (≥ 30 kg/m^2)	84,400 (48.3%)	34,978 (58.4%)	7,330 (51.6%)	10,520 (60.1%)
	Missing	30,655 (17.6%)	8,728 (14.6%)	2,104 (14.8%)	2,733 (15.6%)
Smoking status	Current smoker	33,086 (19.0%)	15,872 (26.5%)	2,708 (19.1%)	5,349 (30.6%)
	Missing	925 (0.5%)	60 (0.1%)	18 (0.1%)	24 (0.1%)
Any psychiatric disorder (hospital admission)		4,990 (2.9%)	6,802 (11.4%)	2,002 (14.1%)	5,711 (32.6%)

All comparisons significant at P < 0.001

Disclosure: **C.R.L. Greene:** None.

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Selection pressures on the ACE2 gene in a Scottish and South Indian type 2 diabetes population

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Background and aims: Host-pathogen interaction studies have identified ACE2 gene as the receptor of the SARS-COV2 spike protein, which is implicated in the pathogenesis of the COVID-19 pandemic. The infection has varying levels of severity within and between populations and between the sexes. Severity also varied in people with co-morbidities like Type 2 Diabetes (T2D). The current study thus aims to analyse whether selection pressures have played a role in the pathogenesis of the disease between a Scottish and South Indian population, and its association with T2D.
Materials and methods: The study populations consisted of Scottish T2D individuals from the Genetics of the Scottish Health Research Register (GoSHARE) study (n=6,681) and South Indian T2D individuals from the Madras Diabetes Research Foundation (MDRF) cohort (n=6,056). Pairwise calculation of F_{ST} was done on the X Chromosome. High F_{ST} Single Nucleotide Polymorphisms (SNPs) from the ACE 2 gene were taken for further analysis. This was followed by tests for selective sweep - Tajima's D and Nucleotide Diversity. Candidate SNP association studies - sex stratified, and sex adjusted, were done on 13 phenotypes affecting T2D including age at onset, anthropometric measurements, blood pressure and lipids, in both the populations independently. Conditional analysis and gene expression studies were also done.
Results: 28 SNPs within the ACE2 gene had high F_{ST} values (F_{ST} >0.25). No evidence was found for a selective sweep as indicated by Tajima's D (D > 0) and Nucleotide diversity (π>0) values. Candidate SNP association analysis showed significant association of these SNPs only in the South Indian population. P value significance was taken as p<0.05 since there was only one independent SNP. 27 SNPs were significantly associated with age at onset in males, 24 in the sex adjusted model (overall). With triglycerides, 16 SNPs were associated in males. 27 SNPs were associated with height, 23 with weight ,25 with waist hip ratio and 5 with BMI overall; 1 each with waist circumference in females and overall. The most significant hits from each association analysis are presented in Table1. Conditional analysis was done for age at onset and triglycerides (sex stratified). In both cases the top SNPs - rs2158083 and rs1978124, respectively, showed significance independent of the others. Gene expression studies showed a higher expression of the gene in males (testis).
Conclusion: The results showed an association of the alleles with only the South Indian cohort. No genotypic association was seen in the Scottish population. These diabetes related alleles have shown positive selection in the South Indian population indicating they may have been beneficial to them in the past. The results also indicate a possible male specific susceptibility to Covid. The results are yet to be confirmed in a Covid-19 infected cohort.

Phenotype	rsid	A1/A2*	BETA_M**	SE_M	P value_M	BETA_F**	SE_F	P value_F	P value_overall
Ageonset - Sex adjusted	rs971249	C/T	1.280	0.773	3.2E-03	-1.040	0.470	0.27	3.9E-03
Ageonset - Sex stratified	rs2158083	T/C	1.280	0.769	3.0E-03	-0.910	0.471	0.34	4.8E-03
Height - Sex adjusted	rs4646120	A/G	-0.090	0.400	6.7E-01	-0.290	0.210	0.45	1.7E-16
Weight -Sex adjusted	rs2316904	T/C	-0.305	0.883	5.4E-01	-1.530	0.531	0.15	2.0E-03
Waist Hip Ratio - Sex Adjusted	rs1978124	C/T	-0.003	0.004	1.6E-01	-0.002	0.002	0.70	6.9E-10
BMI - Sex adjusted	rs4646124	C/T	0.079	0.226	5.5E-01	0.035	0.160	0.92	5.6E-03
Waist Circumference - Sex Adjusted	rs4240157	C/T	-0.175	0.307	3.4E-01	-0.795	0.256	0.02	8.1E-03
Waist Circumference - Sex Stratified	rs4240157	C/T	-0.175	0.307	3.4E-01	-0.795	0.256	0.02	8.1E-03
Triglycerides - Sex stratified	rs1978124	C/T	-10.180	8.160	2.8E-02	9.900	3.730	0.18	2.3E-01

Table1: The most significant hits from the association analysis (*A1=Effect allele,A2= Non-effect allele; **M=Males, F=Females).

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Disclosure: **C. Nangia:** None.

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Sex, race, and ethnicity representativeness in cardiovascular outcomes trials in type 2 diabetes: a meta-epidemiological study

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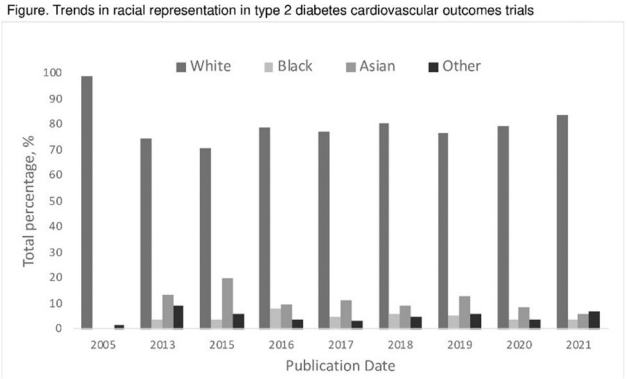
Background and aims: Optimal sex, race, and ethnicity representativeness in clinical trials is necessary to enhance external validity of research findings. We performed a systematic review to evaluate the representation of participants in cardiovascular outcomes trials of type 2 diabetes based on sex, race, and ethnicity.
Materials and methods: We searched Pubmed, Embase, and the Cochrane Library for multi-regional randomised controlled trials assessing the effect of antidiabetic medications on major adverse cardiovascular events in adults with type 2 diabetes. Descriptive statistics were used to summarise demographic data, and chi-square test was used to compare the representation of all race and ethnic groups across eligible trials to the general distribution of these groups in the overall population with type 2 diabetes in the United States (US).
Results: We included 20 trials with 159715 patients. The median number of participating sites per trial was 592 (interquartile range [IQR] 391 to 680), the median number of participating countries per trial was 32 (IQR 23 to 39), and the median age of participants was 64.5 years (IQR 63.3 to 66.1 years). All trials reported demographic data on sex and race (white, black, asian, and other), whereas 12 trials reported data on ethnicity (Hispanic/Latino, not Hispanic/Latino). Across all trials, fewer participants were female comprising 35% (range 22% to 46%, n=56554) of all participants. Most participants were white (77%, n=123013), whereas asian (n=19462) and black (n=7141) participants represented 12% and

4% of the total population, respectively (Figure). In general, these percentages remained consistent in trials published from 2013 onwards (Figure). Compared to the general distribution of racial groups in the overall US population with diabetes, black patients were significantly underrepresented (4% vs 15%, *p*-value <0.05), whereas white patients were overrepresented (77% vs 57%, *p*-value <0.05) in cardiovascular outcomes trials. Hispanic/Latino participants represented 16% of all patients (n=18208), which is comparable to the representation of this ethnic group in the overall diabetic population in the US.

Conclusion: Over the last decade**,** black patients and women are being consistently underrepresented in large cardiovascular outcomes trials in type 2 diabetes. Targeted efforts to increase sex and race representativeness in diabetes research are needed to ensure generalisability of research findings and equity in health care provision.

Figure. Trends in racial representation in type 2 diabetes cardiovascular outcomes trials



Disclosure: **I. Avgerinos:** None.

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GWAS reveals a novel locus associated with kidney function in people of Middle-Eastern decent

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Background and aims: Chronic kidney disease is a significant public health problem that is increasing. Type 2 diabetes is one of the leading causes to kidney failure. Middle Eastern immigrants represent one of the largest immigrant populations to Europe today. Despite having a poor metabolic control and high risk for early onset insulin deficient type 2 diabetes and cardiovascular diabetic complications, they have better kidney function and lower mortality rates in all-cause mortality and cardiovascular-related mortality as compared to native Swedes. This study aimed to understand the underlying genetic basis of this difference by performing GWAS of eGFR and ten type 2 diabetes related traits on people of Iraqi ancestry living in Malmo.

Materials and methods: A total of 1201 residents of Malmo born in Iraq were genotyped, imputed using HRC reference panel, and tested for association with eGFR and ten type 2 diabetes-related traits using a linear mixed model.

Results: Out of the eleven phenotypes tested for association, novel loci for fasting glucose (in *CAMTA1, NDUFA10, TRIO, WWC1, TRAPPC9, SH3GL2 and ABCC11*), quantitative insulin-sensitivity check index (in *METTL16*), eGFR (in *ERBB4*), and HbA1C (in *CAMTA1, ME1, PAK1 and RORA*) was identified at a genome-wide significant level. eGFR also showed a strong signal in a previously reported locus (*CST9)*. It was associated with 107 significant SNPs with an increasing effect on eGFR.

Conclusion: This study, in addition to identifying novel variants, also demonstrated the underlying reason behind the improved kidney function in the Middle Eastern population. The preserved kidney function may be a strong contributor to the survival benefits in Middle Eastern immigrants with type 2 diabetes.

Disclosure: **S.A. Mohamed:** None.

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People with type 1 diabetes of African-Caribbean ethnicity are at increased risk of sight-threatening retinopathy

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Background and aims: There is limited information on the interaction between ethnicity and traditional risk factors on the development of sight-threatening diabetic retinopathy (STDR) in people with type 1 diabetes from ethnically diverse populations. The aim of our study was to describe the risk factors and features of an ethnically diverse cohort of people with type 1 diabetes who develop STDR. STDR was defined by the presence of any moderate to severe non proliferative or pre-proliferative diabetic retinopathy (R2) or proliferative diabetic retinopathy (R3) or maculopathy (M1) in either eye as per UK diabetes eye screening grading.

Materials and methods: Clinical and digital retinal imaging data from 1876 people (50% male, 72.3% Caucasian, 17.4% African-Caribbean, and 10.3% other) with type 1 diabetes as documented from primary care records, without retinopathy at baseline, attending diabetes eye screening in South East London were reviewed. Median duration of follow up was 6 years. Multivariable cox regression analyses was used to estimate hazard ratios for STDR.

Results: The median (interquartile range) age of the cohort was 29 (21, 41) years, duration of diabetes 6.0 (2.0, 12.0) years. Baseline HbA1c was 70.0 (56.8, 94.5) mmol/mol. Cumulative incidence of people with progression from no baseline retinopathy to STDR was 0.1% (95% CI 0.08-0.12) at 5 years, rising to 2.9% (2.5-3.3) at 10 years. The total number of people who developed STDR over 14 years of follow up was 359 (19.1 %). People with STDR had higher baseline HbA1c 77 (60.7, 97.8) vs. 69 (56, 93.9) mmol/mol, raised systolic blood pressure 121 (111, 133) vs. 119 (110, 129) mmHg, longer duration of diabetes 8 (4, 14) vs. 5 (2, 11) years and were more often of African-Caribbean origin (24% vs 15.6%), p<0.05 for all. There were no significant differences in other variables including lipid profiles, body mass index, albuminuria or degree of socio-economic deprivation. In multivariable cox regression analyses, hazard ratio (95% confidence intervals), HbA1c 1.007 (1.004, 1.011), duration of diabetes [1.44 (1.09-1.89) for patients with duration between 10-20 years and 1.52 (1.07-2.16) above 20 years, compared to those with duration of 0-9 years], and African-Caribbean ethnicity 1.43 (1.09-1.88) emerged as independent risk-factors associated with an increased risk of STDR, p< 0.05 for all.

Conclusion: People with type 1 diabetes of African-Caribbean ethnicity are at significantly greater risk of STDR. Further research is required to understand the mechanisms and reasons that may explain this novel observation.

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Disclosure: **A. Mangelis:** None.

OP 26 Beta cells: sensing, signalling and secreting

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Gamma aminobutyric acid-induced calcium signalling in the primary cilium of islet beta cells

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Background and aims: The primary cilium is a rod-like structure that protrudes from the cell surface. It is studded with various receptors and is believed to be a specialised signalling organelle important for probing the local environment. Recent studies have shown that the absence of cilia on β-cells results in aberrant signalling and impaired insulin secretion. Although the primary cilia membrane is continuous with the plasma membrane, it presents with a unique set of receptors and effector proteins. These are selectively granted access to the primary cilium through a gate-keeping mechanism at the cilia base. The cilium is also a unique compartment for $Ca^{2+}$and cAMP signalling. How these small molecules are prevented from freely diffusing between the cilia and cytoplasm is not known, although that would be required for a local signalling function. The best-characterized example of cilia signaling is the hedgehog pathway, which involves activation of the receptor Smoothened and is important for β-cell development and differentiation. This pathway also extensively cross-talk with other pathways to tune the responses, but the identity of these remains largely unknown. The aim of this study was to identify and characterise cilia-specific signaling pathways in β-cells.
Materials and methods: Cilia and cytosolic Ca^{2+} concentration changes were measured using custom-made biosensors and TIRF microscopy. Morphometric analysis of islet cell cilia and distribution of various receptors was performed on fixed islets by immunostaining followed by 3D confocal or STED microscopy.
Results: Transduction of mouse islets with an adenoviral vector encoding a cilia-targeted Ca^{2+} indicator enabled Ca^{2+} imaging within the cilia lumen. We found that elevation of the cytosolic Ca^{2+} concentration by either membrane depolarization of carbachol-mediated release from the ER failed to elevate the cilia Ca^{2+} concentration. In fact, rise of cytosolic Ca^{2+} resulted in a lowering of cilia Ca^{2+} concentration, which was particularly apparent in glucose-stimulated islets, where we observed anti-parallel Ca^{2+} oscillation in the two compartments. The isolation of the cilium against cytosolic Ca^{2+} involved active Ca^{2+} extrusion at the cilia base and was enhanced by membrane depolarization and suppressed by inhibition of the Na/Ca-exchanger. Extended imaging of unstimulated islets revealed prominent cilia Ca^{2+} "flashes" that were synchronized across the islet. Addition of low concentration of GABA (1-10 nM), the $GABA_B$-receptor agonist Baclofen or inhibition of GABA degradation with Vigabatrin increased the occurrence of "flashes" 3-4-fold. Consistent with local GABA action on primary cilia, we find that $GABA_B$-receptors are selectively enriched at the primary cilia base of both mouse and human islet β-cells and become mobile upon agonist binding. GABA stimulation was associated with an acute exit of the hedgehog pathway inhibitor Patched from the β-cell cilia, indicating functional crosstalk between the cilia GABA and hedgehog pathways.
Conclusion: We demonstrate that β-cell cilia are a unique Ca^{2+} compartment that is isolated against cytosolic Ca^{2+} changes, and we identify GABA and $GABA_B$ receptors as a cilia-selective signaling unit that crosstalk with the hedgehog pathway. Signal integration and modulation at the primary cilium may represent an important general mode of regulation of β-cell function within islets.
Supported by: Novo-Nordisk foundation Emerging Excellent Investigator award, Swedish Research Council
Disclosure: **G. Sanchez:** None.

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Involvement of extracellular ATP signalling in the diabetogenic response of pancreatic beta cells

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Background and aims: Chronic exposure of pancreatic beta-cells to elevated nutrient levels impairs their function. While the use of fructose as a sweetener is associated with obesity, fructose alone does not acutely stimulate insulin secretion, as opposed to the chief secretagogue glucose. Upon glucose stimulation, ATP and ADP are co-secreted with insulin and may activate purinergic receptors. We recently reported that chronic exposure to fructose induces extracellular ATP signalling in beta-cell, resulting in the potentiation of physiological glucose-stimulated insulin secretion (GSIS). This effect is mediated by the activation of the calcium-mobilizer purinergic P2Y1 receptor and is associated with the release of cellular ATP through pannexin-1 channels. However, little is known on extracellular ATP signalling responses to nutrient-rich metabolic stresses. Here, we investigated the expression pattern of genes implicated in the extracellular ATP signalling of beta-cells following metabolic stresses.
Materials and methods: INS-1E beta-cells or freshly isolated human islets were exposed for 3-4 days to glucose (5.5-25 mM), palmitate or oleate (0.4 mM), and fructose (5.5 mM). Following these treatments, we analyzed transcripts levels of several components of extracellular ATP signalling in INS-1E cells by qRT-PCR and in human islets by RNA-Seq, including *Panx1*, the ecto-ATPase *Entpd3* and *P2ry1*. NTPD3 was also evaluated at the protein level by immunoblotting. The secretory response of INS-1E beta-cells was monitored during acute 8.3 mM glucose stimulation using online luminescence assay.
Results: Diabetogenic conditions reduced expression of the beta-cell enriched NTPD3 and P2Y1 in INS-1E cells (upon glucotoxicity) and in human islets (upon glucolipotoxicity), while PANX1 expression was preserved. Immunoblot analyses revealed that NTPD3 is an abundant glycosylated protein regulated by glucose in INS-1E cells and human islets. Addition of the ecto-ATPase inhibitor ARL67156 or the P2Y agonist 2MeSADP to naive INS-1E cells (i.e. cultured in standard 11 mM glucose media) potentiated the secretory response to 8.3 mM glucose, mimicking effects of chronic fructose treatment. Conversely, clearance of extracellular ATP and ADP to AMP by apyrase reduced stimulated secretion in naive cells and reversed the potentiated secretory response induced by fructose. INS-1E cells cultured at low 5.5 mM glucose exhibited poor responses to 8.3 mM glucose stimulation and neither chronic fructose treatment nor addition of ARL67156 and/or 2MeSADP did restore such blunted secretory response.
Conclusion: These results suggest that ectonucleotidases and purinergic receptors are key components of the ATP extracellular signalling during physiological conditions in pancreatic beta-cells and may represent putative target during pathophysiological conditions.
Disclosure: **T. Brun:** None.

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A dual GLP-1/GIP agonist may encompass the beneficial effects of both incretins on pancreatic beta cell function in the absence of beta-arrestin2

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Background and aims: Increasing the efficacy of GLP-1 receptor (GLP-1R) agonists by using dual GLP-1/GIP receptor agonist is a new strategy in the treatment of type 2 diabetes. GLP-1R and GIPR are G protein

coupled receptors (GPCRs) known to be positively coupled to cAMP production and PKA/EPAC2 activations. Both GPCRs are also able to recruit the scaffold protein beta-arrestin2 (ARRB2), which may activate new signaling pathways such as the kinases ERK1/2 or Focal Adhesion Kinase (FAK). Our aim is to determine whether Tirzepatide, a dual GLP-1/GIP receptor agonist, has a beneficial effect superior to GLP-1 on primary pancreatic beta cells.

Materials and methods: Experiments were performed in beta cells from 4-month-old *Arrb2*$^{-/-}$ and *Arrb2*$^{+/+}$ male mice. Endogenous PKA (AKAR3) and ERK1/2 (EKAR) activations were assessed by live cell imaging in mouse pancreatic beta cells after adenoviral infection with FRET-based sensors of interest. Insulin secretion was measured on isolated islets by homogenous time-resolved fluorescence. F-actin depolymerization was evaluated by phalloidin staining (Alexa Fluor 488-conjugated phalloidin) and the phosphorylation of FAK by immunofluorescence. GLP-1R endocytosis was assessed by immunofluorescence from 4% formaldehyde fixed and non-permeabilised beta cells.

Results: PKA activation and insulin secretion were significantly increased in response to 10pM-100pM GLP-1 ($p<0.01$) and maximally activated by 100nM-1nM. In contrast, GIP and Tirzepatide were only effective from 1nM ($p<0.01$), indicating, that Tirzepatide is less potent than GLP-1 on GLP-1R, as already reported in non-beta cells. In addition, GLP-1 induced PKA long lasting activation (>25 min) that was associated with a prolonged internalisation of GLP-1R, whereas the reversal of PKA activation upon GIP stimulation was rapid (<5min), suggesting slow versus fast recycling receptors, respectively. Surprisingly, Tirzepatide induced PKA long lasting activation similar to that of GLP-1, but without any GLP-1R internalisation. In *Arrb2*$^{-/-}$ beta cells, PKA and EPAC2 activations by GLP-1 or GIP were not affected despite a strong reduction of insulin secretion in response to GIP (~50%, $p<0.01$) that was caused by a reduction of F-actin depolymerisation (~50%, $p<0.01$) and FAK activation (~50%, $p<0.01$). Tirzepatide, like GLP-1, induced a similar insulin secretion, F-actin depolymerisation and FAK activation in *Arrb2*$^{+/+}$ and *Arrb2*$^{-/-}$ beta cells. By contrast, ERK1/2 activation in response to GLP-1 was strongly reduced by ~50% ($p<0.05$) in *Arrb2*$^{-/-}$ beta cells while GIP and Tirzepatide recruited ERK1/2 independently of ARRB2.

Conclusion: In beta cells, Tirzepatide combines the beneficial effects of GLP-1 and GIP. Our study reports that this dual GLP-1/GIP agonist may overcome the functional consequences of the decrease in ARRB2 expression that can be observed in diabetogenic conditions.

Supported by: SFD research grant

Disclosure: **N. Zaïmia:** None.

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Feeding inhibits the catabolic activity of glutamate dehydrogenase in mouse pancreatic beta cells as revealed by in situ assessment of enzyme activity

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Background and aims: Glutamate dehydrogenase (GDH) is a mitochondrial enzyme playing a key role in the control of insulin secretion. However, its regulation remains unclear, in particular regarding a putative oxidative catabolic activity consuming glutamate. Classical lysate preparations for enzymatic activity assessment might lose the in situ allosteric regulations governing GDH. Here, we developed an *in situ* redox-sensitive nitro blue tetrazolium (NBT) assay to investigate dehydrogenase activity in cryopreserved pancreatic sections. On subsequent sections, DTZ staining and immunohistochemistry provided corresponding mapping of the cells. The aim of the present study was to investigate *in situ* islet GDH activity in cryopreserved pancreatic sections.

Materials and methods: We applied the redox-sensitive nitro blue tetrazolium (NBT) assay to investigate *in situ* pancreatic islet GDH activity. On the same preparations, we measured activities of succinate dehydrogenase for positive control of mitochondrial metabolism, of GAPDH as a readout of glycolytic capacity, and of LDH. The study was conducted in mice of 12 weeks of age. As GDH negative control, we used beta-cell specific GDH knockout mice (Beta-*Glud1*$^{-/-}$). Mice were fed a standard diet and sacrificed under different nutritional states: fed, 6-hour fasting, and overnight starvation. Pancreata were collected and stored at -80 C before the preparation and analyses of cryosections. On the same section series, insulin-containing cells were revealed by DTZ staining.

Results: Measured *in situ*, the NBT-based assay revealed strong activities of both GAPDH and succinate dehydrogenase in insulin-positive cells of cryopreserved islets isolated from fed mice. With prolonged fasting time, GAPDH activity was significantly reduced ($p=0.014$). LDH *in situ*-activity was absent in islets, whatever the nutritional state. Specifically, in insulin-positive cells, the catabolic GDH flux to glutamate oxidation was activated upon overnight starvation, while such GDH activity was suppressed in fed conditions ($p=0.0049$). These GDH readouts were undetectable in Beta-*Glud1*$^{-/-}$ islets. In exocrine non-islet cells, GDH activity was not modulated by the nutritional state.

Conclusion: Overall, our study shows that commonly used assessment of GDH function does not properly reflect *in situ* activity. The catabolic activity of GDH was inhibited by feeding when beta-cells are stimulated, while glycolytic GAPDH and mitochondrial succinate dehydrogenase were active. The beta-cell disallowed LDH was silent in all tested conditions. The increased glutamate-consuming GDH activity observed upon starvation points to an energy-supporting role for GDH in such conditions.

Supported by: Swiss National Science Foundation

Disclosure: **Y. Zhou:** None.

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Alterations in the expression of proteins involved in nuclear-cytoplasmic shuttling of cargo in pancreatic beta cells under the duress of chronic hyperglycaemia

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Background and aims: Recent evidence in the islet beta cell suggests critical regulatory roles for Rac1, a small G protein, in the pathogenesis of metabolic dysfunction under the duress of chronic hyperglycemia (HG). Evidence is also emerging to suggest increased nuclear translocation (i.e., mistargeting) of Rac1 in human islets, rodent islets and INS-1 832/13 cells following exposure to HG. Data from quantitative proteomics studies identified Karyopherin-α2 (KPNA2), a nuclear transport protein, as one of the interacting partners for Rac1 in pancreatic beta cells. Therefore, we determined the effects of HG conditions on the expression and subcellular distribution of specific nuclear-cytoplasmic shuttling proteins in pancreatic beta cells.

Materials and methods: Rat islets were isolated by the collagenase digestion method. Human islets were from Prodo Laboratories. INS-1 832/13 cells were cultured in the presence of low (2.5 mM) or high (20 mM) glucose for 24 hrs. for assessing the effects of HG. Nuclear and cytosolic fractions were isolated from INS-1 832/13 cells using the NE-PER Nuclear and Cytoplasmic Extraction Kit (Thermo Fisher Scientific). Expression of candidate proteins involved in nuclear-cytoplasmic shuttling of cargo was quantified by Western blotting and densitometry.

Results: KPNA2 is expressed in human islets, rat islets and INS-1 832/13 cells. Incubation of INS-1 832/13 cells with HG, but not mannitol (osmotic control), markedly increased (2.5-4-fold) the expression of KPNA2. HG conditions elicited minimal effects on the expression of karyopherin-β1. Subcellular fractionation studies indicated significant increase in KPNA2 expression in both cytosolic and nuclear fractions isolated from HG exposed INS-1 832/13 cells compared to cells exposed

to basal glucose. A significant increase in nuclear association of Rac1 was also seen under these conditions. HG exposure conditions exerted no effects on the expression of Ran, a small G protein involved the shuttling of cargo between the nuclear and cytosolic compartments. However, a significant increase (2.6-fold) in the expression of Ran GTPase-Activating Protein 1 (RanGAP1), which converts Ran-GTP to Ran-GDP, was noted in beta cells exposed to HG. Lastly, HG exposure conditions did not significantly alter the expression of Regulator of Chromosome Condensation 1 (RCC1), a known guanine nucleotide exchange factor that mediates the conversion of Ran-GDP to Ran-GTP.
Conclusion: Our findings provide the first evidence for potential alterations in the expression of nuclear-cytoplasmic shuttling proteins in pancreatic beta cells under the duress of HG. Based on these data, we propose that HG conditions promote RanGAP1-mediated conversion of Ran-GTP to Ran-GDP, which might dictate directionality of nuclear transport by setting up the gradient of RanGAP1-regulated Ran-GDP level in the cytoplasm and RCC1-regulated Ran-GTP level in the nucleus. Molecular and pharmacological studies are underway to determine potential impact of these alterations in beta cells in the cascade of events involved in the transport and inappropriate localization of Rac1 in the nucleus for propagation of signals necessary for HG-induced metabolic dysfunction of the islet beta cell.
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Disclosure: **A. Kowluru:** None.

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What regulates insulin granule mobility in the submembrane space? Role of cAMP and adenine nucleotides

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Background and aims: Submembrane insulin granules are in constant motion, the extent of which varies widely. While about 30% of the granules appear to be attached with very limited mobility, another 30% spend less than 1 s in the immediate submembrane space before disappearing again. The regulation and energetic requirement of granule mobility are largely unknown. Here, we have investigated the functional consequences of modifying cAMP levels and inhibiting the oxidative phosphorylation.
Materials and methods: MIN6-cells were cultured in DMEM-medium and the insulin granules were labelled by transient transfection with hIns-EGFP. The submembrane granules of constantly perifused cells were imaged by TIRF-microscopy and their number and mobility pattern were analyzed by an observer-independent evaluation program. The insulin secretion by statically incubated MIN6-cells was measured by ELISA, the ATP- and ADP-content was measured by luciferase luminometry and the cytosolic Ca2+ concentration ($[Ca^{2+}]_i$) by Fluo4-fluorescence.
Results: After an equilibration period of 45 min during which the cells were perifused with Krebs-Ringer-Medium containing 3 mM glucose, the cAMP levels were initially raised by perifusion with 1 nM GLP-1. Since this left the mobility pattern virtually unaffected, 75 μM IBMX was used. After a wash-out period the stimulation was repeated in the presence of 30 mM glucose. Again, the effect on the parameters of mobility was only modest, but the number of exocytoses was moderately increased. Acute inhibition of oxidative phosphorylation was induced by perifusion with either 10 μM CCCP or 5 mM Na-Azid or 4 μg/ml Oligomycin. Here, clear consequences for the granule mobility pattern appeared, but these were qualitatively and quantitatively different, depending on the inhibitor. The effects were reversible upon wash-out, corresponding to known kinetics of action. The most marked effects were produced by CCCP, which was also the most effective compound to lower the ATP/ADP ratio, to diminish the rates of exocytosis and at the same time to increase $[Ca^{2+}]_i$. Azide came close to CCCP in reducing the number of exocytoses and its effects showed the fastest reversibility. Oligomycin proved to be as effective as the other compounds to inhibit the secretion by potassium depolarization, even though it was the least effective to influence the granule mobility pattern, the ATP/ADP ratio, and $[Ca^{2+}]_i$.
Conclusion: The mobility pattern of submembrane granules does not appear to be regulated by cAMP, thus the known enhancing effect of cAMP on insulin secretion may not depend on the increased delivery of granules to sites of exocytosis. In contrast, the granule mobility pattern was markedly affected by inhibitors of the oxidative phosphorylation, but their effects in addition to the lowering of the ATP/ADP ratio makes it difficult to ascribe a specific role to the level of adenine nucleotides. The effects on mitochondrial function need to be studied in more detail to clarify their relation with granule mobility and exocytosis.
Supported by: DDG
Disclosure: **B. Gaus:** Grants; Deutsche Diabetes Gesellschaft.

OP 27 Prediction tools for outcomes in diabetes

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Prediction models for future complications in type 1 diabetes

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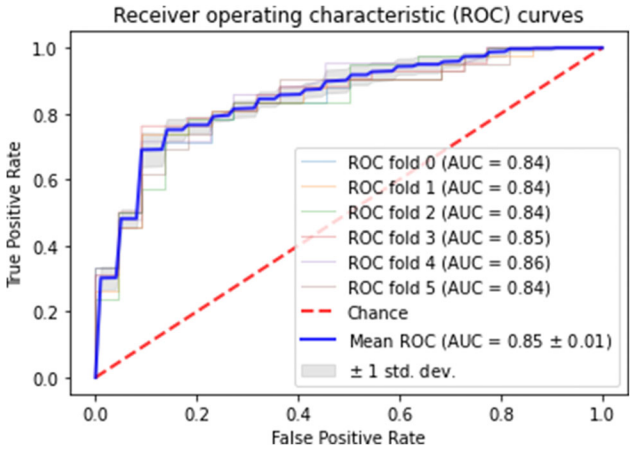
Background and aims: Developing panel that predicts the risk of developing diabetes complications (DC) including diabetic nephropathy (microalbuminuria, macroalbuminuria or ≥30% decline in estimated glomerular filtration rate (eGFR)) and diabetic retinopathy (mild, moderate or severe).
Materials and methods: From a cohort of 537 clinically diagnosed adults with type 1 diabetes followed-up a median of 5.4 years at Steno Diabetes Center Copenhagen, we developed prediction models for DC progression. Exploratory analyses included non-matched participants and both tree and network based algorithms. A $HbA1_c$and diabetes duration matched subset of the participants was classified into two groups: T1D stable (n=128), and T1D with progression to DC (n=189). Two random forest models were compared, first using clinical risk factors (16 features, Model 1) and using clinical risk factors with blood small molecule data (965 features, Model 2).
Results: The major contributors to the DC progression Model 1 included eGFR, Age, diabetes duration, BMI and blood triglycerides. The major contributors to Model 2 included eGFR, three metabolites (2,4-dihydroxy butyric acid (DHBA), 3,4-DHBA, creatinine) and a lipid species sphingomyelin d42:1. The machine-learning algorithm (Model 2) including 5 variables provided the best performance as assessed by net reclassification index (NRI, 5%) with an accuracy of 0.81 and the average receiver operating characteristic (ROC)= 0.85 curve with 6-fold cross validation (**Fig.1**).
Conclusion: A high-performing diabetes complications progression model was developed, with clinical variables and blood metabolites, to predict diabetes complications.



Supported by: This work was supported by theNovo Nordisk Foundation (grant number NNF14OC0013659 PROTON Personalising treatment of diabetic kidney disease; internal funding was provided by Steno Diabetes Center Copenhagen, Gentofte, Denmark.
Disclosure: **N. Al-Sari:** None.

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Validation of the classification for type 2 diabetes into five subgroups: a report from the ORIGIN trial

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Background and aims: Data analyses from Swedish individuals with newly diagnosed diabetes have suggested that diabetes could be classified into five subtypes that differ with respect to the progression of dysglycemia and the incidence of diabetes consequences. We assessed this classification in a multiethnic cohort of participants with established and newly diagnosed diabetes, randomly allocated to insulin glargine versus standard care.
Materials and methods: 7,017 participants from the Outcome Reduction with Initial Glargine Intervention (ORIGIN) trial were assigned to the five predefined diabetes subtypes (namely, severe auto-immune diabetes (SAID), severe insulin-deficient diabetes (SIDD), severe insulin-resistant diabetes (SIRD), mild obesity-related diabetes (MOD), mild age-related diabetes (MARD)) based on the age at diabetes diagnosis, body mass index, glycosylated hemoglobin, fasting C-peptide levels and the presence of glutamate decarboxylase antibodies at baseline. Differences between diabetes subtypes in cardiovascular and renal outcomes were investigated using Cox regression models for a median follow-up of 6.2 years. We also compared the effect of glargine versus standard care on hyperglycemia, defined by having a mean post-randomization glycosylated hemoglobin ≥6.5%, between subtypes.
Results: The five diabetes subtypes were replicated in the ORIGIN trial and exhibited similar baseline characteristics in Europeans and Latin Americans, compared to the initially described clusters in the Swedish cohort. We confirmed differences in renal outcomes, with a higher incidence of events in the SIRD compared to the MARD subtype (i.e., chronic kidney disease stage 3A: HR=1.47, 95%CI [1.30-1.67]; stage 3B: HR=2.20 [1.80-2.69]; macroalbuminuria: HR=1.48 [1.17-1.86]). No differences were observed in the incidence of retinopathy and cardiovascular diseases after adjusting for multiple hypothesis testing. Diabetes subtypes also differed in glycemic response to glargine, with a particular benefit of receiving glargine (versus standard care) in the SIDD subtype compared to the MARD subtype, with a decreased risk of hyperglycemia by 14% (OR=1.37 [1.32-1.43] on glargine; OR=1.51 [1.45-1.58] on standard care; P for interaction subtype*intervention =0.001).
Conclusion: Cluster analysis enabled the characterization of five subtypes of diabetes in a multiethnic cohort. Both the incidence of renal outcomes and the response to insulin varied between diabetes subtypes. These findings reinforce the clinical utility of applying precision medicine to predict comorbidities and treatment responses in patients with diabetes.
Clinical Trial Registration Number: NCT00069784
Supported by: The ORIGIN trial and biomarker project were supported by Sanofi and the CIHR
Disclosure: **M. Pigeyre:** Other; The ORIGIN trial and biomarker project were supported by Sanofi and the Canadian Institutes of Health Research (CIHR).

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Machine learning approaches for prediction of nocturnal hypoglycaemia in patients with type 1 diabetes in a hospital setting

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Background and aims: Nocturnal hypoglycaemia (NH) is a potentially dangerous and underestimated complication of insulin therapy. Minimizing the number of NH events requires reliable prediction.

Machine learning algorithms operating on continuous glucose monitoring (CGM) data opens up new possibilities for hypoglycemia forecasting. In this study we aimed to establish an approach to the short-term prediction of NH in hospitalized patients with type 1 diabetes (T1D) based on sets of clinical and CGM data and machine learning techniques.

Materials and methods: We used a dataset of CGM records obtained from 406 adult T1D patients admitted to tertiary referral hospital. The NH was defined as an episode of interstitial glucose level <3.9 mmol/L for at least 15 min in the interval from 0 to 6 am. CGM-derived metrics, including mean glucose and glucose range, gradient, linear, quadratic and cubic trend coefficients, dynamic time wrapping similarity-based indices, and a number of glucose variability parameters, were used as potential NH predictors. At the next step, clinical parameters were added to the models. In total, we estimated 113 variables. Visualization of CGM data with t-distributed stochastic neighbor embedding technique was performed. The sub-sampling of mostly representative records without NH was applied to get more balanced distribution of classes. To this end, we used the dynamic time wrapping similarity metric between time series. Augmentation methodology, including perturbation with small Gaussian noise and weighted averaging of NH records, was applied for generating artificial CGM records. Random Forest method was used for the NH forecasting and estimation of the predictors. The quality of prediction was evaluated on a non-augmented test sample using Monte-Carlo cross-validation.

Results: Depending on predictor sequence length (30 minutes - 1 hour) and horizon of forecasting (5-30 minutes), we have analyzed 209-256 CGM records with at least one episode of NH and near $4 \cdot 10^4$ records without NH. Cluster analysis has revealed that the CGM data were rather homogeneous: a number of closely spaced clusters have been found. The quality of forecasting did not depend significantly on predictor sequence length; therefore, we used 30 minutes as a baseline. The sensitivity and specificity varied from 98% and 97% respectively for a 5-minute horizon to 85% and 87% for a horizon of 30 minutes. However, the positive predictive values of the models were low (less than 5-10%) due to the unbalanced nature of the data. Mean and minimal glucose levels, linear trend coefficient and downward 5-minute gradient were the most important predictors assessed by Random Forest. Incorporating clinical data into the models, as well as the augmentation procedures, improved the quality of prediction for a horizon of 30 minutes giving an increase in sensitivity and specificity by 3-7%.

Conclusion: The results demonstrate that machine learning based on the clinical and CGM data is a promising approach for prediction of NH events in patients with T1D in a hospital setting.

Supported by: RSF (20-15-00057)

Disclosure: **V.B. Berikov:** None.

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Non-invasive prenatal diagnosis of fetal genotype in pregnant women with GCK-MODY: the impact of precision medicine on antenatal care

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Background and aims: Birthweight and pregnancy outcomes in pregnancies affected by GCK-MODY are determined by fetal genotype. Fetuses who do not inherit the *GCK* variant from their mother are at risk of being large-for-gestational-age (LGA, birthweight >90th percentile for sex and gestational age), whereas fetuses who do inherit the maternal variant have normal birthweights. It is not certain if treatment of maternal hyperglycaemia is beneficial and it may reduce growth in fetuses with GCK-MODY. Accelerated growth of the fetal abdominal circumference (AC) >75th percentile for gestational age may indicate that the fetus has not inherited the maternal variant, but is imprecise. Non-invasive prenatal diagnosis (NIPD) is highly sensitive and specific for identifying fetal genotype and has recently become available. We aimed to investigate the impact of NIPD on management of pregnancies affected by GCK-MODY.

Materials and methods: We investigated 29 international pregnancies affected by maternal GCK-MODY who underwent NIPD at Exeter Genomics Laboratory between July 2019 and March 2021. Of these, 12 women had delivered and had available information on antenatal care and pregnancy outcome.

Results: Women were referred across pregnancy (median 11 weeks, range 5-32 weeks), with the fetal genotype reported within a median of 4 weeks (range 2-15 weeks) from receipt of the first maternal blood sample. Women referred in the first trimester (n=17) received a diagnosis by a median gestational age of 19 weeks (range 15-31 weeks). Of the 12 women who had delivered, 7 had an unaffected fetus and 5 had an affected fetus. Four women with an unaffected fetus underwent treatment for hyperglycaemia with insulin and/or metformin in pregnancy, but only two received treatment until delivery. Three women with an affected fetus received treatment with insulin or metformin in pregnancy, but they were stopped prior to delivery. Insulin was stopped in two women as a result of the affected fetal genotype reported by NIPD. An AC >75th percentile was common, present in 5 of the 7 unaffected fetuses and 4 of the 5 affected fetuses on at least one ultrasound scan performed after 20 weeks gestation. All women with an unaffected fetus had an induction or caesarean delivery before term, whereas all women with an affected fetus had a vaginal delivery, 3 of which were spontaneous. Two babies were born LGA and both were unaffected by GCK-MODY.

Conclusion: We have shown for the first time the impact of NIPD on the management of pregnancies affected by maternal GCK-MODY. Rapid and early diagnosis prior to the start of growth scans is possible and is more reliable; an AC >75th percentile was common in fetuses with and without a GCK-MODY variant, despite only two babies being LGA. We also showed how NIPD could be used to guide treatment decisions, as two women with an affected fetus had their insulin therapy stopped. Diagnosis of an unaffected fetus will help plan delivery decisions, where the risk of LGA is high. NIPD could be used to assess whether treatment of maternal hyperglycaemia reduces birthweight in unaffected fetuses as part of a prospective study, as this remains a key area of practice re*quiring more investigation.*

Supported by: Wellcome Trust, Royal Society, NIHR, University of Exeter

Disclosure: **A.E. Hughes:** Grants; Wellcome Trust GW4-CAT PhD Fellowship, University of Exeter.

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Validation of fear of hypoglycaemia screener: results from the T1D Exchange Registry

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Background and aims: To examine reliability and validity of a newly developed fear of hypoglycaemia (FoH) screener as a practical and actionable tool for in-clinic use in adults with type 1 diabetes (T1D), in accordance with American Diabetes Association's position on psychosocial care.

Materials and methods: In this validation study, adults with T1D were recruited from the T1D Exchange Registry to complete a draft screener online; potential items were previously identified from literature review, and interviews with health care professionals (HCPs) and people with T1D. Standard psychometric analyses assessed reliability and validity

of the screener. The final FoH screener comprised 9 items assessing 2 domains - "worry" (6 items) and "behaviour" (3 items).
Results: The final sample comprised 592 adults with T1D (age 43.1 ± 15.3 years; duration of T1D 24.1 ± 15 years, 66.7% females, 91.6% White, 5.2% Hispanic, self-reported HbA1c 54.1 ± 13.1 mmol/mol [7.1% ± 1.2%]). Approximately 30% of participants reported severe hypoglycaemia in the past 12 months; 33.4% reported impaired awareness of hypoglycaemia. The FoH screener showed internal consistency (Cronbach's α=0.88) and was highly correlated (r=0.71-0.75) with the Hypoglycemia Fear Survey ("worry" and "behaviour" subscales and total scores), confirming reliability. Construct validity of the FoH screener was demonstrated with significant correlations with depression (r=0.44), anxiety (r=0.47), Diabetes Distress Subscales (powerlessness, management distress, hypoglycaemia distress) (r=0.49-0.66). These correlations are considered moderate. Additionally, multivariable regression analysis showed that higher FoH screener scores were significantly associated with higher HbA1c (regression coefficient, β=0.04) and multimorbidities (β=0.03).
Conclusion: This 9-item FoH screener demonstrated good reliability and validity. Further research is planned to assess clinical usability to help appropriately identify patients and assist effective HCP-patient conversations around FoH.
Supported by: Eli Lilly and Company
Disclosure: **J. Liu:** Employment/Consultancy; T1D Exchange.

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Mental illness, ethnicity and civil status are associated with non-attendance in diabetic retinopathy screening among people with type 2 diabetes

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Background and aims: Diabetic retinopathy can cause severe vision loss and blindness, if not detected early and treated. The key to early detection is screening, and in Denmark, a nationwide screening programme for diabetic retinopathy has been implemented. However, current participation in eye screenings is below the recommended levels. The aim of this study was to identify factors associated with non-attendance in screening for diabetic retinopathy among people with type 2 diabetes.
Materials and methods: We performed a retrospective, observational study using individual-level register data from 156,878 people with type 2 diabetes aged 40-70 years at diagnosis. Based on their eye screening history from 2013 till 2018 each person was characterised as either an attender (at least one registered screening) or a never-attender (no registered screening). We compared baseline characteristics between attenders and never-attenders using non-parametric tests. Eleven characteristics were included: Age, gender, ethnicity, place of residence (region), civil status, education, employment, income, diabetes duration, comorbidity and mental health. Among the attenders, we assessed 230,173 screening intervals and defined them as non-attender intervals if the person failed to participate in the eye screening within the recommended interval. Mixed-effects models were used to investigate the impact of the eleven characteristics on the likelihood of having a non-attender interval. Univariable and multivariable analyses including all characteristics were performed.
Results: A total of 42,068 (27%) individuals were identified as never-attenders, having no registered eye screening, and comparisons showed that attenders and never-attenders differed significantly on all included baseline characteristics. Compared to attenders, never-attenders were more frequently divorced, 30% vs. 22% (p<.0001), living in the Capital Region, 36% vs. 28% (p<.0001), mentally ill 10% vs. 5% (p<.0001) and had more comorbidities, 22 % vs. 17 % (p<.0001). For the remaining factors the quantitative differences were modest. Among the 230,173 screening intervals, 62,381 (27%) were identified as non-attender intervals. All characteristics except gender were significantly associated with the likelihood of having a non-attender interval in the univariate analysis. In the multivariate analyses the five factors with largest odd ratios (ORs) for non-attendance were mental illness (OR: 1.50 [1.42, 1.58], p<.0001), non-western descent (OR: 1.42 [1.35, 1.50], p<.0001), divorce (OR: 1.26 [1.22, 1.31], p<.0001), comorbidity (OR: 1.25 [1.19, 1.30], p<.0001) and region with highest ORs for the Capital Region and North Denmark Region.
Conclusion: This is the first national study to outline factors associated with non-attendance among people with type 2 diabetes in the Danish screening programme for diabetic retinopathy. Our findings suggest that both never-attendance and non-attendance are more common among people who are divorced and among people of poorer health. Additionally, non-attendance is more frequent among people of non-western decent. These population subgroups may benefit from targeted interventions aimed at increasing participation in eye screenings.
Disclosure: **G.B. Petersen:** None.

OP 28 Pathogenic mechanisms of complications

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The gut microbiome composition is altered in long-standing type 1 diabetes and associated with disease-related complications

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Background and aims: Type 1 diabetes (T1D) is associated with an increased risk for infections and an increased risk to develop long-term (micro and macro-)vascular complications. Although changes in the microbiome have been linked to the risk of development of T1D, not much is known about the role of the gut microbiome in long-standing type 1 diabetes. We therefore set out to determine differences in the gut microbiome of T1D patients compared to healthy controls and to associate the gut microbiome with diabetes-related parameters.
Materials and methods: 239 T1D patients were included with an average disease duration of 28,4 (15.6) years. Microbiome data were compared to a healthy cohort consisting of 2937 age-,sex- and BMI-matched individuals. Clinical characteristics and faecal samples were collected. Metagenomic shotgun sequencing was performed and the results were associated to T1D-related characteristics such as HbA_{1c}, and macro- and microvascular complications.
Results: 43 bacterial taxa were significantly depleted in T1D, including *S. Alistipes Putredinis* (FDR=$3.0x10^{-12}$). Furthermore, 37 bacterial taxa were significantly enriched in T1D, such as *G. Clostridium* (FDR=$2.0x10^{-5}$). Interestingly, enriched species consisted mainly of opportunistic species *(Clostridiales, Oscillibacter*), whereas a reduction in commensal species was seen (*Dorea sp, Bifidobacterium sp.*). However, no significant difference in diversity of the gut microbiome was found between T1D patients and healthy controls. Several diabetes related factors displayed a significant association with changes in the gut microbiome. Glycaemic control, measured by HbA_{1c} (ranging from 34 to 136 mmol/mol), and disease duration explained a significant part of variation in the gut microbiome (R^2>0.010, FDR<0.05). HbA_{1c} was also significantly associated with the presence of several microbial species. Furthermore, both micro-and macrovascular complications explained a significant part of variation in gut microbiome (R^2>0.0075, FDR<0.05). Presence of nephropathy was strongly associated with several microbial species, consisting of *Clostridiales* (FDR<0.05). Macrovascular complications displayed similar associations with *Clostridiales* (p-value<0.05).
Conclusion: While the diversity is not affected, the composition of the gut microbiome in T1D patients differs significantly from the microbiome of healthy controls. Furthermore, the changes in the gut microbiome are associate with T1D related characteristics and vascular complications. These data suggest that the gut microbiome is not only important in the context of disease development, yet may also be involved in the development of diabetes-associated complications.
Supported by: TIMID project LSHM18057-SGF
Disclosure: **J.I.P. van Heck:** Grants; collaborative TIMID project: LSHM18057-SGF.

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No association between haptoglobin genotype and cerebral small-vessel disease in type 1 diabetes

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Background and aims: It has been proposed that susceptibility to cardiovascular disease in diabetes could partly be ascribed to a variation in the haptoglobin (Hp) gene. Haptoglobin is a protein that binds to free haemoglobin, inhibiting its oxidative activity. Although Hp1 allele has been suggested to associate with stroke and white matter hyperintensities (WMH) in type 1 diabetes, data on these associations are sparse. Our aim was to further evaluate the association between Hp and cerebral small-vessel disease (SVD).
Materials and methods: For this cross-sectional study, we included 179 individuals with type 1 diabetes from the Finnish Diabetic Nephropathy study. A total of 53% were female, mean age was 39±7 years, duration of diabetes 23±10 years, and HbA_{1c} 65±12 mmol/mol. All participants underwent brain MRI and clinical investigation. Hp genotype was determined with two polymerase chain reactions showing the presence (Hp2) or absence (Hp1) of exons 5 and 6. Brain MRIs were assessed for WMHs, cerebral microbleeds (CMB), and lacunar infarcts as signs of SVD and analysed in relation to Hp genotype.
Results: Genotype Hp1-1 was observed in 28 (16%), Hp1-2 in 78 (44%), and Hp2-2 in 73 (41%) of the participants. Participants with Hp1-1 differed from those with Hp2 alleles (Hp1-2 or Hp2-2) only regarding diastolic blood pressure (76±9 vs 80±6 mmHg, p=0.027). The same was seen in comparison across all three genotypes (p=0.019). In analysis of Hp1-1 vs Hp2 allele carriers, we detected no difference in the prevalence of SVD (36% vs 34%, p>0.999), or in any observed manifestation (WMH 29% vs 22%, p=0.595; CMB 25% vs 22%, p=0.904; lacunar infarcts 0% vs 3%, p>0.999). Hp1-1 was not associated with SVD in a logistic regression model adjusted for diastolic blood pressure, age, and diabetic retinopathy (OR 1.09 95% CI 0.44-2.61, p=0.855). When SVD manifestation were analysed separately, Hp1-1 was associated neither with WMH, CMB, nor lacunar infarcts. Correspondingly, comparison of all genotypes yielded no differences in prevalence of SVD (36% vs 37% vs 32%, p=0.758), nor in any observed manifestation (WMH p=0.117, CMB p=0.860, lacunar infarcts p=0.343). Furthermore, no association between the number of Hp2 alleles and SVD was observed.
Conclusion: Although the prevalence of white matter hyperintensities and cerebral microbleeds was high in our cohort of neurologically asymptomatic adults with type 1 diabetes, we observed no association between cerebral small-vessel disease and haptoglobin genotype.
Supported by: Folkhälsan Research Foundation, Academy of Finland, Stockmann Foundation, EVO governmental grants
Disclosure: **M.I. Eriksson:** Grants; Wilhelm and Else Stockmann Foundation, Otto-Malm foundation. Stock/Shareholding; Shareholder in BCB Medical.

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Nox5 in circulating peripheral blood mononuclear cells: a potential biomarker in unstable diabetic vascular and renal disease

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Background and aims: Oxidative stress derived from the human NADPH oxidase 5 (NOX5) plays a critical role in diabetic vascular and renal disease and is expressed and functionally active in monocytes and macrophages. Increased NOX5 expression has been demonstrated in atherosclerotic plaques in those with diabetes and coronary artery disease (CAD) as well as in the glomeruli and mesangial cells in kidney biopsies of diabetic patients. Therefore, we hypothesise that NOX5 expression in circulating peripheral blood mononuclear cells (PBMCs) is increased in patients with diabetes, particularly in those with unstable CAD and chronic kidney disease (CKD).
Materials and methods: 46 males aged 33-83 years underwent elective or emergency coronary angiography/angioplasty at the Alfred Hospital Catheter Laboratory. PBMCs were isolated from whole blood and processed for flow-cytometry to measure NOX5 protein. In parallel, NOX5 was measured in PBMCs by qPCR. To complement the in vivo findings, human macrophages (THP-1) were incubated in low (5 mM) and high glucose (25 mM). NOX5 expression and inflammatory markers were measured by qPCR.
Results: NOX5 protein expression in PBMCs was primarily driven by expression in monocytes (CD 45+/CD14+ cells) and was increased in diabetic and non-diabetic patients with CKD (29.5±4.4 versus 18.2±1.9 AU; p=0.0093) and in diabetic patients with CKD versus without CKD (28.4±4.3 versus 16.5±2.2 AU; p=0.03). CAD with acute presentation was associated with increased NOX5 expression versus elective presentation (26.9±3.3 vs 18.2±2.2 AU; p=0.02), particularly in diabetic patients presenting acutely versus electively (30.2±4.4 vs 15.2±2.4 AU; p=0.0046). A 4-fold upregulation of NOX5 gene was observed in patients with CKD versus patients without CKD irrespective of diabetes or CAD (p=0.018). In vitro, there was a 2-fold increase in NOX5, TNF-alpha, and Interleukin-6 expression in THP-1 cells exposed to high glucose.
Conclusion: The presence of CKD and unstable CAD appear to be the key factors for increased NOX5 protein and gene expression in circulating PBMCs in diabetic patients. These findings are consistent with postulated pathogenic mechanisms whereby NOX5 accelerates vascular and renal inflammation and fibrosis. Measurement of NOX5 in PBMCs may serve as a valuable prognostic marker in patients with clustering diabetic complications and lead to targeted interventions in patients at high cardiovascular and/or renal risk.
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Dicarbonyl stress alters mitochondrial protein homeostasis in endothelial cells

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Background and aims: The main source for advanced glycation end products (AGEs) during hyperglycemic conditions is the reactive dicarbonyl methylglyoxal (MG), a byproduct of glycolysis. AGEs are formed by non-enzymatic glycation of proteins, lipids and DNA. MG-derived hydroimidazolone (MG-H1) is formed in a reaction with arginine side chains of proteins. This modification can lead to misfolding, aggregation and loss-of-function of the protein. Heat shock proteins (Hsp) are part of the protein quality control system (PQS). They are involved in maturation of newly synthesized polypeptides, refolding of misfolded proteins and maintenance of mitochondrial activity. We questioned the effect of MG on mouse cardiac endothelial cells (MCECs), and whether the loss of the stress-inducible Hsp70 (Hspa1a/Hspa1b) in a double knockout cell line (Hspa1a/Hspa1b KO) leads to reduced MG tolerance.
Materials and methods: mRNA was measured by qPCR and protein levels were measured by immunofluorescence. The data for the KO cells was obtained from three independent Hspa1a/Hspa1b KO cell clones.
Results: After treatment with 500 μM MG, Hspa1a/Hspa1b was up-regulated on the mRNA level (Hspa1a: mean= 1.57-fold, n= 4, p= n.s.; Hspa1b: mean= 1.78-fold, n= 4, p= n.s.) and on the protein level (mean= 1.56-fold, n= 3, p= n.s.) in wild-type cells (WT). The two mitochondrial chaperones Hspa9 and Hspd1 were both up-regulated after 500 μM MG treatment on the mRNA level (Hspa9: mean= 1.42-fold, n= 4, p= 0.029; Hspd1: mean= 2.11-fold, n= 4, p= 0.0054) and on the protein level (Hspa9: mean= 1.96-fold, n= 6, p= 0.000014; Hspd1: mean= 2.35-fold, n= 3, p= 0.0017). Compared to WT, treating Hspa1a/Hspa1b KO cells with 500 μm MG resulted only in a slight increase in mRNA expression of Hspa9 (mean= 1.52-fold, n= 3, p= n.s.) and Hspd1 (mean= 1.57-fold, n= 3, p= n.s.), whereas there was a significantly higher increase of Hspa9 (mean= 3.17, n= 3, p= 0.00044) and Hspd1 (mean= 3.84, n= 3, p= 0.00047) protein expression. The mitochondrial fission protein Drp1 was increased 1.39-fold on the mRNA level after 500 μM MG treatment (n= 3, p= 0.0097) in WT cells, indicating a disturbance in mitochondrial homeostasis. Drp1 mRNA levels in KO cells were also increased compared to WT (WT: mean= 1.39, n=3 vs. KO: mean= 2.11, n= 3, p= 0.0080), suggesting an aggravated mitochondrial disturbance. However, mitophagy related proteins Parkin, Bnip3 and Pink1 were all down-regulated (Parkin: mean= 0.45-fold, n= 4, p= 0.0066; Bnip3: mean= 0.39-fold, n= 4, p< 0.000001; Pink1: mean= 0.69-fold, n= 4, p= 0.0025).
Conclusion: In both, WT and Hspa1a/Hspa1b KO cells, mitochondrial stress was present after MG treatment. Drp1 was up-regulated, indicating increased mitochondrial fission and disturbance of mitochondrial homeostasis. As a compensatory mechanism, expression of the mitochondrial heat shock proteins Hspa9 and Hspd1 was significantly induced. In Hspa1a/Hspa1b KO cells, up-regulation of Drp1 and of mitochondrial Hspa9 and Hspd1 was even stronger, indicating pronounced MG-induced mitochondrial stress in the absence of stress-inducible Hsp70.
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Insulin resistance associates with arterial stiffness in type 1 diabetes: a novel component of double diabetes

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Background and aims: The presence of insulin resistance (IR) in type 1 diabetes (T1D), known as double diabetes, increases the risk of both macro- and microvascular complications. When IR occurs, insulin impairs its metabolic effects while preserves its growth/mitogenic activities. In the arterial wall, IR could promote arterial stiffness (AS, or early vascular aging), a well-known cardiovascular risk factor, through several mechanisms (e.g. vasoconstriction, inflammation, hypertrophy, fibrosis - collagen synthesis-). The current study was aimed to evaluate the potential association between IR and AS in well -characterized cohort of people with T1D.
Materials and methods: 179 adults with T1D without established cardiovascular disease were evaluated. IR was assessed by the estimation of the glucose disposal rate -eGDR- in mg/kg/min as follows:

eGDR=21.158-(0.09xWHR)-(3.407xHypertension)-(0.551xHbA1c). Subjects were categorized into four quartiles according to their eGDR (Q1:<6.1; Q2:6.1 to 8.6, Q3 8.6 to 10, and Q4 >10). AS was assessed by the measurement of aortic pulse wave velocity (aPWV).

Results: The characteristics of subjects included in the study are shown in Table 1, stratified by eGDR quartiles. aPWV increased in parallel with IR, i.e., it was negatively correlated with eGDR (r=-0.589; p<0.001). After adjustments for age, sex, diabetes duration, dyslipidemia and microvascular complications, the association between aPWV and eGDR still remained significant (R^2: 0.546. β=-0.218; p=0.001), thus, being IR one of the main independent factors associated with the increase of AS. When each component of the eGDR equation were introduced in the same model separately, only WHR was significantly associated with aPWV (R^2: 0.589. β=0.376; p<0.001).

Conclusion: Insulin resistance measured by eGDR is associated with arterial stiffness in people with T1D and no previous established cardiovascular disease. The main factor explaining this association seems to be abdominal obesity instead of glycemic control or hypertension.

Table 1. Baseline characteristics according to the estimated glucose disposal rate (mg/kg/min) quartiles

	Total (n=179)	<6.1 (n=45)	6.1-8.6 (n=45)	8.6-10.0 (n=44)	>10.0 (n=44)	p for trend
Age (years)	41.1 (13.0)	50.7 (10.6)	43.4 (11.9)	38.0 (11.7)	32.0 (10.0)	<0.001
Women (n, %)	87 (48.9)	11 (24.4)	19 (42.2)	23 (52.3)	34 (77.2)	<0.001
Smokers (n, %)	34 (19.1)	14 (31.1)	10 (22.2)	5 (11.4)	5 (11.4)	0.239
Hypertension (n, %)	49 (27.5)	40 (88.9)	9 (20.0)	0 (0)	0 (0)	<0.001
Dyslipidemia (n, %)	97 (54.5)	33 (73.3)	28 (62.2)	24 (54.6)	12 (27.3)	<0.001
WHR	0.88 (0.81-0.94)	0.94 (0.90-1.0)	0.93 (0.89-0.97)	0.86 (0.82-0.90)	0.77 (0.72-0.82)	<0.001
Diabetes duration (years)	16 (12-23)	18 (14-28)	18 (12-25)	17 (13-22)	13 (8-19)	0.003
Retinopathy (n, %)	41 (23.0)	18 (40.0)	10 (22.2)	7 (15.9)	6 (13.6)	0.016
Nephropathy (n, %)	40 (22.7)	31 (68.9)	8 (17.8)	0 (0)	1 (2.3)	<0.001
HbA1c (%)	7.7 (7.1-8.5)	7.9 (7.2-8.9)	8.1 (7.5-9.1)	7.6 (6.9-8.7)	7.2 (6.7-7.9)	<0.001
aPWV (m/s)	7.1 (6.1-8.3)	8.3 (7.5-9.7)	7.9 (6.6-8.9)	6.7 (6.1-7.6)	6.1 (5.5-6.6)	<0.001

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Wnt regulation and collagen gene expression in the bone of type 2 diabetes elderly women

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Background and aims: Fragility fractures are a skeletal complication associated with type 2 diabetes (T2D), implying disability, hospitalization, impaired quality of life, and increased mortality. The interaction between bone metabolism, higher risk of fractures, and T2D is complex and not fully understood. Increased circulating sclerostin levels and accumulation of advanced glycation end-products (AGEs) are two potential mechanisms underlying low bone turnover and increased fracture risk. Recent data from our group have shown that T2D affects the expression of genes controlling bone formation (SOST and RUNX2) and that accumulation of AGEs not only higher in diabetics but it is associated with impaired bone microarchitecture. We hypothesized that T2D and increased SOST gene expression lead to a downregulation of Wnt β- catenin genes target (transcription factors TCF/LEF1) and AGEs accumulation is correlated to a downregulation of collagen (COL1A1) in bones from T2D subjects. The aim of this study was to investigate gene expression levels of TCF-LEF1, Wnt β -catenin inhibitor Dickkopf -1 (DKK-1), COL1A1, and to evaluate correlation analysis of AGEs bone content previously measured in the same population with above mentioned bone target genes to study underlying mechanisms of increased bone fragility in T2D.

Materials and methods: Bone tissues were obtained from femoral heads of 14 T2D (HbA1c 6.5 ± 1.7 %) and 21 non-diabetic postmenopausal women (Age >65 years) undergoing hip replacement surgery. Biopsies of trabecular bone were isolated from the surgical specimens. RNA was extracted with standard Tryzol protocol and gene expression of TCF-LEF1, DKK-1, and COL1a1 was measured by Real Time- PCR.

Results: Age (non-diabetic 73.2 ± 5.8 years vs diabetic 75.2 ± 8.5 years) and, BMI (non-diabetic 27.7 ± 5.6 kg/m2 vs diabetic 29.9 ± 5.4 kg/m2) did not differ between the two groups. We found that expression levels of TCF-LEF1 were significantly lower in T2D compared to non- diabetic subjects (p=0.002). DKK-1 was not different between groups (p=0.1083), however analysis of correlation showed that DKK-1 increases with age (r2=0.038; p=0.043) and HbA1c (r2=0.503; p=0.048) in T2D subjects. COL1A1 gene expression was lower in T2D compared to controls although significance was not fully reached (p=0.0564). Importantly, correlation analysis of AGEs bone content with LEF1 gene expression levels showed a negative association (r=-0.55; p=0.02), as well as for COL1A1 (r=-0.61; p=0.01).

Conclusion: Our data show for the first time that even in patients with a relatively good glycemic control, T2D affects the expression of Wnt β-catenin target genes and collagen. We also found that accumulation of AGEs in the bone of T2D is negatively associated with alteration in COL1A1 and TCF/LEF1 gene expression. We provide novel insights that may help understand the mechanisms underlying bone fragility in T2D.

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OP 29 Understanding muscle and liver metabolism

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Assessing the association between metabolic flexibility measured upon insulin stimulation and during incremental submaximal exercise

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Background and aims: Metabolic flexibility is defined as the capacity of substrate oxidation to adapt to substrate availability, commonly measured as the change in respiratory exchange ratio (RER). In obese, metabolically compromised individuals, metabolic flexibility is reduced, reflected by a blunted change from fat to glucose oxidation upon insulin stimulation during a hyperinsulinemic-euglycemic clamp. Reduced metabolic flexibility might be due to low mitochondrial oxidative capacity but may also merely be a reflection of reduced insulin-stimulated glucose uptake. As exercise of progressive intensity also requires a gradual switch from fat to glucose oxidation but is not dependent on insulin for its glucose uptake, we here investigate if metabolic flexibility (measured as the change in RER) during progressive exercise in metabolically compromised individuals is similar to the metabolic flexibility measured by hyperinsulinemic-euglycemic clamp. If so, this would suggest that mitochondrial function might be the main determinant of metabolic flexibility.
Materials and methods: Twenty-five (11 males and 14 females) overweight and obese individuals (BMI 31.7 ± 3.3 kg/m^2; 64.7 ± 7.0 years) underwent an incremental submaximal cycling test at 30%, 50%, and 70% of the predetermined maximal power output (Wmax) and a two-step hyperinsulinemic-euglycemic clamp (10 and 40 mU/m^2/min). Substrate oxidation upon insulin stimulation and during exercise was assessed by indirect calorimetry. Metabolic flexibility upon insulin stimulation was measured as the change in RER from the basal state to the high-insulin infusion state, while during incremental cycling, this was measured as the change in RER from 30% to 70% Wmax.
Results: Upon insulin stimulation, the RER showed an average increase of 0.12 ± 0.05 (from 0.78 ± 0.03 during the basal state to 0.89 ± 0.05 during the high-insulin state). During incremental cycling, the mean increase in RER was 0.13 ± 0.04 (0.80 ± 0.03 at 30% to 0.93 ± 0.03 at 70% Wmax). The change in RER upon insulin stimulation did not correlate with the change in RER measured during incremental submaximal cycling (r = -0.075, p = 0.723).
Conclusion: Although metabolic flexibility measured upon insulin stimulation quantitatively matches metabolic flexibility assessed during exercise of progressive intensity these variables did not correlate. This hints towards different underlying mechanisms controlling substrate oxidation upon insulin stimulation and progressive exercise.
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TSC22D4 interacts with Akt to regulate insulin sensitivity

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Background and aims: Transforming Growth Factor β-1 Stimulated Clone 22 D4 (TSC22D4) acts downstream of stress and metabolic signals playing role in glucose and lipid homeostasis. Particularly, hepatic TSC22D4 controls insulin sensitivity and regulates blood glucose levels. In diabetic mouse models, TSC22D4 promotes hyperglycemia and insulin resistance and strikingly, in obese human patients elevated hepatic TSC22D4 expression correlates with insulin resistance. These studies clearly establish a novel connection between TSC22D4 and metabolic regulation, yet the molecular mechanisms of this connection remains elusive. Protein Kinase B/Akt represents one of the very well-established components of the insulin signaling pathway that regulates gluconeogenesis and lipogenesis and contributes to metabolic homeostasis. Our preliminary data show that TSC22D4 interacts with Akt. The aim of this study is to investigate whether TSC22D4 plays role in metabolic regulation by interacting with Akt.
Materials and methods: We performed co-immunoprecipitation (co-IP) experiments to study the regulation of TSC22D4-Akt interaction. In order to map the domains required for TSC22D4-Akt interaction, we performed site-directed mutagenesis to delete conserved domains on TSC22D4. In order to understand the function of TSC22D4-Akt interaction *in vivo*, we generated Adeno-associated viruses (AAV) that contained the vector control, wild type (WT)-TSC22D4 or the TSC22D4 deletion mutant that cannot interact with Akt (ΔD2-TSC22D4). We introduced these AAVs to hepatocyte specific TSC22D4 knock out (TSC22D4$^{Hep-/-}$) mice and subjected them to either chow diet or to high fat diet with high sucrose water and performed glucose and insulin tolerance tests (GTT and ITTs). We measured plasma insulin levels, liver triglycerides, and expression of gluconeogenic and lipogenic genes.
Results: We have shown that glucose and insulin stimulation impaired the TSC22D4-Akt interaction, whereas starvation or mitochondrial inhibition promoted it. Our experiments also indicate that together with its homodimerization domain (i.e. TSC box), TSC22D4 requires its intrinsically disordered region (D2) to interact with Akt. While deletion of D2 domain (ΔD2-TSC22D4) impaired the TSC22D4-Akt interaction; the D2 domain together with TSC box (D2+TSC) was sufficient to maintain the interaction. Furthermore, TSC22D4$^{Hep-/-}$ mice overexpressing ΔD2-TSC22D4 mutant in their livers performed better both in GTT and ITTs; and had lower insulin levels and an improved HOMA-IR index compared to mice overexpressing WT-TSC22D4. Additionally, mice overexpressing ΔD2-TSC22D4 tended to accumulate less triglycerides in their liver compared to mice with WT-TSC22D4 and had slightly lower expression of lipogenic genes. Expression of gluconeogenic genes, however, were not affected.
Conclusion: Here, we identify TSC22D4 as a novel Akt interacting protein. We show that not only glucose and insulin but also energy levels regulate the TSC22D4-Akt interaction. Hence, we define TSC22D4 as a novel signaling molecule that responds to metabolic signals by interacting with Akt and thereby regulates insulin sensitivity.
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C2-ceramide recycling inhibits insulin signalling in muscle cells

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Background and aims: It is well documented that ectopic accumulation of saturated fatty acids (FA) induces lipotoxicity in muscle cells. Saturated FA are metabolized into ceramides (Cer) of which accumulation plays a central role in the development of muscle insulin resistance (IR). Studies involved in the discovery of Cer deleterious actions used short-chain Cer analogues such as C2-Cer (C2cer). Since C18-Cer is the predominant Cer species in myotubes and since Cer synthase 1 (CerS1)-derived C18-ceramide promotes insulin resistance, how can exogenous

C2cer reproduce the harmful action of C18-Cer in myotubes? Our hypothesis is that C2cer is converted into longer chain Cer species that will mediate muscle using a salvage pathway by the action of ceramidase and CerS.

Materials and methods: We treated C2C12 myotubes with C2cer for 2h in the presence or absence of either a CerS inhibitor, Fumonisin B1 (FB1) or a ceramidase inhibitor (ceranib-2) before to quantify intracellular sphingolipids using UHPLC/MS/MS, and to evaluate the insulin response (Akt phosphorylation, GLUT4 translocation and glucose uptake). In addition, we used both Acetyl-CoA Carboxylase (ACC) and FA synthase (FAS) inhibitors to characterize whether FA produced from endogenous lipogenesis participate in the process.

Results: 2 h C2cer incubation of myotubes increased several endogenous long chain Cer species such as C14, C16, C18:1, C18, C24 and C24:1-Cer ($p \leq 0.05$, n=5-6). To test whether generation of these long-chain Cer species from C2cer could happen through a C2cer de-acylation/re-acylation process, we treated myotubes with ceranib-2. After 2 h treatment, ceranib-2 alone induced a 50% accumulation in total endogenous Cer content, without alteration of insulin response ($p \leq 0.05$, n=3-4). In contrast, inhibition of ceramidase activity with ceranib-2 reduced C2cer-induced endogenous Cer build-up and prevented C2cer to inhibit the insulin response in myotubes ($p \leq 0.05$, n=3). To confirm a de-acylation/re-acylation process, we treated myotubes with FB1, and showed that it prevented the generation of endogenous Cer in response to C2cer, suggesting that sphingosine backbone of C2cer is re-used to produce other Cer species ($p \leq 0.05$, n=3). FB1 pre-treatment also prevented the negative action of C2cer by restoring a normal insulin response ($p \leq 0.05$, n=4). Endogenous FA used by CerS could be provided by the lipogenic pathway. To test it, myotubes were incubated with C2cer in the presence of 25 or 5 mM glucose. At 25 mM glucose, C2cer inhibited completely the insulin signal whereas at 5 mM glucose, C2cer action was blunted and the insulin signal remained unaffected ($p \leq 0.05$, n=3). In addition, inhibition of *de novo* lipogenesis through the use of either a competitive inhibitor of ACC, 5-tetradecyloxy-2-furoic acid, or a FAS inhibitor, C-75, prevented the increase in total Cer content in response to C2cer. This was accompanied with the restoration of insulin action in myotubes treated with C2cer ($p \leq 0.05$, n=3-6).

Conclusion: Our study provides important molecular mechanistic data showing for the first time that recycling of exogenous C2cer induced in muscle cells a loss in insulin sensitivity through production of endogenous Cer species. This recycling model also suggests a potential role of the uptake/recycling of circulating Cer, that are increased during obesity, in the installation of muscle insulin resistance.

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The impact of different lipogenic diets on indirect pathway contributions to hepatic glycogen synthesis

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Background and aims: We evaluated the impact of diet composition on hepatic glycogen metabolism in mouse models of diet-induced Non-Alcoholic Fatty Liver Disease (NAFLD). High fat and/or high sugar diets induce moderate weight gain and a mild form of NAFLD associated with impaired hepatic insulin action. In comparison to lipid, little is known about hepatic glycogen metabolism in this setting, in particular the indirect pathway contributions. We analysed the amount of glycogen synthesized in the liver, the contribution of the indirect pathway to glycogen synthesis, and the sources of indirect pathway carbons, including dietary fructose.

Materials and methods: For 18 weeks, 46 C57/BL6 male mice were assigned to 4 different diets: 12 to standard chow (SC), 12 to standard chow with sugar in the drinking water at 30% w/v (HS), 11 to a high-fat chow (HF) and 11 to high-fat chow with sugar in the drinking water at 30% w/v (HFHS). The sugar formulation was 55/45% fructose/glucose mimicking high-fructose corn syrup 55 (HFCS-55). During the final evening, deuterated water (^2H_2O) was administered and the fructose component of the HFCS-55 formulation was enriched to 20% [U-^{13}C]fructose. Animals were allowed to feed *ad libitum* overnight. The following morning, livers were collected, and their glycogen extracted, digested and derivatized to mono-acetone glucose (MAG) for ^{2}H- and ^{13}C-NMR analysis. Total glycogen levels were assayed and the contribution of the indirect pathway to glycogen synthesis, including inputs from trioses phosphate (triose-P) and anaplerosis, were calculated using the ^{2}H-enrichment of MAG positions 5 and 6. The contribution of fructose to the indirect pathway was estimated by quantifying ^{13}C-enrichment of glycogen from [U-^{13}C]fructose.

Results: Postprandial glycogen levels did not differ between the 4 diets. In HF mice, the indirect pathway accounted for 58±8% of overnight glycogen synthesis, a significantly higher fraction compared to the other three diets (SC = 32±8%, HS = 37±9%, HFHS = 40±4%, $p<0,05$). For both HF and SC mice, the majority of indirect pathway carbons were derived via anaplerosis, while for HS and HFHS mice, a substantial fraction of indirect pathway carbons were derived from triose-P sources, the entry point of fructose metabolites into the gluconeogenic pathway. Recruitment of HFCS-55 fructose by the indirect pathway was confirmed by the observation of [4,5,6-$^{13}C_3$]- and [5,6-$^{13}C_2$]glycogen isotopomers by ^{13}C-NMR for HS and HFHS groups.

Conclusion: While postprandial hepatic glycogen levels were not affected by high fat and/or high sugar feeding, the sources of glycogen synthesis were strongly influenced by diet. High fat feeding promoted a high indirect pathway contribution, possibly compensating for impaired direct pathway activity secondary to hepatic insulin resistance. Although HFCS-55 provided additional indirect pathway precursors via fructose metabolites, direct pathway flux was preserved, possibly by fructose-mediated activation of glucokinase.

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Disclosure: **A. Reis-Costa:** None.

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Transcriptional repression of the iron exporter ferroportin via the PI3K-AKT-Foxo1 signalling pathway may explain liver iron overload in patients with type 2 diabetes

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Background and aims: Iron and glucose homeostasis are tightly interlinked. Patients and mice with type 2 diabetes (T2DM) show elevated serum iron and ferritin levels, while patients with the iron overload disease hereditary hemochromatosis are prone to diabetes. In this study we investigated iron content and localization in the human diabetic liver and asked the question whether alterations in iron export via ferroportin (Fpn) explain altered iron homeostasis.

Materials and methods: Iron content and localization were analyzed in liver biopsies of 23 patients with T2DM and 25 patients without T2DM by Perl's-Prussian-blue staining. Kupffer cells were identified by CD68 staining. To establish a cellular model of insulin resistance we applied palmitate (200 μM) and insulin (100 nM) to Hepa1-6 cells. Expression of

mRNA and proteins involved in insulin signaling and iron homeostasis were analyzed by qPCR and western blotting. The PI3K and Foxo1 signaling pathways were blocked by pharmacological inhibition (Wortmannin and AS1842856, respectively) and RNAi.

Results: We show increased liver iron content in patients with T2DM compared to controls, whereby iron accumulates in hepatocytes, Kupffer cells or both. The percentage of CD68 positive Kupffer cells was not different. To study molecular mechanisms of iron accumulation in the diabetic liver, we established a cellular model of insulin resistance. We treated the hepatocytic cell line Hepa1-6 with palmitate and insulin, either alone or in combination. Insulin resistance was indicated by higher basal p-AKT levels and a blunted response to short-term insulin treatment. Similar to observations in T2D patients, insulin resistance in Hepa 1-6 cells was associated with cellular iron excess (e.g. increased ferritin, low transferrin receptor 1). Interestingly, treatment of insulin-resistant Hepa1-6 cells with exogenous iron (i.e. ferric ammonium citrate; FAC) resulted in aggravated iron accumulation. Systemic iron homeostasis is controlled by the hepcidin/ferroportin regulatory axis. We show that in conditions of insulin resistance the iron exporter ferroportin is downregulated at the mRNA and protein level in a hepcidin-independent manner. Reduced ferroportin transcription seems to be controlled by the PI3K signaling pathway, as inhibition by Wortmannin or silencing of AKT blocked the insulin-mediated Fpn response. Insulin inactivates the transcription factor FOXO1 via the AKT pathway. Consistently, the specific Foxo1 inhibitor AS1842856 as well as RNAi of Foxo1 reduced Fpn mRNA expression.

Conclusion: Our findings suggest that the iron exporter ferroportin is a novel target of the PI3K-AKT-Foxo1 signaling pathway in hepatocytes. We speculate that decreased expression of ferroportin may explain liver iron overload in patients with T2D.

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Ciprofibrate decreases net hepatic glucose uptake and tends to decrease net myocardial glucose uptake in prediabetic male volunteers

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Background and aims: Type 2 diabetes mellitus is characterized by reduced (hepatic) insulin sensitivity and reduced cardiac function. PPARαtreatment has beneficial effects on metabolism and regulates cardiac energy metabolism and function as established in pre-clinical animal studies. The objective of the current study was to determine whether the PPARα agonist ciprofibrate would improve cardiac and hepatic metabolism in volunteers with prediabetes.

Materials and methods: Ten male volunteers with prediabetes (BMI 29.1 ± 2.9 kg/m^2, age 62.2 ± 9.3 years) were administered with 100 mg/day placebo and ciprofibrate in a randomized double-blind crossover study for 5 weeks. Investigations included cardiac and hepatic glucose uptake during a hyperinsulinemic euglycemic clamp by ^{18}F-FDG positron emission tomography, cardiac function and structure with MRI and ultrasound, cardiac energy status (PCr/ATP ratio), whole body insulin sensitivity, intrahepatic lipid content, body composition; all analyzed by two-tailed paired sample t-tests with $p < 0.05$ considered as statistically significant.

Results: As expected, serum triglycerides were significantly decreased by ciprofibrate treatment indicating a therapy response in all volunteers (2.15 ± 1.32 mmol/l in placebo vs. 1.57 ± 1.05 mmol/l in ciprofibrate, p = 0.014). Ciprofibrate had no effect on fasting free fatty acids (FFA), cholesterol, fasting glucose and insulin levels. Ciprofibrate treatment decreased net hepatic glucose uptake (Ki 0.9 ± 0.2 versus 0.7 ± 0.2 ml/100ml/min in placebo and ciprofibrate, p = 0.039) and increased hepatic lipid content (7.1 ± 6.9 % versus 10.4 ± 10.0 % in placebo and ciprofibrate, p = 0.021). Myocardial net glucose uptake tended to decrease (0.049 ± 0.039 versus 0.040 ± 0.024 ml/100ml/min/mIU insulin in placebo and ciprofibrate, p = 0.098) upon ciprofibrate, but ciprofibrate had no effect on cardiac function and cardiac energy status. Whole body insulin sensitivity and body composition were unchanged upon ciprofibrate treatment.

Conclusion: This study indicates that 5 weeks of treatment with the PPARα ligand ciprofibrate decreases insulin-stimulated glucose uptake in the liver, with a similar tendency in the heart. The increase in liver fat was not associated with a decline in whole body insulin sensitivity, nor with reduced cardiac function parameters or cardiac energy status.

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OP 30 GLP-1 receptor agonism: putative mechanisms of benefit

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Combination very low dose sulphonylurea and DPP4 inhibitor have a potent glucose lowering effect through augmentation of beta cell function without increase in hypoglycaemia

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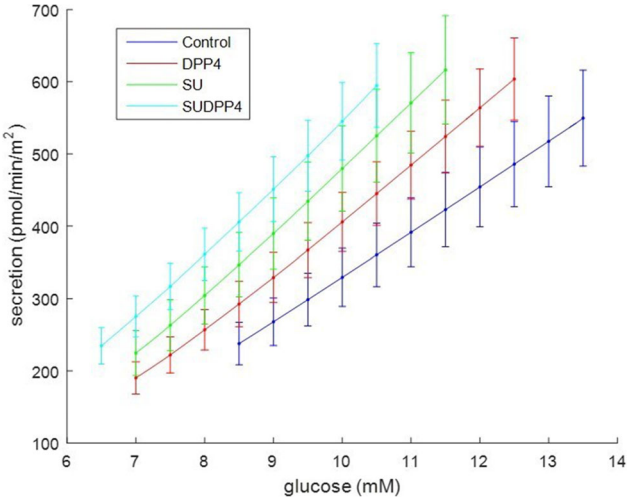
Background and aims: The global prevalence of type 2 diabetes (T2DM) will surpass 600 million by 2035, with highest prevalence contributed by low and middle-income countries. We must readdress our use of cheaper generic therapies such as sulphonylureas (SU) and those due to come off patent such as DPP4 inhibitors. Negative aspects of SU, particularly hypoglycaemia, weight gain and reduced durability may be avoided by their use at low dose. We have previously shown that 20mg standard release gliclazide reduces plasma glucose through augmentation of the classical incretin effect, increased beta-cell glucose sensitivity and late phase incretin potentiation. We further hypothesise potential synergy between low dose SU in combination with a DPP4 inhibitor.

Materials and methods: 30 participants with T2DM (HbA1c <64mmol/l) treated with diet or metformin monotherapy were recruited to a single-centre, open-label, randomised crossover study. Participants completed four, 14-day blocks in a random order: control, gliclazide 20mg once daily (SU), sitagliptin 100mg (DPP4), or combination (SUDPP4). A 2-hour MMT was conducted at the end of each block. Beta cell function was modelled through the relationship between insulin secretion and glucose concentration. The primary outcome was the effect of treatment on beta cell glucose sensitivity. Continuous glucose monitoring (CGM) parameters were explored as a secondary outcome.

Results: Linear mixed model estimates showed a potent additive glucose lowering effect: mean glucose from area under the curve (mean 95% CI) (mmol/l): Control 11.5 (10.7 - 12.3), DPP4 10.2 (9.4 - 11.1), SU 9.7 (8.9 - 10.5), SUDPP4 8.7 (7.9 - 9.5) ($p < 0.001$). Glucose sensitivity (pmol min^{-1} $m^{-2}mM^{-1}$) mirrored this additive effect: Control 71.5 (51.1 - 91.9), DPP4 75.9 (55.7 - 96.0), SU 86.3 (66.1 - 106.4), SUDPP4 94.1 (73.9 - 114.3) ($p = 0.04$ SUDPP4 Only). The plot of insulin secretion against glucose is shown in figure 1, which shows progressive increase in insulin secretion at the same glucose concentrations (i.e. increased glucose sensitivity) in favour of combination DPP4 and SU. Mean gliclazide concentrations (mean (SD)) (ng/ml) were: SU 662 (408), SUDPP4 603 (355) respectively ($p = 0.31$). Glucose time in range <3mmol/l on CGM (%) was unaffected: Control 1 (2 - 4), DPP4 2 (3 - 6), SU 1 (0 - 4), SUDPP4 3 (2 - 7) ($p = 0.648$).

Conclusion: Combination low dose gliclazide with a DPP4 inhibitor has potent glucose lowering effect through augmentation of beta cell function. Glucose reduction was achieved at gliclazide concentrations far below those achieved with standard therapeutic doses (~600ng/ml vs >5000ng/ml C_{MAX} with 80mg gliclazide daily). A double-blind randomised controlled trial is merited to formalise efficacy and safety of this combination, which may avoid negative aspects of SU and provide pharmacoeconomic benefit in a world of rising costs of diabetes care.



Clinical Trial Registration Number: NCT04192292
Supported by: This research is funded by the Wellcome Trust New Investigator Award held by ERP
Disclosure: **R.L.M. Cordiner:** None.

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Emotional eating is associated with reduced sensitivity to the central effects of GLP-1 receptor agonist treatment

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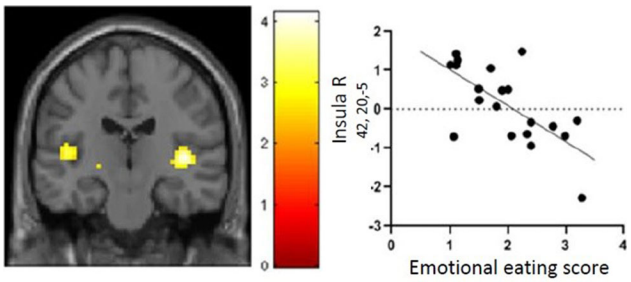
Background and aims: The weight loss induced by GLP-1 receptor agonists (GLP-1RAs) is related to suppressed appetite signaling in the brain and increased satiety, which leads to a reduced food intake, via direct and indirect actions on the CNS. However, there is considerable variation in individual responses to GLP-1RAs. It is unclear why some patients respond with weight loss and others do not. It has been suggested that emotional eaters are less sensitive to the acute central effects of GLP-1RAs. Therefore, the aim of this study was to investigate if individuals with higher emotional eating scores are less sensitive to longer term effects of GLP-1 RA on central responses to food cues.

Materials and methods: We performed secondary analysis of a randomized crossover study in obese patients with type 2 diabetes ($n = 20$, mean age 59.3 ± 4.1 years, mean BMI 32 ± 4.7 kg/m^2), consisting of two periods of 12-week treatment with either liraglutide 1.8 mg or insulin glargine. Using functional MRI, we assessed the relation between emotional eating and the effects of the GLP-1RA liraglutide after 10 days and 12 weeks of treatment on regional brain responses to visual food stimuli and to actual food.

Results: At baseline, higher emotional eating scores were associated with stronger brain responses to the anticipation of chocolate milk in the insula (right p=0.03, left p=0.05), and with less response to chocolate milk receipt in the insula (right p=0.004, left p=0.023). After 10 days of treatment, compared to insulin glargine, higher emotional eating scores were associated with less pronounced GLP-1RA induced reductions in brain responses to food pictures in the amygdala (p=0.01), bilateral insula (p=0.048) and left caudate nucleus (p=0.02). In addition, higher emotional eating scores tended to be associated with less pronounced GLP-1RA increases in brain responses to food reward in the left caudate nucleus and left insula. After 12 weeks of treatment, there were no significant associations between emotional eating scores and liraglutide-induced changes in CNS responses to food cues.

Conclusion: Our findings indicate that individuals with higher emotional eating scores, who are more vulnerable to cravings and less sensitive to rewarding properties of food, may be less sensitive to GLP-1RA treatment. These insights may help to optimize treatment strategies for obesity and to select patient groups with better efficacy of GLP-1RA treatment.



Association between emotional eating and brain responses to food stimuli at baseline. Coronal brain slice showing negative correlation in right insula between emotional eating and brain responses to receipt of chocolate versus receipt of tasteless solution. Left side of the slice is the left side of the brain. The color scale reflects the *T*-value of the functional activity. Results are presented at a threshold of $P < 0.05$, FWE corrected on the basis of cluster extent. In the graph on the right, the BOLD signal intensity (arbitrary units) is plotted as a function of emotional eating score.

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Liraglutide decreases postprandial fibroblast growth factor 19 and glucagon-like peptide 2, and increases postprandial cholecystokinin in individuals with obesity

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Background and aims: Treatment with liraglutide as well as other glucagon-like peptide 1 (GLP-1) receptor agonists is associated with slightly increased risk of gallbladder-related disorders, which have been proposed to be a consequence of altered gallbladder motility; thus, liraglutide seems to delay postprandial gallbladder refilling. The gut hormones cholecystokinin (CCK), fibroblast growth factor 19 (FGF19) and glucagon-like peptide 2 (GLP-2) are known to regulate gallbladder motility, and changes in postprandial concentrations of these hormones could explain the altered gallbladder motility.
Materials and methods: In a single-centre, double-blinded, 12-week trial 52 participants with obesity were randomised 1:1 to once-daily subcutaneous liraglutide (escalated from 0.6 mg to 3.0 mg with 0.6-mg weekly increments) or placebo. We evaluated gallbladder dynamics using ultrasonograpy during 4-hour liquid meal tests (600 kcal, 23.7 g fat) at baseline, after the first dose of study drug and following 12 weeks of treatment and showed a liraglutide-induced deceleration of postprandial gallbladder refilling. Postprandial plasma responses of hormones known to modulate gallbladder motility (CCK, FGF19, GLP-2) were secondary endpoints. The primary endpoint of the study, maximum postprandial gallbladder ejection fraction, was reported previously.
Results: Baseline characteristics were similar between groups (50% male, age 47.6±10.0 years, body weight 99.0±15.7 kg, BMI 32.6±3.4 kg/m^2 (mean±SD)). Compared to placebo, liraglutide reduced postprandial FGF19 responses after first dose (AUC 24.8 vs 48.0 ng/ml×min with treatment ratio (TR) [95% CI] 0.52 [0.39; 0.69]) and following 12 weeks of treatment (AUC 33.7 vs 48.5 ng/ml×min, TR 0.69 [0.52; 0.93]). Liraglutide also reduced postprandial GLP-2 responses (AUC 3,650 vs 4,894 pmol/l×min, TR 0.75 [0.62; 0.90]) following first dose as well as after 12 weeks (AUC 3,760 vs 4,882 pmol/l×min, TR 0.77 [0.60; 0.99]). Compared to placebo, liraglutide increased postprandial responses of CCK after first dose (AUC 762 vs 670 pmol/l×min (TR 1.14 [0.97; 1.33]) and following 12 weeks of treatment (AUC 873 vs 628 pmol/l×min (TR 1.39 [1.12; 1.73]).
Conclusion: Treatment with liraglutide caused increased postprandial plasma CCK concentrations and decreased plasma FGF19 and GLP-2 concentrations compared to placebo, which may explain the delayed postprandial gallbladder refilling observed in individuals with obesity treated with liraglutide.
Clinical Trial Registration Number: NCT02717858
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Disclosure: **A. Brønden:** None.

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Short-term treatment with liraglutide does not improve cardiac diastolic function in patients with type 2 diabetes: a randomised double-blind placebo-controlled trial

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Background and aims: The cardioprotective effect of glucagon-like peptide-1 (GLP-1) receptor agonists in patients with type 2 diabetes is still not well explained. In these patients, diastolic dysfunction is significant and linked to outcome, and the cardioprotective effect of GLP-1 receptor agonists may be by improving diastolic function. This study aimed to investigate if short-term treatment of liraglutide a GLP-1 receptor agonist improves left ventricular diastolic function.
Materials and methods: In an investigator-initiated double-blind randomized placebo-controlled trial the effect of 18 weeks of treatment with liraglutide on diastolic function was assessed in type 2 diabetes patients and echocardiographic signs of diastolic dysfunction (echo-Doppler determined E/e´≥9 or/and lateral e´≤10 cm/sec). Primary outcomes were improved left ventricle filling (the early peak filling rate, ePFR) and left atrium ease of emptying (the passive emptying fraction, LA_{PEF}), assessed by cardiac magnetic resonance imaging at rest and during chronotropic stress (glycopyrrolate 4 μg/kg; a cholinergic receptor

antagonist increasing heart rate, and thereby inducing chronotropic stress without also affecting contractility). Secondary outcomes included left ventricular and left atrial volumes and systolic function, measures of aortic stiffness, and echocardiographic diastolic parameters.

Results: Forty patients were randomized to liraglutide s.c. 1.8 mg/day (n=20) or placebo (n=20). Liraglutide reduced HbA1c (-0.47% 95%CI (-0.88 to -0.06)) and weight (-2.9kg 95%CI (-4.6 to -1.2)), both p<0.03). Liraglutide did not change ePFR at rest -24±60 vs. -6±46 ml/sec, during stress 2±58 vs. -2±38 ml/sec, or the changes from rest and stress 12.9 ±72.5 vs. 4.7±104.0, all p>0.05. LA_{PEF} decreased with liraglutide during stress (median(Q1,Q3)) -3.1(-9.0, 1.1) vs. 1.0(-2.9, 6.1) %, p=0.049, but no changes were evident at rest -4.3(-7.9, 1.9) vs. -0.6(-3.1, 2.2) %, p=0.19, or for the changes from rest to stress -1.7±8.4 vs. 0.8±8.2, p=0.4. All secondary outcomes were unchanged by liraglutide.

Conclusion: Short-term treatment with liraglutide did not improve diastolic function in patients with type 2 diabetes and echocardiographic signs of diastolic dysfunction. This suggests that the cardioprotective effect seen in long-term studies of liraglutide is not related to the improvement of left ventricular diastolic function.

Table 2 Effect of treatment with liraglutide vs. placebo on secondary outcomes

	Treatment effect		Delta estimates (95%CI)	P-value
	Liraglutide	Placebo		
LA maximum volume (mL)	-7.2±20.1	-1.6±12.7	-5.6(-16.4 to 5.2)	0.3
Lateral e´ (cm/sec)	-0.2±1.5	0.09±1.9	-0.3 (-1.5 to 0.8)	0.6
Average E/e'	0.7 (-0.1, 1.5)	0.1 (-1.3, 2.4)	0.4 (-1.2 to 1.9)	0.6
LV end-diastolic volume (mL)	-15.5±22.2	-9.9±22	-5.6(-19.6 to 8.4)	0.4
LV end-systolic volume (mL)	-1.7±12.6	-1.9±13.4	0.2(-8.2 to 8.5)	1
LV mass (g)	-1.0 (-9.3, 8.0)	7.5 (-3.0, 15.3)	7.0 (-3.0 to19.0)	0.14
LV myocardium native T1	1.5 (-10.5, 14.5)	-1.0 (-47.0, 47.0)	4.0 (-34.0 to 49.0)	0.8
LV myocardial perfusion index	-0.41 (-0.71, -0.15)	-0.15 (-0.22, -0.10)	-0.21 (-0.74 to 0.28)	0.3
LV ejection fraction (%)	-4.5 (-6.3, 1.3)	1.0 (-4.5, 2.0)	-2.0 (-6.0 to 2.0)	0.3
PWV_{Total} (m/s)	1.0 (-1.2, 2.0)	0.3 (-0.7, 2.0)	0.2 (-1.7 to 2.1)	0.9
Ascending aorta distensibility (%)	2.69 (-7.22, 9.49)	0.27 (-8.15, 9.33)	0.19 (-9.83 to 7.84)	1

Data presented as mean ± standard deviation or as median (Q1, Q3) or as number (%) as appropriate. LA; left atrium, e´; early diastolic myocardial velocity, E; late mitral inflow, LV; left ventricle, $PWV_{Total;}$ pulse wave velocity for the total aorta

Clinical Trial Registration Number: NCT02655770
Supported by: During this work, ASB has received funding from the local research committee at NSR hospital, the regional research committee of Region Zealand [13–000835], and the Danish Heart Association [16-R107-A6790-22002, and 18-R125-A8444-22110]. Novo Nordisk supported the study by an unrestricted grant covering the costs of CMR scans and blood analyses. Further, Novo Nordisk provided free study medication and matching placebo pens. None of the funding sources played any role in the process of conduction, interpretation of results or publishing the study. The corresponding author had full access to all data and had the final responsibility for the decision to submit for publication.
Disclosure: **A.S. Bojer:** None.

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Glucose-dependent insulinotropic polypeptide (GIP) contributes to sitagliptin-mediated improvement of beta cell function in patients with type 2 diabetes

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Background and aims: In patients with type 2 diabetes, dipeptidyl peptidase 4 (DPP-4) inhibitor treatment improves glycaemic control by raising the active levels of the insulinotropic gut hormones glucagon-like peptide 1 and glucose-dependent insulinotropic polypeptide (GIP). The potentiating effect of these hormones on glucose-stimulated insulin secretion seen in healthy persons was deemed lost in type 2 diabetes, as exogenous GIP was shown to have a poor or even absent insulinotropic effect in these patients. Using the highly selective GIP receptor antagonist GIP(3-30)NH_2, we investigated endogenous GIP's contribution to the insulinotropic effect of DPP-4 inhibition (assessed by insulin secretion rate (ISR) relative to plasma glucose) in patients with type 2 diabetes.

Materials and methods: In a double-blind, placebo-controlled, crossover study, 12 patients with type 2 diabetes underwent two randomised 12-13-day treatment courses with DPP-4 inhibitor (sitagliptin 100 mg once-daily) and placebo, respectively, with an interposed 1-3-week washout period. In the end of each treatment period, two randomised 5-hour liquid mixed meal tests with infusion of GIP(3-30)NH_2 (1,200 pmol/kg/min) or saline (placebo) were performed.

Results: We included eight men and four women (mean ± SD; BMI 27.4 ± 2.6 kg/m^2, HbA_{1c} 54 ± 15 mmol/mol) in the study. During placebo treatment, GIP(3-30)NH_2 lowered postprandial serum C-peptide (determined as difference in baseline-subtracted AUC (ΔbsAUC%) ± SEM: −31 ± 9%, $p = 0.005$) and increased postprandial plasma glucose excursions (ΔAUC% ± SEM: 7.3 ± 2.8%, $p = 0.017$) compared to saline. Sitagliptin increased concentrations of active GIP(1-42) (ΔAUC% ± SEM: 153 ± 21%, $p < 0.0001$) and lowered fasting plasma glucose from 8.7 to 7.6 mmol/L ($p < 0.0004$), whereas baseline-subtracted postprandial glucose excursions were unchanged compared to placebo treatment. We calculated the percentage reduction in $AUC_{ISR}/AUC_{glucose}$ ratio to evaluate the contribution of GIP to the insulinotropic effect of sitagliptin. The maximal potential of GIP, quantified as the AUC during DPP-4 inhibitor treatment including the contribution of GIP (*sitagliptin treatment + saline*) minus the physiological meal-response without GIP (*placebo treatment + GIP(3-30)NH_2*) was specified to be 100% of what GIP could potentially mediate. Thus, GIP(3-30)NH_2 caused a reduction in $AUC_{ISR}/AUC_{glucose}$ ratio equivalent to 37 ± 12% of the increments due to sitagliptin.

Conclusion: We demonstrate an insulinotropic and glucose-lowering effect of endogenous GIP in patients with type 2 diabetes and show that endogenous GIP is responsible for more than one third of the improved beta cell function observed during DPP-4 inhibitor treatment.

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Supported by: EFSD and Novo Nordisk A/S Programme for Diabetes Research in Europe 2017, AP Moller Foundation and Novo Nordisk Foundation
Disclosure: **S. Stensen:** None.

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Semaglutide reduces hsCRP levels across different treatment settings: post hoc analyses of SUSTAIN and PIONEER trials

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Background and aims: Limited information is available on the effects of glucagon-like peptide-1 receptor agonists on high-sensitivity C-reactive protein (hsCRP). This exploratory analysis aimed to determine the effect of the two formulations of semaglutide vs comparators on hsCRP.

Materials and methods: This analysis included trials where hsCRP data were available (SUSTAIN 3 and PIONEER 1, 2, 5). Subjects with type 2

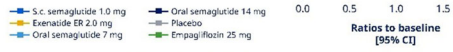
diabetes (T2D; N=2,482) and chronic kidney disease (PIONEER 5 only) received once-weekly s.c. or once-daily oral semaglutide or comparators (see **Figure**). Mediation analyses assessed the direct and indirect effect of change in HbA_{1c} and body weight (BW) on hsCRP; the percentage mediated by these parameters was calculated. These mediation analyses were performed using mixed models for repeated measurements, with change in hsCRP and change in the mediator as the outcomes. All hsCRP measurements were analysed by trial.

Results: Geometric mean baseline hsCRP was similar across trials (range 2.7-3.0 mg/L). Semaglutide significantly reduced hsCRP from baseline in all trials and vs all comparators (except vs placebo in PIONEER 5; **Figure**). Some of the semaglutide effect on hsCRP was mediated by a change in HbA_{1c} (percentage mediated: 30-39%), whereas BW played a lesser role (5-35%), except in PIONEER 5 (HbA_{1c} 26%; BW 50%).

Conclusion: Semaglutide reduced hsCRP levels vs comparators in subjects with T2D, partially mediated indirectly via the effect on HbA_{1c} and, to a lesser extent, BW; however, there may be a direct semaglutide effect. The observed reductions in hsCRP in subjects with T2D suggest a possible anti-inflammatory effect of semaglutide. Ongoing trials will provide further insights into the impact of semaglutide as an anti-inflammatory drug and its cardiovascular effects.

Changes from baseline, ratios to baseline and estimated treatment ratios for hsCRP with semaglutide vs comparators in SUSTAIN 3 and PIONEER 1, 2 and 5 trials

Change in hsCRP by trial	N	Change from baseline (mg/L)	Ratio to baseline [95% CI]	p-value vs baseline	ETR [95% CI] to comparator	p-value for ETR
SUSTAIN 3						
S.c. semaglutide 1.0 mg	404	−1.98	0.55 [0.49;0.61]	<0.0001	0.75 [0.65;0.88]	0.0002
Exenatide ER 2.0 mg	405	0.22	0.72 [0.65;0.81]	<0.0001		
PIONEER 1						
Oral semaglutide 7 mg	175	−1.45	0.72 [0.63;0.82]	<0.0001	0.72 [0.59;0.88]	0.0016
Oral semaglutide 14 mg	175	−0.39	0.76 [0.66;0.87]	<0.0001	0.76 [0.62;0.93]	0.0075
Placebo	178	−0.06	0.99 [0.86;1.15]	0.9207		
PIONEER 2						
Oral semaglutide 14 mg	411	−1.64	0.63 [0.57;0.70]	<0.0001	0.70 [0.61;0.80]	<0.0001
Empagliflozin 25 mg	410	−1.19	0.91 [0.83;1.00]	0.0443		
PIONEER 5						
Oral semaglutide 14 mg	163	−0.50	0.82 [0.70;0.95]	0.0105	0.83 [0.67;1.03]	0.0839
Placebo	161	−0.43	0.99 [0.85;1.15]	0.8919		



'On-treatment without rescue medication' data from the full analysis set. Changes from baseline and ratios to baseline were analysed using a mixed model for repeated measurements with treatment as categorical fixed effect and baseline value as covariate, all nested within visit, and an unstructured residual covariance matrix. The ratio to baseline and the corresponding baseline value were log-transformed prior to analysis. Trial durations: 56 weeks for SUSTAIN 3, 26 weeks for PIONEER 1 and 5, and 52 weeks for PIONEER 2.
ETR, estimated treatment ratio; exenatide ER, exenatide extended release; hsCRP, high-sensitivity C-reactive protein.

Clinical Trial Registration Number: NCT01885208; NCT02906930; NCT02863328; NCT02827708
Supported by: Novo Nordisk A/S
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OP 31 Modelling diabetes long term complications

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Mir-34a mediates progression of liver injury from NAFLD to fibrosis in diabetes associated liver fibrosis

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a multifactorial disease that encompasses a spectrum of pathological conditions, ranging from simple steatosis (NAFL), nonalcoholic steatohepatitis (NASH) and fibrosis/cirrhosis which could end up to hepatocellular carcinoma and liver failure. Diabetes is an independent risk factor in the development of liver fibrosis. However, the precise mechanisms by which diabetes worsens liver function is poorly understood. The aim of this study was to determine the mechanistic role of miR-34a at different disease stages of liver injury and wound healing in diabetic context.

Materials and methods: A mouse model that highly resembles the pathological development in patients with liver cirrhosis was established by treating the mice with a dose (200μg) of streptozotocin (STZ) at day 2 after born to induce diabetes and followed by feeding a high fat diet (HFD) after weaning to induce NAFLD and liver fibrosis. Liver tissues and primary hepatocytes were collected from the mice at the age of 6, 8 and 12 weeks and subjected to histological analysis, microRNA deep sequencing, q-RT-PCR and immunoblotting analysis.

Results: Histological analysis revealed the pathological development of hepatic steatosis at week 6, NASH at week 8 and liver fibrosis at week 12, a series of liver injury similar to clinical patients at different disease stage. Importantly, in the liver tissues from mice treated with STZ/HFD, lacking insulin induced significant upregulation of miR-34a which was further exacerbated by the subsequent HFD feeding. Upregulated miR-34a targeted the hepatic nuclear factor 4α (HNF4α), a verified true target of miR-34a, and inhibited expression of HNF4α at both mRNA and proteins ($P<0.05$). These pathological changes disturbed expression of genes involved in mitochondrial fatty acid β-oxidation and hepatic very low density lipoprotein (VLDL) metabolism, indicated by the decreased mRNA expression of PPARα and its downstream target genes CPT-1α and ACOX-1 and the upregulation of apolipoprotein B and MTP, respectively. Consequently, lipotoxicity generated from the accumulated lipids in hepatocytes induced metabolic inflammation and compromised hepatic insulin signaling. Moreover, upregulation of miR-34a further enhanced expression of pro-fibrogenic cytokines TGFβ1 and TGFβ2 in both hepatocytes and hepatic stellate cells (HSC) which stimulated expression of fibrosgenic genes, α-SMA and Col1A1, resulting in accumulation of extracellular matrix (ECM) components in the liver and inducing fibrosis. *In Vitro*, transfection of miR-34a into a mouse hepatocyte AML12 inhibited expression of insulin signaling molecules IRS-1 and PI3K ($P<0.05$). More importantly, incubation of the miR-34a transfected cells with insulin can inhibit activation of TGFβ signaling and mitigate fibrogenic gene expression, suggesting the essential role of insulin in preventing liver injury and fibrosis.

Conclusion: Our study, for the first time, demonstrates that upregulation of miR-34a induced by insulin deficiency and HFD target multiple metabolic pathways, including lipoprotein metabolism, oxidative stress and fibrogenesis, to induce liver fibrosis/cirrhosis via mediation of HNF4α. This novel finding may lend support to the development of miR-34a and HNF4α as pharmaceutical targets for the treatment of NAFLD and hepatic fibrosis.

Disclosure: **Q. Su:** None.

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An siRNA strategy to silence apolipoprotein CIII in the fight against the metabolic syndrome

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Background and aims: Apolipoprotein CIII (apoCIII) is increased in obesity-induced insulin resistance and type-2 diabetes. Emerging evidence support the advantages of small interfering RNAs (siRNAs) to target disease-causing genes. We aimed to develop a new RNA-based platform for *in vivo* silencing of apoCIII and investigate its protective effects against the metabolic syndrome.
Materials and methods: We used 12-week-old male and female B6.Cg-Lep^{ob}/ Lep^{ob}J (*ob/ob*) mice on a C57BL6/j background from our own breeding colony. Before start of treatment body weight (BW) and blood glucose (BG) were determined to randomize and assign the mice to two different groups: i) apoCIII-siRNA (n = 5); and ii) control-siRNA treatment (n = 5). The siRNAS were given intravenously (i.v.), at a dose of 0.008 mg/kg, for three consecutive days every 15 days for eight weeks. Plasma apoCIII, BW and BG were monitored during the study. After the last treatment plasma lipoprotein lipase (LPL) activity in pre- and post-heparin injected mice (0.2 international units per g of BW) was determined and intraperitoneal glucose tolerance test (IPGTT) and glucose-stimulated insulin secretion (GSIS) were performed. At the end of the study samples for lipid profiles, apoCIII gene expression and protein levels and off-target effects were taken. Data are expressed as mean ±s.e.m. Statistical comparisons between the two groups of mice were performed using the Mann-Whitney U *t*-test. When two independent variables were considered, 2-ANOVA followed by Bonferroni´s *post-hoc* test was used. Statistical significance was defined as $P < 0.05$.
Results: Our results show that circulating apoCIII was progressively lowered upon administration of apoCIII-siRNA (apoCIII reduction: 47.8±4.5%; 2-ANOVA; $F_{1,72}$=11.71; $P < 0.001$). Plasma LPL activity was higher in mice with reduced apoCIII levels (LPL activity fold-increase: 8.2±2.1; 2-ANOVA; $F_{1,24}$=24.06; $P < 0.0001$). The increased plasma LPL activity resulted in lower levels of triglycerides (apoCIII-siRNA: 141.9±26.9 mg/dL; control-siRNA: 264.7±34.9 mg/dL Mann-Whitney U *t*-test; $P < 0.05$). Decreasing apoCIII induced a progressive reduction in weight gain (weight gain reduction: 8.8 ±2.1%; 2-ANOVA; $F_{1,119}$=5.97; $P < 0.05$) and non-fasting BG levels (BG reduction: 35.8±6.8%; 2-ANOVA; $F_{1,119}$=27.27; $P < 0.0001$), as well as improved IPGTT and GSIS (2-ANOVA; $F_{1,35}$=34.33; $P < 0.0001$ and $F_{1,35}$=11.86; $P < 0.05$, respectively). At the end of the study, it was confirmed that apoCIII gene and protein levels were reduced in liver from siRNA-treated mice (liver apoCIII mRNA reduction: 34.8±4.3%, $P < 0.001$; liver apoCIII protein reduction: 40.7±8.7%, $P < 0.05$; Mann-Whitney U *t*-test), compared to the control group. To test the specificity of the siRNA the expression of apoCIII was analyzed in duodenum, the second largest source of the apolipoprotein, and the levels were unaffected. Furthermore, since the apoCIII gene is located within a gene cluster with apoAI, apoAIV and apoAV we confirmed that there were no off-target effects of the siRNA on these apolipoproteins.
Conclusion: Our data demonstrate that obese, insulin resistent and hyperglycemic mice, treated during eight weeks with siRNA targeting the apoCIII gene, decrease in BW, improve their insulin sensitivity, lipid- and glucose homeostasis without any observed side effects. Therefore, our siRNA strategy to decrease apoCIII might become a new tool in the fight against the metabolic syndrome.
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Disclosure: **P. Recio-López:** None.

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Loss of Hsp70 leads to increased albuminuria in a STZ-induced diabetic mouse model

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Background and aims: The stress-inducible heat shock protein 70 (Hspa1a/Hspa1b) is part of the protein quality control system. It is involved in the maturation of newly synthesized polypeptides, stabilization and refolding of misfolded proteins as well as disaggregation of protein aggregates. Posttranslational modifications, for example by glycation during hyperglycemia, can lead to misfolding, aggregation and loss-of-function of the protein. The resulting advanced glycation endproducts are associated with the development of diabetic complications. The aim of this study was to investigate the effect of the loss of Hspa1a/Hspa1b in a STZ-induced diabetic mouse model in terms of the development of diabetic nephropathy.
Materials and methods: Diabetes was induced at the age of 10 weeks and mice were sacrificed at the age of 28 weeks. Urine was collected for 24hrs just before organ collection and albumine and creatinine were measured *via* ELISA. Protein expression was analyzed *via* Western Blot and Immunohistochemistry (IHC). Morphologic changes of diabetic nephropathy were assessed by IHC and electron microscopy.
Results: Hspa1a/Hspa1b knockout mice (KO) did not differ from wild-type mice (WT) in the control or STZ-treated group in respect to bodyweight or blood glucose levels. However, STZ-treated KO mice (KO+STZ) revealed to have a significantly higher albumine/creatinine ratio (ACR) compared to STZ-treated wild-type mice (WT+STZ) and to controls (KO-STZ) (KO+STZ: 15.73 mg/g; WT+STZ: 9.83 mg/g; KO-STZ: 6.77 mg/g; vs. WT+STZ p=0.04, vs. KO-STZ p=0.03; n=3-5 per group). Preliminary data suggest that both, KO+STZ and WT+STZ, show atrophic proximal tubuli in the outer layers of the kidney parenchyma with thickening of the basal membrane. Furthermore, WT+STZ mice seem to have decreased Hspa1a/Hsapa1b protein expression compared to WT-STZ mice.
Conclusion: Loss of the stress-inducible Hspa1a/Hspa1b leads to increased ACR in a STZ-induced diabetic mouse model. Interestingly, both KO+STZ and WT+STZ mice showed atrophic proximal tubuli in the outer region of the kidney parenchyma. Furthermore, we observed a downregulation of Hspa1a/Hspa1b in WT+STZ mice, which is in line with preliminary human data, where Hspa1a/Hspa1b expression seems to be lost in the course of diabetic nephropathy.
Supported by: German Research Foundation (DFG, CRC1118)
Disclosure: **J. Zemva:** Grants; DFG (CRC1118).

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Involvement of gut-hormones in regulating female reproductive function in obese and incretin receptor knockout animal models

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Background and aims: Metabolism and reproduction are interdependent with the hypothalamus acting as major control centre. Recent studies suggest correlation between energy intake and reproductive dysfunction such as polycystic-ovary-syndrome (PCOS) and ovulatory disturbances. Metabolic peptides including glucose-dependent-insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1) and Peptide YY (PYY) have been suggested to play a role in the regulation of reproductive function. The present study evaluates disturbed reproductive health, altered gut/reproductive hormone receptor expression and fertility outcome induced by high-fat feeding and global knockout (KO) of GLP-1 or GIP receptors in rodent models.
Materials and methods: Female Wistar rats (4-weeks-old) fed with high-fat diet for 20-weeks were used with regular monitoring of metabolic parameters. Consecutive estrous cycles were observed using vaginal smears and rats were bred with normal diet fed Wistar males to assess fertility. At week 20, gut and reproductive gene expression in ovaries and adrenals were assessed using PCR. In a second series, effects of global GIPR and GLP-1R deletion on estrous cycling and reproductive outcomes were evaluated in C57BL/6 mice.
Results: Female rats after 20 weeks of high-fat feeding displayed significantly ($p<0.05$ to $p<0.001$) increased body weight and plasma insulin without

change of blood glucose and HbA1c. 50% of the high-fat fed Wistar rats had prolonged average cycle length (≥7days). Fertility outcome showed 48% reduction in litter size and 16% were unable to attain pregnancy after prolonged high-fat feeding. 35% of pups born to high-fat-fed rats were eaten by their mothers or born dead whilst this phenomenon was absent with control fed rats. H&E staining revealed morphological changes in the ovaries with significant ($p<0.01$) increases in the number of cysts. Ovarian expression of *Amh, Npy2R and GcgR* genes were downregulated ($p<0.01$) while *Glp-1r* and *Insr* (insulin R) genes were upregulated ($p<0.05$-$p<0.001$) in high-fat rats. Expression of *Glp-1R, Gipr*, *Gshr*, *Insr, Amh, Esr-1, Npy2R* and *GcgR* genes were also upregulated ($p<0.01$ to $p<0.001$) in high-fat fed rat adrenals. In the transgenic model study, female GIPR and GLP-1R KO mice exhibited significantly ($p<0.05$ and $p<0.01$) deranged estrous cycling compared to wild-type controls. 50% and 16% of female GIPR and GLP-1R KO mice respectively produced litters with wild-type males across three breeding cycles. Consistent with functional role of incretin receptors in pregnancy outcome, litter size was significantly ($p<0.001$ to $p<0.05$) decreased in $GIPR^{-/-}$ and $GLP\text{-}1R^{-/-}$ mice.

Conclusion: High-fat feeding and incretin hormone receptor deletion disrupts reproductive function in females. This suggests important interactions between gut and reproductive hormones at level of ovaries and adrenals. Taken together with previous observations, these data suggest that incretin receptor modulation could represent a novel means for treating female reproductive disorders.

Supported by: DUK RD Lawrence Fellowship and UU strategic funding

Disclosure: **D. Khan:** None.

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Adiporon improves endurance capacity and decreases ectopic lipid deposition in middle-aged obese mice

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Background and aims: Obesity and ageing go hand in hand with loss of endurance capacity, insulin-resistance and features of the metabolic syndrome. Two additional burdens associated with this syndrome are non-alcoholic fatty liver disease (NAFLD)/steatohepatitis (NASH) and myosteatosis, whose severity increases with age. Myosteatosis is known to negatively correlate with muscle performance. Adiponectin (ApN) is an insulin-sensitizing, lipid-lowering hormone, which is decreased in the metabolic syndrome. Muscle and liver are two of its main target tissues. The aim of this study is to investigate whether an active ApN receptor agonist, AdipoRon (AR) could protect the muscle and the liver of middle-aged obese mice.

Materials and methods: Three groups of male mice were studied up to 14 months of age: one received a normal diet (ND), another a high fat diet (HFD) and the last a HFD combined with AR given orally (30 mg/kg/d scaled up to 50 mg/kg during the last part of the study; HFD+AR). Treadmill tests and micro-computed tomography (MCT) were carried out *in vivo*. Tissue analyses were performed by (immuno)histochemistry *ex vivo*.

Results: AR did not markedly alter diet-induced-obesity. Yet, this treatment, even when given at a low dose, rescued exercise endurance of obese mice during treadmill tests ($P < 0.05$), while improving insulin sensitivity only at the higher dose ($P < 0.05$). Density of dorsal muscles and liver, which was measured *in vivo* before the end of the study by MCT, was decreased in obese mice, suggesting fatty infiltration. This decrease was less pronounced in the HFD+AR group. These data were confirmed by (immuno)histochemistry. AR significantly decreased steatosis and cellular ballooning in the liver ($P < 0.05$), thus decreasing the NAFLD activity score. AR strikingly reversed (and actually even over corrected) intramyocellular lipid (IMCL) accumulation either due to ageing in oxidative fibers (types I and IIb, soleus) or to HFD in glycolytic ones (types IIx and IIb, extensor digitorum longus) ($P < 0.05$). Size of subsarcolemmal lipid droplets, known to be associated with adverse metabolic outcomes, was reduced as well.

Conclusion: In conclusion, AR enhances muscle endurance in obese mice, an event that precedes the improvement in insulin sensitivity. AR also protects obese middle-aged mice against burdens associated with the metabolic syndrome: NASH and myosteatosis, the effects on muscle being particularly impressive.

Supported by: FNRS, SFD

Disclosure: **C. Selvais:** None.

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Analysis of the hypothalamic transcriptome controlling counter-regulatory responses to hypoglycaemia

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Background and aims: The development of hypoglycaemia during the treatment of diabetes mellitus by insulin injection is a major risk and one of the principal difficulties associated with the maintenance of normoglycaemia. After a hypoglycaemic episode, the probability of having subsequent episodes of hypoglycaemia increases. These defects in the counter-regulatory response (CRR) to hypoglycaemia are due to the suppression of the hormonal secretion of glucagon and catecholamines to restore normal glucose levels. The role of the central nervous system (CNS) is essential to activate it, but the modulators involved in this process and the mechanisms underlying the response to hypoglycaemia are poorly understood. In this context, we aim to identify novel regulators of hypoglycaemia counter-regulation in the murine brain in the context of diabetes and recurrent hypoglycaemia.

Materials and methods: A model of type-2 diabetes mellitus (T2DM) in mice exposed to acute (AH) or recurrent hypoglycemia (RH) was used. Counter-regulation was assessed by glucagon secretion. Single nuclei RNA sequencing (snRNAseq) was performed in the hypothalami of AH and RH mice. Differential expression and pathway analyses were performed in an overall dataset and in the different cell types resulting from the clustering identification.

Results: Recurrent hypoglycemia driven by insulin injections significantly impaired glucagon secretion in T2DM mice (AH, 74.96 ± 8.42 pg/mL vs RH, 49 ± 6.57 pg/mL, $p<0.05$). We studied the transcriptional profile of the hypothalami from AH and RH T2DM mice through snRNAseq. This data revealed 299 differentially expressed genes between AH and RH (FDR<0.05); 274 of these mRNAs were part of the neuron subpopulation. A subsequent analysis was done using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) to determine the associated functions of these differentially expressed genes. This analysis revealed that the there was a strong association of these genes to mechanisms controlling synapse organization, regulation of membrane potential, neurotransmitter secretion and vesicle-mediated transport suggesting that RH impairs the connectivity of the cells altering the brain function in response to hypoglycaemia.

Conclusion: Here, we first established a mouse model of RH and impaired glucagon secretion in T2DM. Transcriptomic analysis of hypothalami revealed that RH and defective insulin-induced glucagon secretion were associated with important changes in genes controlling synaptic plasticity; surprisingly no changes in the expression of metabolic or glucose signaling-related mRNAs were detected. Thus, this investigation suggests that hypoglycemia-associated autonomic failure may be caused by a general defect on synaptic activity rather than in glucose sensing pathways.

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Disclosure: **J. Castillo-Armengol:** Grants; It has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 777460.

OP 32 Benefits of GLP-1: from traditional to non-traditional complications

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Liraglutide may induce impaired diastolic heart function by activation of sympathetic tonus: A group effect of this class of drugs?

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Background and aims: Glucagon-like-peptide 1 receptor agonists (GLP1-RAs) are a group of drugs not only used in treatment of type 2 diabetes but also to treat obesity. The widely used GLP1-RAs semaglutide and liraglutide have been shown to increase heart rate, maybe due to increased sympathetic activation. We have shown that liraglutide reduces heart rate variability, reduces diastolic heart function (e'), and increases heart rate in obese people with newly diagnosed type 2 diabetes and stable coronary heart disease. We suggest that activation of the sympathetic tonus as reflected in increased heart rate and decreased heart rate variability may be associated with impaired diastolic function of the heart.
Materials and methods: Out of 41 people with stable coronary artery disease and newly diagnosed type 2 diabetes 30 people completed a 12 plus 12 weeks over-crossed placebo-controlled clinical trail with 2 weeks of washing out of antidiabetic therapy before and between the intervention periods. The people taking part in the study undertook 24 hours HOLTER monitoring and a transthoracic echocardiography at weeks 0, 12, 14 and 26. They were treated by metformin as backbone and liraglutide titrated to 1.8 mg q.d. and corresponding placebo.
Results: Heart rate increased by 11% (p=0.003) and heart rate variability (SDNN) decreased independent of the increase in heart rate by 25% (p<0.001) during treatment with liraglutide. Diastolic filling of the heart as measured by e' was impaired by 10% (p<0.02) during liraglutide therapy. It was observed that the increase in mean heart rate during 24 hours was strongly associated with decrease in e' (R^2= 16%, p<0.01). The decrease in SDNN during sleep (from midnight to 2 am.) was also associated with decrease in e' (R^2=10%, p<0.02).
Conclusion: Activation of the sympathetic tonus during therapy with the GLP1-RA, liraglutide may result in increased heart rate and reduced heart rate variability. Our data suggest an association between increased heart rate and reduced heart rate variability and impaired diastolic function of the heart in obese people with stable coronary heart disease and newly diagnosed type 2 diabetes undergoing therapy with liraglutide. It should be addressed whether this observation is a group effect of GLP1-RAs, and if so, whether drug classes with anti-chronotropic and lusitropic effects will abolish this effect on heart rate and heart rate variability with possibly positive effects on diastolic function of the heart of people with type 2 diabetes.
Clinical Trial Registration Number: NCT01595789
Supported by: Novo Nordisk A/S, Danish Heart Foundation, A.P. Møller Foundation
Disclosure: **S.B. Haugaard:** Grants; From Novo Nordisk A/S.

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Liraglutide reduces cardiac adipose tissue in type 2 diabetes: results from the LiraFlame randomised controlled trial

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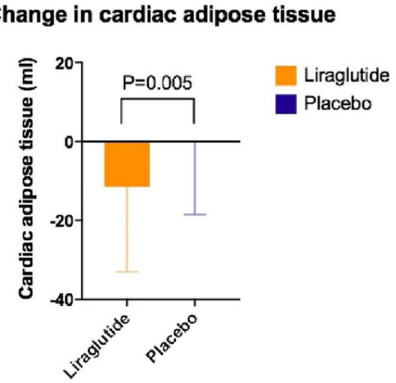
Background and aims: An increased amount of cardiac adipose tissue is associated with a higher risk of cardiovascular disease and mortality in persons with type 2 diabetes. Recent studies have shown that treatment with GLP-1 receptor agonists can reduce the risk of major adverse cardiovascular events. We hypothesized that 26 weeks of treatment with liraglutide was associated with a reduction in cardiac adipose tissue as compared to placebo.
Materials and methods: We conducted a randomized placebo-controlled, double-blind, parallel clinical study, where participants with type 2 diabetes were randomized to treatment with liraglutide 1.8 mg/day or placebo for 26 weeks. Computed tomography was performed before and at end-of-treatment to evaluate the cardiac adipose tissue volume, which was automatically quantified. We report the results of a secondary endpoint evaluating the change in cardiac adipose tissue.
Results: A total of 102 participants were included and randomly assigned to receive liraglutide (n=51) or placebo (n=51). The participants had a mean age of 66.4 (SD 8.2) years, 15.7% were females and median diabetes duration was 10.9 [IQR 5.7 - 18.2] years. Baseline clinical characteristics were balanced between the two treatment groups, except for triglycerides. From baseline to end-of-treatment mean change in HbA_{1c} was -5.1 (95% CI -8.1, 2.0) mmol/mol in the liraglutide group and -0.1 (-1.9, 1.7) mmol/mol in the placebo group. The mean change in body weight was -3.7 (-4.8, -2.6) kg in the liraglutide group and -0.18 (-0.76, 0.40) kg in the placebo group. At baseline, the mean (SD) cardiac adipose tissue volume was comparable between the liraglutide and the placebo group [232.6 (112.8) vs 227.0 (103.2) ml, p=0.80]. From baseline to end-of-treatment the mean cardiac adipose tissue change was -11.5 (95% CI: -17.6, -5.4) ml in the liraglutide group (p<0.001) and -0.01 (-5.3, 5.3) ml in the placebo group (p=1.00) (Figure 1). The reduction in cardiac adipose tissue was significantly larger in the liraglutide group compared to the placebo group (mean difference: -11.4 (-19.4, -3.3) ml, p=0.005).
Conclusion: Treatment with liraglutide for 26 weeks was associated with a reduction in cardiac adipose tissue compared to placebo, suggesting a possible mechanism of the cardioprotective benefits of liraglutide observed in outcome studies.

Figure 1: changes in cardiac adipose tissue in the liraglutide and the placebo treated group



Changes in cardiac adipose tissue is presented as mean with standard deviation. P value for the group-wise comparison of participants treated with liraglutide or placebo was calculated using unpaired samples t-test

Clinical Trial Registration Number: NCT03449654
Supported by: NN
Disclosure: **T. Hansen:** None.

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Positive impact of liraglutide on pulmonary function in patients with type 2 diabetes: data from the randomised cross-over LIRALUNG study

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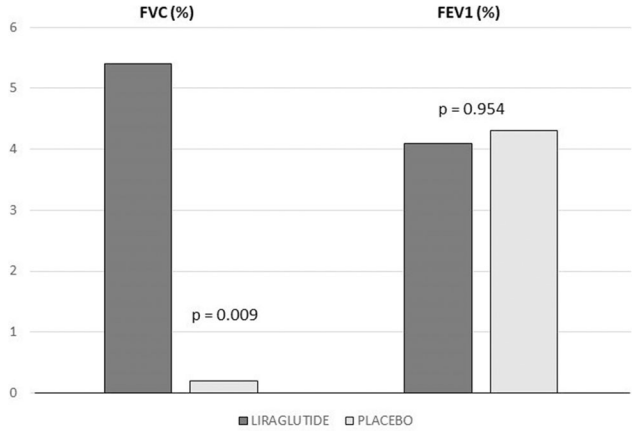
Background and aims: There is experimental evidence that glucagon-like peptide 1 receptor (GLP-1R) agonists ameliorates lung fibrosis and stimulates surfactant production. Therefore, our aim was to evaluate the effect of liraglutide (a GLP-1R agonist) on pulmonary function and the serum levels of surfactant in type 2 diabetes.
Materials and methods: A double-blind, randomized, crossover, placebo-controlled clinical trial comprising 76 patients with a baseline forced expiratory volume in first second <90% of predicted. Liraglutide was administered for 7 weeks (2 weeks of titration plus 5 weeks at 1.8 mg daily). This short duration was intentional in order to minimize weight loss as a potential confounding factor. Lung function parameters included: FEV1, FVC, peak expiratory flow (PEF), maximum mid-expiratory flow (FEF_{25-75}). Serum surfactant protein D was also assessed.
Results: Liraglutide significantly reduced HbA1c [Δ: -1.2% (95%CI: -1.5 to -0.9)] and the BMI [Δ: -0.5 kg/m^2 (-0.8 to -0.2)] and exerted a positive impact on forced vital capacity [ΔFVC: 5.2% of predicted (0.8 to 9.6)] in comparison with placebo. No differences in the other pulmonary variables were observed between groups. Participants under liraglutide treatment also experienced a decrease in serum surfactant protein D [196.4 (128.2 to 271.4) to 169.6 (108.1 to 233.6), p=0.038]. The absolute change in FVC correlated with the final serum surfactant protein D in participants receiving liraglutide (r=-0.313, p=0.036). Stepwise multivariate regression analysis showed that the final serum surfactant protein D independently predicted changes in FVC.
Conclusion: Liraglutide exerts a positive impact on FVC and surfactant in patients with type 2 diabetes.



Treatment effect (liraglutide vs. placebo) on spirometric values from baseline to 7 weeks in the LIRALUNG study.

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Effect of subcutaneous semaglutide on features of the metabolic syndrome in patients with non-alcoholic steatohepatitis

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Background and aims: Non-alcoholic fatty liver disease and non-alcoholic steatohepatitis (NASH) are closely linked to insulin resistance, visceral obesity and features of the metabolic syndrome. Treatment options for patients with NASH are currently limited. Here, the effects of semaglutide on parameters associated with metabolic syndrome are reported in patients with NASH.
Materials and methods: In a 72-week phase 2 trial, 320 patients with NASH and fibrosis stage F1-F3 were randomised to subcutaneous semaglutide (0.1, 0.2 or 0.4 mg once daily) or placebo. Treatment with semaglutide 0.4 mg led to a significantly greater proportion of patients having NASH resolution without worsening of fibrosis (primary endpoint). Changes in metabolic and inflammatory parameters are reported here for all randomised patients during the in-trial period; HbA_{1c} and fasting plasma glucose are reported in patients with T2D only, and HOMA-IR and adipose tissue insulin resistance index (Adipo-IR: fasting plasma insulin × free fatty acids) in patients not treated with insulin at baseline. Correlations with primary endpoint (NASH resolution) are reported in all patients on-treatment at week 72 (semaglutide 0.4 mg and placebo combined).
Results: A total of 320 patients were randomised to treatment (semaglutide 0.1 mg, n=80; 0.2 mg, n=78; 0.4 mg, n=82; or placebo n=80), of whom 62% (n=199) had type 2 diabetes and 49% (n=158) were treated with insulin at baseline. Semaglutide 0.4 mg was associated with significantly greater improvements than placebo in waist circumference, HbA_{1c}, fasting plasma glucose, HOMA-IR, Adipo-IR, HDL cholesterol, triglycerides and high-sensitivity C-reactive protein (Table). Changes in non-HDL cholesterol and blood pressure were not significantly different between semaglutide 0.4 mg and placebo. Improvements in waist circumference, HbA_{1c} (both p<0.001), fasting plasma glucose (p=0.006), HOMA-IR (p=0.017), Adipo-IR (p=0.015) and HDL cholesterol (p<0.001) were correlated with achieving NASH resolution without worsening of fibrosis (primary endpoint).
Conclusion: Once-daily subcutaneous semaglutide 0.4 mg in patients with NASH, in addition to weight loss and improved glycaemic control, resulted in significant improvements in multiple features of the metabolic syndrome. Changes from baseline in metabolic parameters were correlated with resolution of steatohepatitis.

		Semaglutide 0.4 mg	Placebo	ETD/ETR (95% CI)	P-value		With NASH resolution	Without NASH resolution	P-value†
Estimated change from BL at week 72*	Waist circumference, cm	−11.0	−1.3	−9.7 (−12.2, −7.3)	<0.0001	Mean ± SD change from BL (semaglutide 0.4 mg and placebo groups combined)	−8.8 ± 8.2	−3.5 ±6.6	<0.001
	HbA_{1c}, % (T2D)	−1.2	−0.01	−1.1 (−1.5, −0.8)	<0.0001		−1.2 ± 0.9	−0.5 ± 1.1	<0.001
	Fasting plasma glucose, mmol/L (T2D)	−2.1	−0.3	−1.9 (−2.7, −1.1)	<0.0001		−2.1 ± 2.3	−1.1 ± 2.7	0.006
Ratio to BL at week 72*	HDL-cholesterol, mmol/L	1.1	1.0	1.06 (1.02, 1.11)	0.0064		0.1 ± 0.1	0.0 ± 0.2	<0.001
	Non-HDL-cholesterol, mmol/L	0.9	0.9	0.97 (0.91, 1.05)	0.4514		−0.2 ± 0.7	−0.3 ± 0.9	0.981
	Triglycerides, mmol/L	0.7	1.0	0.75 (0.67, 0.84)	<0.0001		−0.4 ± 1.1	−0.2 ± 0.8	0.116
	hs-CRP, mg/L	0.4	0.9	0.46 (0.35, 0.61)	<0.0001		−1.8 ± 9.3	−1.3 ± 7.2	0.212
	HOMA-IR (no insulin at BL)	0.6	0.9	0.65 (0.52, 0.83)	0.0004		−3.0 ± 4.7	−1.7 ± 8.9	0.017
	Adipo-IR (no insulin at BL)	0.5	0.9	0.57 (0.44, 0.75)	<0.0001		−32.6 ± 55.6	−13.0 ± 68.7	0.015

*Based on ANCOVA with multiple imputation of missing data from placebo group. †P values from Wilcoxon two sample test. BL, baseline; ETD, estimated treatment difference; ETR, estimated treatment ratio.

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Multi-target engagement effect of a novel long-acting Glucagon/GIP/GLP-1 triple agonist (HM15211) in animal model of NASH

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Background and aims: NASH is a complex disease progressed by multiple mechanism. To date, no approved drug is available due to unsatisfied efficacy. Of note, recent studies demonstrate advantages of targeting multiple aspects of this disease. To aid multi-targeting and provide a novel treatment option with improved efficacy, HM15211, a long-acting Glucagon/GIP/GLP-1 triple agonist, has been developed. Here, enhanced anti-steatosis effect by HM15211 were investigated in diet induced obese mice (DIO mice) and potential benefits of HM15211 was evaluated by comparing its therapeutic effects with NASH drug candidates in high-fat diet induced mouse model of NASH.
Materials and methods: To investigate the anti-steatosis effect of HM15211, HM15211 was administered to DIO mice and liraglutide was used as comparator. After 4 weeks treatment, hepatic triacylglycerol (TG), and blood TG and cholesterol were measured. To unveil the MoAs for improved hepatic lipid metabolism by HM15211, expression level of genes involved in β-oxidation and de novo lipogenesis was determined by qPCR analysis. Next, to evaluate potential benefits of HM15211 on NASH, AMLN-diet induced NASH mice were administered either with HM15211 or obeticholic acid (OCA) for 12 weeks and histological analysis and blood ALT level as well as hepatic TG analyzed at the end of treatment. Additional study was performed to compare therapeutic effect of HM15211 with incretin analogs such as acylated GLP-1 or GLP-1/GIP in AMLN-diet induced NASH mice, followed by histological analysis.
Results: In DIO mice, greater reduction in hepatic TG (-63.6%, -80.6% *vs.* vehicle for liraglutide, HM15211) and blood TG (-33.4%, -73.7% *vs.* vehicle for liraglutide, HM15211) were confirmed for HM15211 treatment compared to liraglutide, which was well correlated with favorable reprogramming of hepatic lipid metabolism-related gene expression. In AMLN mice, HM15211 treatment more efficiently normalized hepatic lipid contents (-48.9%, -93.0% *vs.* vehicle for OCA, HM15211) and steatosis score (2.9, 2.1, 0.1 for vehicle, OCA, HM15211) compared to OCA treatment. Consistently, HM15211 treatment significantly reduced histological score for lobular inflammation (1.6, 1.1, 0.9 for vehicle, OCA, HM15211) and ballooning (1.4, 0.7, 0.0 for vehicle, OCA, HM15211) along with blood ALT (546, 349, 138 U/L for vehicle, OCA, HM15211) even greater than OCA treatment. Notably, while all individuals (7/7) treated with HM15211 achieved NASH resolution criteria, only 14.3% (1/7) achieved it after OCA treatment. Similarly, HM15211 treatment was associated with greater reduction in all subcomponents of NAS when compared to acylated GLP-1 or GLP-1/GIP treatment in AMLN mice.
Conclusion: Considering more benefits of HM15211 over other incretin analogs and FXR agonist for NASH treatment, HM15211 may be a novel therapeutic option for NASH. Efficacy study in biopsy proven NASH patients is ongoing to assess the clinical relevance of these finding.
Disclosure: **J. Choi:** None.

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In patients with fatty liver, higher fasting GLP-1 levels are associated with increased insulin resistance and reduced beta hydroxybutirate

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a spectrum of diseases ranging from simple steatosis to more aggressive forms and is a major risk factor for type 2 diabetes (T2DM). Treatment with glucagon-like peptide 1 (GLP-1) receptor agonists ameliorates NAFLD. However, less is known about GLP-1 levels in NAFLD. Thus, we investigate whether fasting levels of GLP-1 were able to distinguish NAFLD patients with different severity and metabolic phenotypes.
Materials and methods: We studied 40 nondiabetic adults with NAFLD, diagnosed by liver biopsy (n=25) or ultrasound (Table). Besides clinical biochemistry tests (Table) we measured metabolic fluxes by tracer infusion, HOMA-IR, hepatic insulin resistance (Hep-IR = endogenous glucose production•insulin), adipose tissue IR (AT-IR = NEFA•insulin), GLP-1 by ELISA and lipidomic profile by LC-MS.
Results: The cohort comprised two groups one with BMI ≤ 30 and one with BMI ≥ 35 (Table). Despite the remarkable difference in BMI, glucose, GLP-1, lipids, liver enzymes, HOMA-IR and Hep-IR and NAFLD Activity Score (NAS) were not different while insulin and AT-IR were higher in obese (Table). To assess the impact of fasting GLP-1 on disease status, we divided our cohort according to the median values of GLP-1 into a low group (GLP-1 < 57.5 pmol/l) and a high group (GLP-1 ≥ 57.5 pmol/l). The two groups had similar age and BMI (Table 1). However, insulin, liver enzymes, and NAS were significantly increased in the high GLP-1 group as compared to the low GLP-1 group (Table). Compared to the low GLP-1, the high GLP-1 group showed increased NAS and insulin resistance as measured by HOMA-IR, Hep-IR and AT-IR (Table). We assessed the circulating metabolite β-hydroxybutyrate as a marker of hepatic lipid mitochondrial oxidation that was reduced in the high vs. low GLP-1 group (0.06 ± 0.04 vs. 0.11 ± 0.11 $p<0.05$). Lastly, preliminary lipidomic analyses highlighted among the best lipid discriminants the triacylglycerol with saturated and monounsaturated fatty acids this being in agreement with the elevated *de novo* lipogenesis observed in IR.
Conclusion: In NAFLD patients, increased fasting GLP-1 levels associate with a worst metabolic phenotype as shown by higher NAS, circulating liver enzymes, and insulin resistance.

Clinical data	BMI		GLP-1	
	Low	High	Low	High
N (M/F)	11/2	18/8	11/9	18/2
Age (years)	49 ± 13	42 ± 9	43.3 ± 12.8	44.6 ± 10.7
Body mass index (Kg/m2)	26.3 ± 2.1	44.4 ± 5.4***	38.5 ± 9.5	38.0 ± 10.1
Fasting insulin (mU/L)	13.1 ± 5.7	21.7 ± 13.4*	13.0 ± 7.1	24.2 ± 13.6**
Fasting GLP-1 (pmol/L)	62 ± 27	56 ± 20	41 ± 11	75 ± 16***
TAG (mg/dL)	113 ± 40	107 ± 31	101 ± 36	116 ± 32
AST (U/L)	32.9 ± 14.2	27.1 ± 12.1	24 ± 10	34 ± 14***
ALT (U/L)	51.4 ± 27.9	38.4 ± 27.0	27 ± 11	58 ± 30***
HOMA-IR	3.2 ± 1.5	5.0 ± 3.3	3.1 ± 1.6	5.7 ± 3.3**
Hep-IR	6.3 ± 3.8	8.6 ± 6.4	5.0 ± 3.3	10.3 ± 6.4***
AT-IR	8.2 ± 3.7	13.2 ± 7.8*	8.3 ± 5.1	14.4 ± 7.5**
NAS	3.8 ± 1.1	3.2 ± 1.5	2.7 ± 1.3	4 ± 1.0**

Data are mean ± SD. Differences between the two groups were analysed by student t-test and significance accepted at $p < 0.05$. *p<0.05, **p<0.01, ***p<0.001. BMI, Body Mass Index; GLP-1, Glucagon-like peptide 1; NAS, NAFLD Activity Score; IR= insulin resistance; Hep = liver ; AT = adipose tissue; TAG = triglycerides.

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Disclosure: **G. Mocciaro:** None.

OP 33 Diabetic foot problems: from prediction to treatment

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Metabolomic risk predictors of diabetic foot ulcers

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Background and aims: Diabetic foot ulcers (DFUs) are among the most severe complications associated with diabetes. The severity is highlighted by the evidence of increased mortality and morbidity and more than 70% of all amputations being preceded by a DFU. In recent years, the main strategy of dealing with DFUs has shifted towards prevention. Even though prevention has been successful in lowering the incidence of DFUs, the lifetime risk of DFUs for individuals with diabetes is still 15-33% warranting research on risk markers with the potential of predicting DFUs. In this study, we investigated the association between circulating plasma metabolites and DFUs in subjects with type 1 diabetes (T1D).
Materials and methods: Plasma metabolites (n=75) and clinical characteristics from 637 individuals with T1D recruited from Steno Diabetes Center Copenhagen as part of a cross-sectional study were assessed. Baseline characteristics, DFU diagnoses at baseline and longitudinal data on development of DFU were retrieved from electronic patient journals. Associations between single metabolites and DFU were evaluated by linear regression analyses at baseline and by Cox proportional hazards model at follow-up. Models were fitted with and without adjustments (age, gender, body mass index, systolic blood pressure, cholesterol, HbA_{1c}, smoking, statin, triglycerides, eGFR and urinary albumin excretion) and corrected for multiple testing.
Results: Participants had a mean age of 54 (IQR 46, 62) years, 55% were male (n=348), diabetes duration 35 (25, 44) years, HbA_{1c} 64 (56, 72) mmol/mol and eGFR 85 (64, 102) ml/min/1.73m^2. In total 19 participants had DFU at baseline, and a further 108 developed DFU during a median follow-up of 10 years. In the crude model, 11 metabolites at baseline were associated with future risk of DFU. After adjustment, higher levels of ribonic acid exhibited a significantly elevated risk of future DFUs (HR 1.38(1.06-1.8) *p<0.05)*. Figure 1 shows DFU-free survival of participants stratified by ribonic acid at baseline measurement.
Conclusion: In this study, we identified several circulating metabolites associated with future risk of DFU in individuals with T1D. After adjustment for potential confounders and multiple testing, a sugar derivate (i.e. ribonic acid) retained significant association to future development of DFUs. Previous studies have demonstrated that ribonic acid is related to other complications (i.e. kidney disease and retinopathy) and this study adds to theses findings as well as the growing evidence of predicting DFUs via measurements of plasma metabolites.

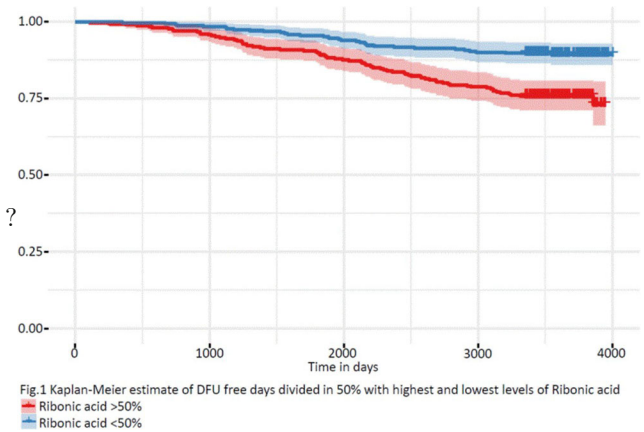

Fig.1 Kaplan-Meier estimate of DFU free days divided in 50% with highest and lowest levels of Ribonic acid

Disclosure: **J. Hedegaard Andersen:** None.

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IDR-1018 peptide improves wound healing in vitro and when topically applied to skin wounds in a model of type 1 diabetes

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Background and aims: The role of skin antimicrobial peptides in wound healing, highly dysregulated in diabetes, is under intense investigation. We aimed to evaluate the impact of synthetic peptide IDR-1018 in wound healing, using an *in vitro* assay followed by pathway analysis of proteomics data. In addition, the effect of topical dermal treatments of IDR-1018 on wound healing kinetics, angiogenesis, and autophagy during wound healing in diabetic mice was evaluated.
Materials and methods: Human keratinocyte cells (HaCaT) were cultured in DMEM medium and Human Umbilical Vein Endothelial cells (HUVEC) were grown in endothelial cell basal medium. For scratch migration assays, cells were stimulated with 25 µg/mL IDR-1018, media, 500 ng/mL EGF, or 25 µg/mL free amino acids. Endothelial tube-formation assay was done to estimate in vitro angiogenesis. As an inflammation model, TNFα stimulated HaCaT cells underwent proteomics followed by pathway analysis for Gene ontology (GO: BP, GO: CC) categories. Diabetes was induced in C57BL/6 mice using low dose streptozotocin injections for 5 consecutive days. Wounds were topically treated with 25 µg, 12.5 µg IDR-1018 or saline. Wound-healing kinetics were evaluated up to day 10. Angiogenesis, inflammation, and autophagy of ubiquitinated cargo in murine diabetic skin were assessed by immunofluorescence for PECAM-1, TNFα and SQSMT1 (p62) in mouse skin sections 10 days after full thickness wounding.
Results: There were no proliferative effects of IDR-1018 on HaCaT nor HUVEC cells. The IDR-1018 treatment had an inhibitory effect on HaCaT cell migration (0.58-fold ± 0.28, NS), whereas the migration of the HUVEC cells was enhanced (2.3-fold ± 1.1, NS). In the tube-formation assay IDR-1018 increased the number of branches (101 branch points ± 13) compared with untreated HUVEC cells (57 branch points ± 14, p<0.0001). TNFα levels in skin were increased by high dose IDR-1018 (2-fold ± 0.6, p<0.05). In terms of biological processes, the majority of significantly altered GOBP categories were related to over-representation of proteins targeting to ER (FDRlog10=4.5) and cellular protein catabolic processes (FDRlog10=4.2) while majority of GO:CC were related to cell-cell (FDRlog10= 2.1) and cell-substrate adherence (FDRlog10=2.8). The high dose of IDR-1018 (25 µg/wound) promoted faster wound closure on days 9 and 10 in mice wounds (23 % ± 14 and 11 % ± 8, p<0.05) compared to saline (43 % ± 17 and 25 % ± 17, p<0.05). The number of blood vessels was elevated with low dose (2.6-fold ± 1.3, p<0.05). Autophagy was elevated in high dose (18±3 cells) compared to low dose (10±1 cells, p<0.01) and compared to saline (13±2 cells, p<0.05).
Conclusion: IDR-1018 peptide promoted angiogenesis in HUVEC cells. Moreover, exogenous delivery of IDR-1018 peptide to wounds accelerated wound closure in diabetic mice, leading to an enhanced vascularization and a decreased autophagy. Our findings suggest that antimicrobial peptide IDR-1018 may improve the healing outcome under diabetic conditions.
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Disclosure: **M. Petkovic:** None.

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Protein tyrosine phosphatase 1B inhibition promotes diabetic wound healing via activation of the antioxidant enzyme heme oxygenase 1

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Background and aims: Diabetic foot ulcers are a leading cause of hospital admissions for people with diabetes in the developed world. Protein tyrosine phosphatase 1B (PTP1B) is a negative regulator of the insulin signaling pathway and it is upregulated in diabetes and diabetic foot ulcers. Evidence has emerged that PTP1B is involved in the pathology of diabetic ulcers which indicates that its inhibition may be a potential therapeutic approach. Our main aim was to evaluate the role of PTP1B inhibition in diabetic wound healing and study the underlying mechanisms.
Materials and methods: Diabetes in male C57BL/6 mice was induced with streptozotocin (STZ), i.p. injections (50 mg/Kg), for 5 consecutive days. After 6 weeks of diabetes, two 6 mm excision wounds per mouse were created in the dorsum and the wounds were treated topically, twice a day, up to day 3 with: vehicle (control); 1 μg of Trodusquemine (PTP1B inhibitor); 1 μg of Protoporphyrin IX zinc (II) (heme oxygenase 1 inhibitor - HO1i) and PTP1Bi+HO1i. The wounds were measured every day up to day 10 post wounding. The skin was collected at day 3 and 10 post wounding. Immunohistochemistry was used for the detection of M1 macrophages, with CD68 and TNF-alpha, or M2 macrophages, with CD68 and CD206. Immunohistochemistry was also used to evaluate the vascularization of the skin with the endothelial cell marker CD31, the proliferation with ki67, and the levels of heme oxygenase 1 (HO1). The levels of PTP1B were measured by western blot and the activity of HO1 was evaluated by the production of bilirubin. The production of reactive oxygen species (ROS) was measured with dihydroethidium (DHE).
Results: PTP1B levels were significantly increased in unwounded (474 ±150 % of control, $p<0.01$) and wounded skin (day 3 post wounding, 398 ±68 % of control, $p<0.01$) of diabetic mice when compared to healthy animals. The treatment with PTP1B inhibitor improved diabetic wound healing progression when compared to non-treated wounds (day 10, 1.1 ±0.2% and 7.2±1.4% of original wound, $p<0.01$). Moreover, PTP1B inhibition decreased the inflammatory environment in the wounds with a decrease in M1/M2 ratio (day 3, 0.7±0.1 and 1.6±0.2, $p<0.01$). In addition, the increase in angiogenesis (147.4±5.5 % of control, $p<0.01$) and cell proliferation (141.1±5.9 % of control, $p<0.01$), after 10 days of wound induction, was also observed. The oxidative stress, in diabetic wounds after 3 days post wounding, was significantly decreased by PTP1B inhibition (68.1±6.1 % of control, $p<0.01$) and the increase in HO1 levels (219.4±20.7 % of control, $p<0.01$) and activity (170.4±15.7 % of control, $p<0.01$) was also observed. These effects of the inhibition of PTP1B were reverted by HO1 inhibition.
Conclusion: PTP1B inhibition promotes wound healing in diabetes with decrease in the inflammatory environment and the oxidative stress which improves the regenerative capacity of the skin with an enhance in angiogenesis and proliferation, through an increase of the antioxidant defense HO1. This study suggests that PTP1B is a target of interest for the treatment of diabetic foot ulcers.
Supported by: Diabetes UK, SPD/GIFT
Disclosure: **E.C. Leal:** None.

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A M1/M2-macrophages-regulating new drug for diabetic foot ulcers with poor-controlled HbA_{1c} risk factor in an International Phase 3 study

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Background and aims: The diabetic foot ulcers (DFUs) has been a life-threatening and complication of diabetes mellitus. DFU chronicity is attributed by the stalled healing progress in inflammation stage due to hyperglycemia triggering overabundance of inflammatory macrophages and the dysregulated M1/M2 macrophages in the lesion. Restoring the balance of M1/M2 macrophages plays a critical role in orchestrating the healing process of DFUs. Recognizing the therapeutic potential by regulating M1/M2-macrophage ratio in DFUs, we aim to evaluate the efficacy and safety of ON101, a topical new drug with M1/M2-macrophage-regulating mechanism versus a hydrocolloid dressing in a multicentre, randomized, controlled, evaluator-blind phase 3 study in 21 clinical/medical centers across the US, China, and Taiwan by following *Guidance for Industry Chronic Cutaneous Ulcer and Burn Wounds - Developing Products for Treatment*, the International Conference on Harmonization guidelines, and investigational new drug programs.
Materials and methods: 236 eligible patients with post-debrided DFUs between 1 - 25 cm^2, present for ≥4 weeks and Wagner Grade 1 or 2, were randomized 1:1 to receive ON101 or the hydrocolloid dressing for up to 16 weeks followed by a 12-week follow-up. Standardized digital photo-taking, study materials, standard of care instruction and blinded evaluation were implemented thoroughly. The primary outcome was incidence of complete healing, defined as complete re-epithelization at 2 consecutive visits during the treatment period assessed by the full analysis set (all participants with post-randomization data collected). The secondary outcome was time to complete ulcer healing. Safety outcomes included assessment of the incidence of adverse events, clinical laboratory values, and vital signs.
Results: Baseline demography and medical history between the two groups were well balanced. The incidence of complete healing as the primary endpoint in overall group in full analysis set was 60.7% by ON101 and 35.1% by the comparator during the treatment period ($p=0.0001$). Subgroup analysis on the primary efficacy variable was also conducted on DFU patients with poor-controlled baseline HbA_{1c} risk factor (defined □9% according to definition of ADA) and found a significant odds ratios in favor of the ON101 group ($p=0.0353$). Time to complete ulcer healing as the secondary endpoint was noted faster in the ON101 group ($p=0.002$). No clinically significant changes or differences between 2 treatment groups in hematology, biochemistry (including HbA_{1c} and fasting glucose), or vital signs. Related treatment of emergent adverse events was reported in 7 patients (5.7%) in the ON101 group and 5 (4.4%) from the comparator group. None of the serious adverse events was related to study drug while there was a case of osteomyelitis reported to be related to the comparator.
Conclusion: Hyperglycemia is an underlying cause of the chronicity of DFUs where M1-to-M2 macrophage transition delays and inflammation stage is prolonged. ON101, recently approved by Taiwan Food and Drug Administration, has been shown with clinically robust efficacy and well-tolerated safety profile in overall DFUs, as well as in DFU patients with poor glycemic control. This indicates that ON101 provides not only a new prospect in DFU management but a differentiated approach towards active-healing.
Clinical Trial Registration Number: NCT01898923
Supported by: Trial sponsorship by Oneness Biotech Company Limited
Disclosure: **M. Kuo:** Employment/Consultancy; Oneness Biotech Company Limited.

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Long-term outcomes of autologous cell therapy, angioplasty and conservative therapy in patients with chronic limb-threatening ischaemia and diabetes

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Background and aims: Long-term clinical outcomes of revascularization, especially by autologous cell therapy (ACT), in diabetic patients

with chronic limb-threatening ischemia (CLTI) remain unclear. The aim of our study was to compare the mortality and amputation rates of patients with diabetic foot (DF) and CLTI treated by ACT with patients treated by repeated percutaneous transluminal angioplasty (re-PTA) and patients treated conservatively.

Materials and methods: One-hundred and thirty patients with DF and CLTI (defined as transcutaneous oxygen pressure - $TcPO_2$ < 30 mmHg after unsuccessful standard revascularization) treated in our foot clinic over 7 years were enrolled into the study. Forty-five patients were treated by ACT, 43 patients underwent re-PTA and 42 patients were treated conservatively and formed the control group. Mortality and major amputation rate were assessed over a 5-year follow-up period.

Results: Patients in all groups did not differ significantly in demographic characteristics. The frequency of comorbidities (hypertension, ischemic heart disease, end-stage kidney disease) also did not differ significantly among the groups. Patients in ACT and control groups had significantly more severe angiographic findings according to Graziani classification than the re-PTA group (5.0±0.9 and 5.1±0.8 vs. 3.5±1.1, p<0.001), but there were no differences in baseline values of $TcPO_2$ among all groups. The rate of major amputation after 5 years was significantly lower in ACT and re-PTA groups in comparison with control group (28.9% and 20.9% vs. 64.2%, p=0.011 and 0.002 respectively). There was a trend to lower mortality in ACT group and significantly lower mortality in re-PTA group in comparison with control group (35.6% and 25.6% vs. 61.9%, p=0.09 and 0.012 respectively).

Conclusion: Our study showed significantly lower long-term amputation rate and increased survival in patients treated by ACT and re-PTA in contrast to patients treated conservatively. Bone-marrow derived autologous cell therapy is a promising method for the treatment of CLTI in diabetic patients, comparable with re-PTA, even in patients with no-option CLTI.

Supported by: the Ministry of Health of the Czech Republic, grant no. 00023001

Disclosure: **M. Dubsky:** None.

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Diabetes is not associated with major amputation after open vascular surgery for chronic limb-threatening ischaemia: a nationwide propensity score analysis

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Background and aims: The risk of major amputation is higher after urgently planned endovascular therapy for chronic limb-threatening ischemia (CLTI) in patients with diabetes mellitus (DM). The aim of this nationwide cohort study was to compare outcomes between patients with and without DM following urgently planned open revascularization for CLTI from 2010 to 2014.

Materials and methods: Out of 1537 individuals registered in the Swedish Vascular Registry, 569 were registered in the National Diabetes Register. A propensity score adjusted Cox regression analysis was conducted to compare outcome between the groups with and without DM. Median follow-up was 4.3 years and 4.5 years for patients with and without DM, respectively.

Results: Patients with DM more often had foot ulcers (p=0.034) and had undergone more previous amputations (p=0.001) at baseline. No differences in mortality, cardiovascular death, major adverse cardiovascular events (MACE), or major amputation were observed between groups. The incidence rate of stroke was 70% higher (95% CI 1.11-2.59; p=0.0137) and the incidence rate of AMI 39% higher (95% CI 1.00-1.92; p=0.0472) among patients with DM in comparison to those without.

Conclusion: Open vascular surgery remains a first-line option for a substantial part of patients with CLTI, especially for limb salvage in patients with DM. The higher incidence rate of stroke and AMI among patients with DM following open vascular surgery for infrainguinal CLTI require specific consideration preoperatively with the aim of optimizing medical treatment to improve cardiovascular outcome postoperatively.

Supported by: Region Skåne, Hulda Almroth foundation, ALF

Disclosure: **E. Lilja:** None.

OP 34 SGLT2 inhibition: putative mechanisms of benefit

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The SGLT2 inhibitor ertugliflozin causes a switch of cardiac substrate utilisation leading to reduced cardiac mTOR-signalling, unfolded protein response and apoptosis

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Background and aims: SGLT2 inhibitors reduce hospitalization for heart failure in patients with and without diabetes. The underlying mechanisms remain incompletely understood but might relate to the induction of a fasting like response with low blood glucose and insulin levels and increased ketone bodies. The study aimed to investigate underlying signaling pathways.
Materials and methods: Cardiac hypertrophy was induced by transverse aortic constriction (TAC) surgery in 20-week-old C57Bl/6J mice. Mice were treated with the SGLT2 inhibitor ertugliflozin (225 mg/kg chow diet) or vehicle for a period of 10 weeks.
Results: Ertugliflozin significantly improved left ventricular systolic and diastolic function (dp/dt_{max} $p<0.001$ and dp/dt_{min} $p<0.01$; by millar catheter with dobutamine stress) and reduced myocardial fibrosis ($p=0.17$) and hypertrophy ($p=0.09$). This was paralleled by the expected fasting like response with lower glucose and insulin levels (HOMA-IR $p<0.05$) and increased ketone body concentrations ($p<0.05$). As a consequence cardiac insulin signaling (AKT-phosphorylation Thr(308) was reduced ($p<0.01$) by ertugliflozin with less insulin-dependent glucose transporter GLUT4 expression ($p<0.05$) while fatty acid transporter CD36 ($p<0.001$) and the ketone body catabolizing key enzyme beta-hydroxybutyrate dehydrogenase BDH-1 were increased ($p<0.01$) in addition to AMPK-signaling ($p<0.01$). This led to downstream inhibition of the mTOR pathway with reduced p70S6K ($p<0.05$)-, 4E-BP1 ($p<0.05$)- and ULK1 ($p<0.01$)-phosphorylation. MTOR signaling critically mediates cardiac hypertrophy, endoplasmic reticulum stress, unfolded protein response (UPR) and adverse cardiac remodeling. Consistently, we found ertugliflozin to reduce ATF6 ($p<0.05$) and elf2α phosphorylation ($p=0.0611$) as well as downstream signaling (ATF4 $p<0.01$; CHOP $p<0.001$). This let to reduced caspase 3 ($p<0.05$), collagen I ($p<0.01$) and IL-1b expression indicating less apoptosis, fibrosis and left ventricular remodeling with consequential reduction of BNP expression ($p<0.001$) in response to SGLT2 inhibition.
Conclusion: The SGLT2 inhibitor ertugliflozin improves left ventricular function in a murine model of cardiac hypertrophy. Mechanistically, this was associated with a metabolic switch of cardiac substrate utilization with reduced cardiac insulin- and increased cardiac AMPK-signaling leading to reduced cardiac mTOR-signaling, unfolded protein response and apoptosis.

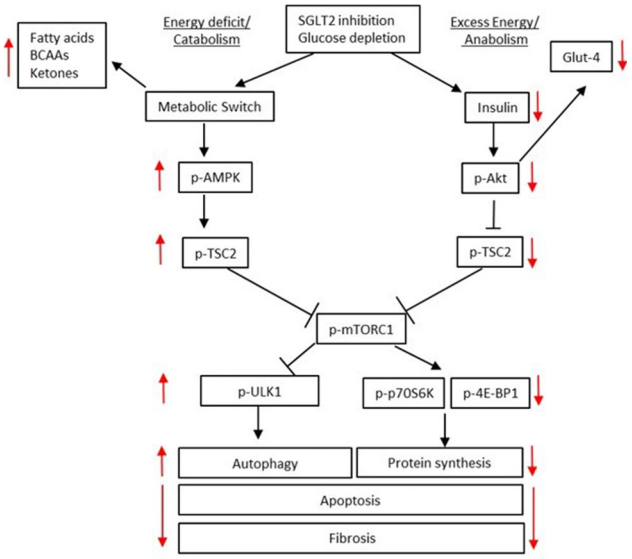

Fig.1: schematic illustration of the signaling pathway.

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Disclosure: **P.A. Mann:** Grants; Michael Lehrke from MSD. Lecture/ other fees; Michael Lehrke received fees from MSD.

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Empagliflozin induced white adipocyte browning and modulated mitochondrial dynamics in KK Cg-Ay/J mice and mouse adipocytes

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Background and aims: White adipose tissue (WAT) browning is a promising target to prevent and/or treat obesity. Empagliflozin has emerged as an agent with weight-loss potential in clinical and in vivo studies, but the mechanisms underlying its effect are not fully understood. Here, we investigated whether empagliflozin could induce WAT browning and mitochondrial alterations in KK Cg-Ay/J(KKAy) mice and explored the relevant mechanisms involved on its effects.
Materials and methods: Eight-week-old male KKAy mice were administered empagliflozin or saline for 8 weeks and compared with control C57BL/6J mice. Mature 3T3-L1 adipocytes were treated in the presence or absence of empagliflozin. Mitochondrial biosynthesis, dynamics, and function were evaluated by gene expression analyses, fluorescence microscopy, and enzymatic assays. The roles of adenosine monophosphate-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor-γ coactivator-1-alpha (PGC-1α) were determined through AICAR (5-Aminoimidazole-4-carboxamide1-β-D-ribofuranoside)/Compound C and RNA interference, respectively.
Results: Empagliflozin substantially reduced the bodyweight of KKAy mice. Mice treated with empagliflozin exhibited elevated cold-induced thermogenesis and higher expression of uncoupling protein 1 (UCP1) and other brown adipose tissue signature proteins in epididymal and perirenal WAT, which was indicative of browning in these WAT depots. At the same time, empagliflozin enhanced fusion protein mitofusin 2 (MFN2) expression, while decreasing the fission markers phosphorylated dynamin-related protein 1(Ser616) (pDRP1(Ser616)) in epididymal WAT and perirenal WAT. Empagliflozin also increased mitochondrial biogenesis and fusion, improved mitochondrial integrity and function,

and promoted browning in 3T3-L1 adipocytes. Furthermore, we found that AMPK signaling activity was indispensable for empagliflozin-induced browning and mitochondrial biogenesis and that PGC-1α was required for empagliflozin-induced fusion.
Conclusion: Our results suggest that empagliflozin is a promising anti-obesity treatment, whose activity made immediate through WAT browning, mitochondrial biogenesis, and regulated mitochondrial dynamics.
Supported by: This work was supported by the National Natural Science Foundation of China (no. 81970697, 81470187) and a grant from the Natural Science Foundation of Tianjin (no. 18JCYBJC26100 and 18JCZDJC35500).
Disclosure: **L. Chen:** None.

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SGLT2 inhibition improves beta cell function and glucose tolerance, but does not affect glucose or FFA uptake in skeletal muscle

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Background and aims: SGLT2 inhibition induces an insulin-independent lowering of plasma glucose. This leads to reduced insulin levels and an increase in circulating free fatty acids (FFA). Isolated elevation of FFA *reduces* insulin sensitivity in skeletal muscle, whereas SGLT2 inhibition *improves* insulin sensitivity, presumably by a reduction in glucotoxicity. This apparent paradox could be explained by a reduction in glucotoxicity, but other factors relating to skeletal muscle substrate utilization may contribute to improvement in insulin sensitivity. Therefore, we aimed to investigate the effects of SGLT2 inhibition on glucose metabolism and skeletal muscle substrate metabolism. We measured the effects of SGLT2 inhibition on beta cell function and glucose tolerance together with substrate utilization and intracellular signalling in skeletal muscle.
Materials and methods: 13 metformin-treated individuals with type 2 diabetes were randomized to once-daily empagliflozin 25 mg or placebo for four weeks in a crossover design with a one-week wash-out between the two treatment periods. At the end of each treatment period participants were studied after an overnight fast.Insulin secretion and action were measured using the oral minimal model. Skeletal muscle glucose and FFA uptake was measured using ^{18}F-FDG PET/CT and ^{11}C-palmitate PET/CT, respectively. Regulation of intracellular signalling pathways involved in lipid and glucose metabolism were measured in biopsies from the vastus lateralis muscle.
Results: Four weeks of empagliflozin treatment increased total beta cell responsivity (ϕ) [20 ± 8 vs. 14 ± 9 10^{-9}min^{-1} ($p < 0.01$)]. In addition, empagliflozin improved glucose tolerance [S_I: [9 (IQR: 10.7) vs. 3.28 (IQR: 4.82) 10^{-4} dL/kg/min per µmol/mL ($p < 0.01$)], glucose effectiveness [GE: 2.6x10^{-2} ± 2.9x10^{-3} vs. 2.4 x10^{-2} ± 2.8x10^{-3} dL/kg/min, (p=0.02)], and the disposition index (DI_{total}) [275 (IQR: 187) vs. 65.6 (IQR: 54.4) 10^{-14} dL/kg/min^2 per pmol/L ($p < 0.01$)]. Empagliflozin did not affect FFA or glucose uptake in skeletal muscle. Protein content of AKT, GLUT4, CD36 and HK-II was unaffected by empagliflozin and no effect was observed on skeletal muscle lipoprotein lipase activity.
Conclusion: 4 weeks of empagliflozin treatment improves beta-cell function. Glucose tolerance is also improved, at least in part by the insulin-independent loss of glucose in the urine. Empagliflozin does not appear to have major effects on glucose and FFA uptake or intracellular pathways involved in substrate uptake and insulin signalling in skeletal muscle in the postabsorptive state.
Clinical Trial Registration Number: EUDRA-CT nr: 2017-001779-22
Disclosure: **J.H. Voigt:** None.

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Urinary proteomics and the effect of dapagliflozin treatment in persons with type 2 diabetes and diabetic kidney disease: a randomised crossover trial

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Background and aims: Nearly 40% of persons with type 2 diabetes develop diabetic kidney disease which places a major economic burden on modern health care systems. Sodium-Glucose-Co-Transporter-2 inhibitors (SGLT2i) have emerged as a novel way to treat persons with type 2 diabetes and diabetic kidney disease. However, the mechanisms by which SGLT2i work, remain partly unclear. The current study aims to better understand SGTL2i function by observing the changes induced by SGLT2i treatment in the urinary proteome.
Materials and methods: The study is a double-blinded, randomized, placebo-controlled, crossover trial in which 32 participants with type 2 diabetes and diabetic kidney disease were treated with 10 mg of dapagliflozin for 12 weeks or matching placebo on top of standard treatment. All participants had albuminuria (Urine Albumin-to-Creatinine Ratio ≥ 30 mg/g) and received Renin-angiotensin-aldosterone system blockade treatment. The urinary proteomics data from after placebo and dapagliflozin periods were analyzed using Wilcoxon signed-rank test adjusted for multiple testing using Benjamini-Hochberg method. All results with $p < 0.05$ were considered statistically significant.
Results: The participants had a mean (SD) age of 63 (8) years, 88% males, diabetes duration 15.9 (4.7) years, BMI 33.7 (5.4) kg/m^2, HbA1c 10.8 (3.2)%, median (IQR) Urine Albumin-to-Creatinine Ratio 154 (94-329), eGFR 85.5(19.1) ml/min/m^2, respectively. 36 urinary peptide fragments belonging to 18 unique proteins changed significantly due to dapagliflozin, of which 24 decreased and 12 increased multifold. Concentrations of albumin and alpha-1-antitrypsin fragments decreased, while collagen alpha-1 (I) & (II) and collagen alpha-3 (I) fragments increased, as expected and in confirmation with previous findings. Decreases were also seen in the concentrations of alpha-1-glycoprotein, cornulin, and peptidase inhibitor 16 fragments, while increases were seen in keratin (type II) cytoskeletal 1, apolipoprotein C-III, and E3 ubiquitin-protein ligase fragments. All of the changes except the changes in two of the collagen alpha-1(III) chain fragments were observed when the urinary proteome of 50 healthy controls was compared to that of 110 participants with diabetic kidney disease and type 1 diabetes from an independent cohort, further supporting the role of these peptides in the development of diabetic kidney disease.
Conclusion: We identified and validated differential urinary peptide patterns in response to SGLT2i (dapagliflozin) on individuals with type 2 diabetes and diabetic kidney disease. The findings highlight the potential renoprotective effects of SGLT2i and help elucidate the response pathways involved in SGLT2i treatment.
Clinical Trial Registration Number: NCT02914691
Supported by: AZ supports the study by a research grant
Disclosure: **T.K.E. Rönkkö:** Grants; Research grant from AstraZeneca.

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Effect of SGLT2 inhibition on ketone bodies in patients with stable chronic heart failure

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Background and aims: Recent studies indicate that sodium glucose cotransporter (SGLT)-2 inhibition increases levels of ketone bodies in the blood in patients with type 1 and 2 diabetes. Other studies suggest that in patients with chronic heart failure (CHF), increased myocardial oxygen demand can be provided by ketone bodies as a fuel substrate. Experimental studies report that ketone bodies, specifically beta-hydroxybutyrate (β-OHB) may increase blood pressure by impairing endothelium-dependant relaxation, thereby leading to increased vascular stiffness. In our study we assessed whether the SGLT 2 inhibition with empagliflozin increases ketone bodies in patients with stable CHF and whether such an increase alters blood pressure and vascular function.
Materials and methods: In a prospective, double blind, placebo controlled, parallel-group single centre study 71 patients (67.6 ± 8.9 years) with CHF (left ventricular ejection fraction 39.0 ± 8.2 %) were randomized (2:1) to the SGLT-2 inhibitor empagliflozin 10mg orally once daily or to placebo. After a run-in phase off any SGLT-2 inhibitors for at least 10 weeks, we evaluated blood pressure with a 24h ambulatory blood pressure (ABP) monitoring device, vascular stiffness parameters by the SphygmoCor system (AtCor Medical, Sydney, NSW, Australia) and fasting metabolic parameters, including β-OHB by an enzymatic assay (Beckman Coulter DxC 700 AU). The same measurements were repeated 12 weeks after treatment. In 21 of the 74 patients levels of β-OHB were beneath the lower border of our assay (<0.05mmol/l) therefore being excluded from the subsequent analysis.
Results: In patients with stable CHF, treatment with empagliflozin (n=36) was followed by an increase of β-OHB by approximately 33 % (p=0.017), as well as a significant fall in 24 h systolic (p=0.038) and diastolic (p=0.003) ABP, weight reduction (p=0.003) and improvement of central systolic blood pressure (p=0.008) and central pulse pressure (p=0.008). The increase in β-OHB was related to a lower decrease of empagliflozin-induced 24h systolic (r=0.321, p=0.069) and diastolic (r=0.516, p=0.002) ABP and less improvement of central systolic blood pressure (r=0.470, p=0.009) and central pulse pressure (r=0.391, p=0.033). No significant changes were seen in any of these parameters in the placebo group (n=17).
Conclusion: In patients with stable CHF ketone bodies as assessed by β-OHB increased after treatment with empagliflozin. This increase led to an attenuation of the beneficial effects of empagliflozin on blood pressure and vascular parameters.

	β-OHB	24h systolic ABPM	24h diastolic ABPM	cSBP	cPP
Baseline	0.12 ± 0.22 mmol/l	120.78 ± 16.23 mmHg	72.19 ± 9.38 mmHg	119.08 ± 15.23 mmHg	42.67 ± 9.07 mmHg
12 weeks	0.16 ± 0.18 mmol/l	117.46 ± 11.97 mmHg	70.91 ± 7.56 mmHg	112.69 ± 10.52 mmHg	38.50 ± 7.65 mmHg
p-value	0.017	0.038	0.003	0.008	< 0.001

Clinical Trial Registration Number: NCT03128528
Supported by: Boehringer Ingelheim
Disclosure: **R. Pietschner:** Grants; supported by Boehringer Ingelheim.

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Effects of SGLT2 inhibition on lipid storage and lipolysis in adipose tissue in type 2 diabetes

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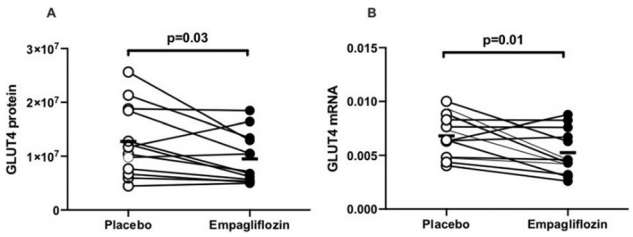
Background and aims: SGLT2 inhibition induces an insulin-independent reduction in plasma glucose causing increased lipolysis and subsequent lipid oxidation by energy-consuming tissues. However, it is unknown whether SGLT2 inhibition also affects lipid storage in adipose tissue.The objective of this study was to elucidate the effects of SGLT2 inhibition on the balance between storage and release of fatty acids in adipose tissue.
Materials and methods: 13 individuals aged 50-70 years with type 2 diabetes treated with metformin were investigated in a randomized, double-blind, placebo-controlled crossover trial after a four-week intervention period with once daily empagliflozin 25 mg and placebo.The main outcome measures were adipose tissue fatty acid uptake, lipolysis rate and clearance measured by ^{11}C-palmitate PET/CT and adipose tissue glucose uptake measured by ^{18}F-FDG PET/CT. Protein and gene expression of pathways involved in lipid storage and lipolysis were measured in biopsies of abdominal subcutaneous adipose tissue.
Results: Subjects were weight stable during the study (94.6±9.6 vs. 95.2±9.7 kg, p=0.15). SGLT2 inhibition did not affect FFA uptake in abdominal subcutaneous adipose tissue (0.31±0.23 vs. 0.25±0.15 µmol/100g/min, p=0.32), but increased FFA uptake in visceral adipose tissue by 27% (p<0.05). Both the lipolysis and FFA clearance rate remained unaffected by SGLT2 inhibition. Interestingly, SGLT2 inhibition reduced GLUT4 protein ($9.51x10^6$±$4.58x10^6$ vs. $12.8x10^6$±$6.46x10^6$ arbitrary units (AU), p=0.03) and mRNA content (0.49 (95% CI 0.36-0.62) vs. 0.66 (95% CI 0.55-0.79) AU, p=0.01) in abdominal subcutaneous adipose tissue (figure). However, adipose tissue glucose uptake was not different in subcutaneous (0.35±0.36 vs. 0.77±0.88 µmol/g/min, p=0.16) or visceral adipose tissue (1.08±1.03 vs. 0.99±0.87 µmol/g/min, p=0.79) after SGLT2 inhibition compared to placebo.
Conclusion: SGLT2 inhibition reduces GLUT4 gene and protein expression in abdominal subcutaneous adipose tissue, indicating a reduced channeling of substrate for lipid storage.



Clinical Trial Registration Number: EudraCT nr.: 2017-001779-22
Supported by: The Novo Nordisk Foundation, the Danish Diabetes Academy supported by the Novo Nordisk Foundation, the Health Research Fund of Central Denmark Region and Steno Diabetes Center Aarhus.
Disclosure: **K.M. Lauritsen:** None.

OP 35 Omics and more for type 2 diabetes and complications

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CpG sites associated with insulin resistance and related novel variants suggest a possible mechanism linking insulin resistance and cardiometabolic traits

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Background and aims: Insulin resistance increases during pregnancy and reaches similar levels seen in type 2 diabetes (T2D), providing a valuable T2D model. We aimed to identify methylation patterns in CpG sites related to insulin resistance, explore their associations with genetic variants, and the relationship of these variants with cardiometabolic traits.

Materials and methods: In EPIPREG, 294 European (EUR) and 162 South Asian (SA) pregnant women had both DNA methylation (EPIC beadchip) and HOMA-IR data at gestational week 28±2. We performed an EWAS of HOMA-IR using linear mixed models, adjusted for age, smoking, cell composition, and using ethnicity as a random effect, accepting a 5% false discovery rate (FDR). We performed methylation quantitative trait loci (mQTL) analysis in cis (1Mb) and trans using imputed genotypes (EUR=187 and SA=135). The mQTLs were performed separately in EUR and SA for each of the six CpG sites. We used linear models adjusted for age, smoking and cell composition as covariates, p<5e-08 was considered significant. Next, we selected specific mQTLs to test for association with HOMA-IR and other cardiovascular related phenotypes using simple linear models. The selection was done as follows: For the CpG sites that showed mQTLs in both ethnic groups, the common SNP with the smallest p-value in both ethnicities was chosen, if there were no common mQTLs between CpG sites across ethnicities; we selected the most significant SNP from each ethnic group.

Results: Six CpG sites were inversely associated with HOMA-IR (Table 1). The results persisted after adjustment for BMI. We observed mQTLs for 5/6 CpG sites in EUR and 3/6 in SA (Table1). In EUR, rs16893889 was positively associated with HOMAI-IR (p=0.039), Gestational diabetes mellitus (GDM) (p=0.048), and both systolic (p=0.001) and diastolic blood pressure (p=0.001). Both rs34964576 and rs1108902 were negatively associated with HOMA-IR (p=0.039 and p=0.039, respectively) and Fasting glucose (p=0.020 and p=0.043, respectively). rs7006759 showed positive associations with HOMA-IR (p=8.46e-06), fasting glucose (p=0.015), GDM (p=0.012), BMI (p=0.002), systolic (p=0.004) and diastolic (p=0.002) blood pressure. In SA, rs16893889 was positively associated with HOMA-IR (p=0.042), rs1108902 with diastolic blood pressure (p=0.030), and rs6641912 with HOMA-IR (p=0.044) and HbA1c (0.043).

Conclusion: We discovered six CpG sites associated with insulin resistance, independently of BMI. The mQTLs were associated with HOMA-IR and other related variables, suggesting that insulin resistance and related traits may be explained by an interplay between genotype and DNA methylation.

Table 1: Summary of the EWAS, mQTL and SNP-phenotype association analyses.

CpG Information			mQTL analysis results				SNPs tested for association*		
ID	Gene	Associated trait/function of the gene	cis-mQTL (EUR)	cis-mQTL (SA)	trans-mQTL (EUR)	trans-mQTL (SA)	SNP ID	Gene	Associated trait/function of the gene
cg02988288	*TXNIP*	Type 2 diabetes	0	0	34	0	rs34964576 (T/C)[2]	*SLC2A1*	Glucose transport
cg19693031	*TXNIP*	Type 2 diabetes	0	0	28	35	rs1108902 (G/A)[1]	*SLC2A1*	Glucose transport
cg26974062	*TXNIP*	Type 2 diabetes	0	0	24	1	rs34964576 (T/C)[2]	*SLC2A1*	Glucose transport
							rs6641912 (A/C)[3]	*N/A*	Not known
cg04861640	*ZNF187*	Diabetic nephropathy	186	272	0	0	rs16893889 (A/G)[1]	*ZNF187*	Diabetic nephropathy
cg06690548	*SLC7A11*	Fasting Insulin	0	0	35	0	rs7006759 (T/C)[2]	*PSD3*	Type 2 diabetes
cg10318066	N/A	Not reported	0	0	0	0	N/A	N/A	N/A

*For the CpG sites that showed mQTLs in both ethnic groups, we tested the common SNP with the smallest p-value in both ethnicities, if there were not common mQTL between CpG sites across ethnicities, we took the most significant SNP from each ethnic group. [1]Common SNP in SA and EUR, [2]Most significant SNP in EUR, [3]Most significant SNP in SA.

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Novel biomarkers for glycaemic deterioration in type 2 diabetes: an IMI RHAPSODY study

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Background and aims: Type 2 diabetes is a progressive multifactorial disease which presently affects >400m worldwide, with numbers expected to increase to >700 m by 2045. Biomarkers for the disease, which provide a deeper understanding of the disease process, are therefore eagerly sought.

Materials and methods: We have deployed a multi-omics approach in large cohorts of patients with existing type 2 diabetes to identify biomarkers for disease progression across three molecular classes, metabolites, lipids and proteins.

Results: A Cox regression analysis for association with time to insulin requirement in 2,973 patients in the DCS, ANDIS and GoDARTS cohorts identified homocitrulline, isoleucine and 2-aminoadipic acid, as well as the bile acids glycocholic and taurocholic acids, as predictive of more rapid deterioration. Increased levels of eight triacylglycerol species, and lowered levels of the sphingomyelin SM 42:2;2 were also predictive of disease progression. Of ~1,300 proteins examined in two cohorts, levels of GDF-15/MIC1, IL-18RA, CRELD1, NogoR, FAS, and ENPP7 were associated with faster progression, whilst SMAC/DIABLO, COTL1, SPOCK1 and HEMK2 predicted lower progression rates. Strikingly, identified proteins and lipids were also associated with diabetes incidence and prevalence in external replication cohorts. Implicating roles in disease compensation, NogoR/RTN4R improved glucose tolerance in high fat-fed mice and tended to improved insulin signalling in liver cells whilst IL-18R antagonised inflammatory IL-18 signalling towards nuclear factor kappa-B *in vitro*. Conversely, high NogoR levels led to islet cell apoptosis.

Conclusion: This comprehensive, multi-disciplinary approach thus identifies novel biomarkers with potential prognostic utility, provides evidence for new disease mechanisms, and identifies potential therapeutic avenues to slow diabetes progression.

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Mirror effects of rare OPRD1 variants on the aetiology of type 2 diabetes and obesity

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Background and aims: Opioid consumption (i.e. opium or heroin) affects metabolic homeostasis with contradictory effects (i.e. by decreasing adiposity, but increasing hyperglycemia), but the mechanisms linking opioid consumption and metabolic alterations are unknown. In rodents, the δ opioid receptor (DOP), encoded by *OPRD1*, is mainly expressed in the central nervous system, although in humans, RNA-seq analyses demonstrated that *OPRD1*, in contrast to other opioid receptor genes, is also expressed in pancreatic islets and β cells. Importantly, DOP as an inhibitory G-protein coupled receptor is a privileged drug target. Here, we aimed to perform large-scale functional genetics to decipher the putative link between *OPRD1* mutations and metabolic disorders.
Materials and methods: *OPRD1* was sequenced in 6,971 adult participants. To assess the functional activity of each variant, we performed SRE-luciferase assays on HEK293 cells overexpressing each variant, followed by DOP activation by its agonists deltorphin II (DII) or DPDPE during 5 hours. In parallel, we performed western blots and immunofluorescence assays on these cells to assess expression and cellular localization of each mutant. We then performed association studies between each cluster of rare gain- (GoF) or loss-of-function (LoF) variants, and various metabolic traits assessed in our cohort. We also performed association analyses between the frequent GoF variant encoding p.I52V and metabolic traits in 34,812 individuals from the AMP T2D Knowledge Portal. Finally, in human pancreatic EndoCβH1 cells, we performed a glucose-stimulated insulin secretion assay coupled to DOP activation by DII and *OPRD1* overexpression.
Results: We identified 31 rare variants (minor allele frequency [MAF] < 1%) and 3 frequent variants (MAF ≥ 1%). Through luciferase assays, we identified 7 GoF variants increasing DOP activity in response to both agonists, including the frequent p.I52V variant, and 12 rare LoF variants decreasing it. Immunofluorescence assays showed that all the mutants were effectively expressed and localized at the plasma membrane, except for two LoF mutants (p.P14R and p.G36E). Western blots showed that these mutants tended to have a lower expression than wild-type DOP, while the other DOP mutants were expressed at the same magnitude as wild-type DOP. Association analyses highlighted that LoF variants were associated with increased overweight/obesity risk (P=0.0054; OR=11) but decreased hyperglycemia risk (P=0.054; OR=0.23), whereas GoF variants were associated with improved lipid metabolism. Association analyses performed in the AMP T2D Knowledge Portal confirmed this mirror effect, as the p.I52V variant that is only frequent in Africans, associates with increased type 2 diabetes risk (P=3.6×10^{-6}; OR=2), but also with lower body mass index (P=0.0038; β=-0.37±0.18), and improved lipid metabolism. Finally, in EndoCβH1 cells overexpressing *OPRD1* (as wild-type cells poorly expressed *OPRD1*), DOP agonist DII significantly inhibits glucose-stimulated insulin secretion.
Conclusion: This study highlights DOP as a major metabolic link between opioids and type 2 diabetes. DOP agonists and/or antagonists should be considered as new tools to improve metabolic homeostasis.
Disclosure: **S. Meulebrouck:** None.

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Multi-phenotype association analysis reveals shared biological pathways between type 2 diabetes and depression

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Background and aims: Type 2 diabetes (T2D) and depression are two common multi-factorial diseases with a growing global disease burden. Epidemiological evidence suggests a bi-directional relationship between the two. Furthermore, genome-wide association analysis (GWAS) results of the two diseases show a positive genetic correlation between T2D and depression. Various hypotheses suggest common pathways involved in the shared predisposition to T2D and depression, however, we are currently unable to adequately explain their shared pathophysiology.
Materials and methods: Using the UK Biobank, we employed the MTAG software to perform a multi-phenotype GWAS (MP-GWAS) of T2D and depressive phenotypes to increase the power for loci identification, improve effect size estimates and provide suggestions for multi-phenotype effects, such as pleiotropy. We compared two diagnostic criteria for depression: clinically diagnosed major depressive disorder (MDD) (n = 91,796; 5,357 cases, 86,439 controls) and depressive symptoms based on self-report assessed using the PHQ-9 questionnaire (n = 153,567) and evaluated how each performs in a MP-GWAS with T2D (n = 482,985; 19,344 cases, 463,641 controls). We applied on the results functional transcriptome-wide analysis (TWAS) based on various tissue prediction models in the GTEx database.
Results: In single-phenotype GWAS of T2D, depressive symptoms and MDD in the UK Biobank we identified 92, three and zero genome-wide significant loci (P-value=5x10^{-8}) respectively. In contrast, MP-GWAS increased the number of significantly associated loci for depressive symptoms from three to eight, while none were identified for MDD in MP-GWAS. Seven of the eight loci identified for depressive symptoms after MP-GWAS were established T2D loci including *TCF7L2, CDKAL1* and *IGF2BP2*. Moreover, the risk alleles at these shared loci were the same for T2D and depressive symptoms. TWAS results revealed enrichment of significant gene associations in the same tissues for depressive symptoms and T2D including the frontal cortex (*CDKAL1*), whole blood (*HLA-DRB1*), adipose subcutaneous tissue (*IRS1*), and muscle (*HLA-DRA*). These genes are involved in immune system and glucose-insulin signalling pathways, important for the shared pathogenesis of T2D and depression.
Conclusion: In this first large-scale multi-phenotype of T2D and depressive phenotypes, we show that depressive symptoms based on self-report perform better than MDD in identification of potential multi-phenotype effects with T2D. The shared loci between depressive symptoms and T2D support a role of insulin function and signalling and, immune system pathways in the pathophysiology of T2D and depression boosting our understanding of their shared pathogenesis.
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Serum magnesium is inversely associated with heart failure, atrial fibrillation and microvascular complications in type 2 diabetes

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Background and aims: In this study, we investigated whether serum magnesium (Mg^{2+}) was prospectively associated with macro- or microvascular complications in people with T2D, and whether this association was mediated by glycemic control (HbA_{1c}).
Materials and methods: This study was performed in 4,348 participants of the Diabetes Care System cohort, a prospective cohort of people with T2D. We analyzed the association of serum Mg^{2+} per 0.1 mmol/L increment with macrovascular disease and mortality (acute myocardial infarction (AMI) (n=91), coronary heart disease (CHD) (n=169), heart failure (HF) (n=155), cerebrovascular accident (CVA) (n=118), peripheral arterial disease (PAD) (n=113)), atrial fibrillation (AF) (n=157), and microvascular complications (n=1,285) (chronic kidney disease (CKD) (n=1,093), diabetic retinopathy (n=114) and diabetic foot complications (n=1,147) using Cox regression, adjusted for confounders. Mediation analysis was carried out to assess whether glycemic control mediated these associations.
Results: The average baseline serum Mg^{2+} was 0.80 ± 0.08 mmol/L, 9.1% of the participants had hypomagnesemia (<0.7 mmol/L Mg^{2+}). Adjusted for cardiovascular confounders, serum Mg^{2+} was during 6.2 years of follow-up prospectively, inversely, associated with HF 0.76 (95% CI: 0.62; 0.93) and AF 0.59 (95% CI: 0.49; 0.72). The association of serum Mg^{2+} with AF was independent of fatal and non-fatal HF. The associations with AMI, CHD, CVA and PAD were not significant. Serum Mg^{2+} was during 5.1 years of follow-up prospectively, inversely, associated with overall microvascular events with a hazard ratio of 0.85 (95% CI: 0.78; 0.91), 0.89 (95% CI: 0.82; 0.96) for CKD, 0.77 (95% CI: 0.61; 0.98) for diabetic retinopathy and 0.85 (95% CI: 0.78; 0.92) for diabetic foot. Glycemic control partly mediated the associations of serum Mg^{2+} with overall microvascular events, diabetic retinopathy and diabetic foot, but not with macrovascular events.
Conclusion: Serum Mg^{2+} is prospectively, inversely, associated with the risk to develop HF, AF, and with the occurrence of microvascular endpoints, CKD, diabetic retinopathy and foot complications, in T2D. HbA_{1c} partly mediated the association of serum Mg^{2+} with microvascular complications.
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Genome-wide meta-analysis and omics integration identifies novel genes for diabetic kidney disease

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Background and aims: Diabetes is the leading cause of kidney disease, with heritability studies suggesting a substantial genetic contribution to diabetic kidney disease (DKD). Our aim was to identify novel genetic factors and genes contributing to DKD, by performing meta-analysis of previous genome-wide association studies (GWAS) on DKD from the DNCRI SUMMIT consortia, and by integrating the results with various renal transcriptomics datasets.
Materials and methods: We performed GWAS meta-analyses for ten phenotypic definitions of DKD, including up to 26,785 individuals with either type 1 or type 2 diabetes. Meta-analysis results were integrated with estimated quantitative trait locus (eQTL) data from human glomerular (N=119) and tubular (N=121) samples to perform transcriptome-wide association study (TWAS) with MetaXcan. Gene expression was studied in human transcriptomics data from nephrectomy samples (433 tubule and 335 glomerulus samples) and kidney biopsies from Pima Indian cohort (67 glomerular and 48 tubule-interstitial tissues) and association was tested with relevant pathological phenotypes.
Results: A novel low frequency intronic variant (rs72831309) in the *TENM2* gene was associated with combined chronic kidney disease (CKD) and DKD phenotype (odds ratio 2.08, 95% confidence interval 1.62 - 2.67, $p = 9.82\times10^{-9}$). Gene-level analysis with MAGMA and PASCAL identified 10 genes associated with DKD phenotypes (*PTPRN - RESP18, COL20A1, DCLK1, EIF4E, GPR158, INIP - SNX30, LSM14A,* and *MFF*; $p < 2.3\times10^{-6}$). TWAS indicated that tubular *AKIRIN2* expression was higher in DKD than in diabetic controls ($p = 1.1\times10^{-6}$).
For many of the identified genes, renal expression was associated with relevant pathological phenotypes: For example, *TENM2* expression in tubular nephrectomy samples correlated with higher eGFR (r = 0.287, p = 2.34×10^{-9}) and less fibrosis (r = -0.292, p = 4.68×10^{-9}). Also, tubular *DCLK1* expression correlated with fibrosis both in the nephrectomy samples (r = 0.345, p = 1.55×10^{-12}), and in kidney biopsies (r = 0.52, p = 0.0003). Finally, tubular *SNX30* expression correlated with higher eGFR (r = 0.341, p = 7.63×10^{-13}) and with lower level of fibrosis (r = -0.534, p < 2×10^{-16}). In line with this, renal *SNX30* expression was associated with higher eGFR also in a TWAS in the general population (p=0.046). Furthermore, the top SNPs within the *DCLK1, AKIRIN2*, *SNX30* and 3 other gene regions were significant methylation quantitative trait loci (mQTL) in 188 healthy kidney samples (p<2.2×10^{-11}).
Conclusion: GWAS meta-analysis and integration with renal transcriptomics data points to novel genes contributing to pathogenesis of DKD.
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Disclosure: **N. Sandholm:** None.

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Impact of the fasting plasma glucose titration target on the success of basal insulin titration in insulin-naive patients with type 2 diabetes: a systematic analysis

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Background and aims: Basal insulin titration algorithms differ in their fasting plasma glucose (FPG) targets. We compared beneficial (glycaemic control) and adverse outcomes (hypoglycaemia, weight gain) of basal insulin titration performed with different FPG titration targets (TT).
Materials and methods: A PubMed literature search retrieved 42 publications reporting clinical trials on titrating basal insulin in 17643 insulin-naïve patients with type 2 diabetes on a defined background of oral glucose-lowering medications (OGLM) and reporting relevant outcomes. 61 individual study arms were grouped by FPG TT (a: ≤ 5.0; b: 5.01-5.6; c: ≥ 5.61 mmol/l). For all subgroups, weighted means and their standard deviations were calculated for baseline and end-of-treatment FPG, HbA_{1c}, target achievement, incidence of any or severe hypoglycaemic events, and insulin doses, and compared by ANOVA and *post hoc* comparisons or contingency table analysis.
Results: Achieved FPG and HbA_{1c} after titration (study end) were lower with more ambitious titration targets (a. 6.17 [6.11; 6.24]; b. 6.67 [6.63; 6.71]; c. 6.93 [6.79; 7.06] mmol/l and a. 7.08 [7.05; 7.11]; b. 7.19 [7.18; 7.21]; c. 7.32 [7.26; 7.37] %; $p < 0.01$ for all comparisons). Accordingly, HbA_{1c} target achievement (≤ 7.0 or 6.5 %) was highest with FPG TT a, intermediate with b, and worst with c ($p < 0.01$). The basal insulin dose achieved by titration was highest in subgroup a. ($p < 0.01$). The incidence of (severe or any) hypoglycaemic episodes was not higher with lower FPG TTs, and body weight gain (1 to 2 kg across all subgroups) was not more prominent as well. However, overall, only 29.1 % (95 % CI: 28.5-29.8 %) reached their individual FPG TT.
Conclusion: Aiming for a lower FPG titration target improves glycaemic control without increasing the risk for adverse events like hypoglycaemia or increased body weight. More stringent titration should take more patients to ambitious targets.
Disclosure: **J. Wolters:** None.

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Advancing therapy in basal insulin users with type 2 diabetes: better clinical outcomes with iGlarLixi vs premix BIAsp 30 in the SoliMix trial

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Background and aims: iGlarLixi, a fixed-ratio combination of insulin glargine 100 U/mL and lixisenatide, offers an alternative treatment advancement option to premix insulin for uncontrolled basal insulin-treated T2D. SoliMix, an open-label, multicentre study, was the first to compare these two insulin coformulations.
Materials and methods: SoliMix randomised adults with T2D and HbA_{1c} 58-86 mmol/mol (7.5-10.0 %) on basal insulin + oral antihyperglycaemic drugs (OADs; metformin ± sodium-glucose co-transporter-2 inhibitors) to once-daily iGlarLixi or twice-daily premix biphasic insulin aspart 30 (BIAsp 30; 30% insulin aspart + 70% insulin aspart protamine). The two primary efficacy endpoints were non-inferiority in HbA_{1c} reduction or superiority in bodyweight change from baseline to Week 26 of iGlarLixi vs BIAsp 30.
Results: Baseline characteristics were similar between those randomised to iGlarLixi vs BIAsp 30 (49% vs 51% female; mean BMI 29.7 vs 30.0 kg/m^2; mean HbA_{1c} 71 vs 70 mmol/mol [8.6 vs 8.6 %]). In both groups, mean age was 59.8 years and mean duration of diabetes was 13.0 years. Both primary efficacy endpoints were met (**Table**), as well as iGlarLixi superiority vs BIAsp 30 in HbA_{1c} reduction. Mean ± SD HbA_{1c} reduced from 71 ± 7 mmol/mol (8.6 ± 0.7 %) for iGlarLixi and 70 ± 7 mmol/mol (8.6 ± 0.7 %) for BIAsp 30 at baseline to 56 ± 12 mmol/mol (7.3 ± 1.1 %) and 58 ± 11 mmol/mol (7.5 ± 1.0 %) at Week 26. Significantly more participants reached HbA_{1c} target (<53 mmol/mol [<7 %]) without weight gain, and HbA_{1c} target without weight gain and without hypoglycaemia, in the iGlarLixi group than in the BIAsp 30 arm. Incidence (**Table**) and rates (data not shown) of hypoglycaemia (ADA Level 1 or 2) were lower with iGlarLixi vs BIAsp 30. Gastrointestinal adverse events were more common with iGlarLixi (10.4%) vs BIAsp 30 (2.3%).
Conclusion: Based on better glucose control with weight benefit and less hypoglycaemia, once-daily iGlarLixi is a favourable alternative to twice-daily premix BIAsp 30 for advancing therapy in people with T2D uncontrolled on basal insulin + OADs.

Table

	iGlarLixi (N=443)	BIAsp 30 (N=444)	LS mean difference (97.5% CI)*	p-value for non-inferiority	LS mean difference (95% CI)*	p-value for superiority
	LS mean change from BL to W26 ± SE					
HbA_{1c}, mmol/mol	-14.2 ± 0.7	-11.5 ± 0.7	-2.6 (-4.5, -0.9)	p<0.001[‡]	-2.6 (-4.3, -1.1)	p<0.001[‡]
HbA_{1c}, %	-1.30 ± 0.06	-1.05 ± 0.06	-0.24 (-0.41, -0.08)	p<0.001[‡]	-0.24 (-0.39, -0.10)	p<0.001[‡]
Bodyweight, kg	-0.70 ± 0.20	+1.15 ± 0.20			-1.86 (-2.28, -1.43)	p<0.001[‡]
	n (%) at W26				OR (95% CI)	
HbA_{1c} target + no weight gain[§]	122 (27.5)	55 (12.4)			2.83 (1.98, 4.04)	p<0.001[‡]
HbA_{1c} target + no weight gain[§] + no hypoglycaemia[#]	86 (19.4)	31 (7.0)			3.40 (2.19, 5.28)	p<0.001[‡]
HbA_{1c} target	187 (42.2)	141 (31.8)			1.65 (1.25, 2.19)	
ADA Level 1 hypoglycaemia incidence	114 (25.8)	170 (38.5)			0.55 (0.42, 0.74)	
ADA Level 2 hypoglycaemia incidence	28 (6.3)	57 (12.9)			0.45 (0.28, 0.73)	
ADA Level 3 hypoglycaemia incidence	1 (0.2)	2 (0.5)			0.50 (0.04, 5.56)	

All endpoints were analysed in the ITT population except for ADA Level 1–3 hypoglycaemia which were analysed in the safety population (iGlarLixi N=442, BIAsp 30 N=441).
*Primary endpoint was assessed at 0.025 for non-inferiority on HbA_{1c} change from baseline then 0.05 for other endpoints, as per the multiple testing approach, the alpha was distributed to subsequent tests. †Primary efficacy endpoint, non-inferiority of HbA_{1c} reduction assessed using a margin of 3.3 mmol/mol (0.30 %).
‡Secondary endpoint was assessed at the alpha 0.05 level. §at Week 26. #Plasma glucose <3.9 mmol/l (<70 mg/dl) during the treatment period.
HbA_{1c} target was <53 mmol/mol (<7 %). ADA Level 1 hypoglycaemia: <3.9 mmol/l [<70 mg/dl] and ≥3.0 mmol/l [≥54 mg/dl]. ADA Level 2 hypoglycaemia: <3.0 mmol/l [<54 mg/dl]. ADA Level 3 hypoglycaemia: a hypoglycaemic event with severe cognitive impairment (hypoglycaemic unconsciousness) requiring external assistance for recovery.
BIAsp 30, biphasic aspart insulin 30 (30% insulin aspart + 70% insulin aspart protamine); BL, baseline; iGlarLixi, a fixed-ratio combination of insulin glargine 100 U/ml and the glucagon-like peptide-1 receptor agonist, lixisenatide; LS, least squares; ITT, intention-to-treat; W, week.

Clinical Trial Registration Number: 2017-003370-13
Supported by: Sanofi
Disclosure: **C. Trescoli:** None.

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Real-world persistence, adherence, healthcare resource utilisation, and costs in people with type 2 diabetes switching from basal insulin (BI) to 2nd- vs 1st-generation BI

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Background and aims: Basal insulin (BI) is a mainstay of treatment for type 2 diabetes (T2D). However, many people need to change their BI for a variety of clinical, personal, or economic reasons. This retrospective, real-world observational study using the Optum Clinformatics claims database compared outcomes in people switching from prior BI to either 2nd-generation (2nd-gen) BI (insulin glargine 300 U/mL [Gla-300]) or 1st-generation (1st-gen) BI (insulin glargine 100 U/mL [Gla-100] or insulin detemir [IDet]).
Materials and methods: Data were included from adults (≥18 years) with T2D who had received prior BI (NPH, Gla-100, IDet) in the 6-month baseline period, and switched to either the 2nd-gen BI, Gla-300 or a 1st-gen BI (Gla-100 or IDet) (treatment switch=index date) between April 1, 2015 and August 31, 2019. Participants were followed from index date for 12 months or plan disenrollment or death. Cohorts were propensity score matched (PSM) on baseline demographic/clinical characteristics. Outcomes were persistence (days on treatment without discontinuation), adherence (proportion days covered), and all-cause, diabetes-related, and hypoglycaemia-related healthcare resource utilisation and costs.

Results: After PSM (n=3077/cohort; mean age 68 years, 52% female), cohorts were well balanced except for 1 variable (hospitalisation) with standardised difference >0.1, which was adjusted in models as a covariate. During the 12-month follow-up period, participants who received Gla-300 vs 1st-gen BI had greater persistence with (adjusted p=0.0001) and adherence to (adjusted p=0.0006) therapy (**Table**). All-cause hospitalisations and emergency room (ER) visits were significantly lower for Gla-300 vs 1st-gen BI (both p<0.0001; **Table**), as were diabetes-related hospitalisations (21.5 vs 29.1 per 100 patient-years [P100PY]) and ER visits (54.8 vs 74.2 P100PY]), and hypoglycaemia-related ER visits (2.9 vs 5.7 P100PY); all p<0.0001. Costs for all-cause hospitalisations, ER visits and total healthcare were numerically lower for Gla-300 vs 1st-gen BI (**Table**), as were diabetes-related hospitalization ($5,626 vs $6,210), ER ($1,087 vs $1,543), and total healthcare costs ($22,613 vs $25,165). Similarly, hypoglycaemia-related hospitalization ($199 vs $333), ER ($46 vs $92) and total healthcare costs ($713 vs $1,326) were also numerically lower for Gla-300 vs 1st-gen BI, while pharmacy costs were numerically higher (all-cause: **Table**; diabetes-related: $7,093 vs $5,178).

Conclusion: Switching to Gla-300 was associated with significantly better persistence, adherence, and—despite numerically higher pharmacy costs—lower all-cause healthcare resource utilisation versus switching to a 1st-gen BI, in people with T2D previously treated with BI therapy. This is the first study to assess these outcomes in tandem.

Table

Outcome	2nd-gen BI (Gla-300), n=3077		1st-gen BI, n=3077		
Persistence; adherence	***n***	***%***	***n***	***%***	***Adjusted p-value***
Persistence, 90[th] centile	1399	45.5	1296	42.1	0.0001
Adherence, ≥80% PDC	1316	42.8	1176	38.2	0.0006
All-cause HRU	***Events, n***	***P100PY***	***Events, n***	***P100PY***	***Adjusted p-value***
Hospitalisations	1261	45.3	1763	65.9	<0.0001
ER visits	3119	111.9	3978	148.8	<0.0001
All-cause costs[a]	***≥1 event, n (%)***	***Cost PPPY, $***	***≥1 event, n (%)***	***Cost PPPY, $***	***Difference, $[b]***
Hospitalisations	674 (21.9)	12,996	805 (26.2)	16,375	−3,379
ER visits	1159 (37.7)	1,767	1243 (40.4)	2,437	−670
Pharmacy	–	13,688	–	10,642	+3,046
Total healthcare	–	41,255	–	45,316	−4,061

[a]Statistical analysis not available at time of abstract submission; [b]Gla-300–1st-gen.
HRU, healthcare resource utilisation; P100PY, per 100 patient-years; PDC, proportion of days covered; PPPY, per person, per year.

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Disclosure: **E. Wright:** Employment/Consultancy; Abbott Diabetes Care, Bayer, Boehringer Ingelheim, Lilly, Sanofi. Honorarium; Abbott Diabetes Care, Avion, Bayer, Boehringer Ingelheim, Lilly, Sanofi. Lecture/other fees; Abbott Diabetes Care, Avion, Bayer, Boehringer Ingelheim, Lilly, Sanofi. Stock/Shareholding; Abbott. Other; Investigator: Abbott Diabetes Care.

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Glycaemic improvement in 3,436 people with type 1 diabetes using the Omnipod DASH® Insulin Management System over first 90 days of use

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Background and aims: Outcome data describing real-world use of various devices by people with type 1 diabetes (T1D) are important to support clinical decision-making. This retrospective study characterized patient-reported clinical outcomes of people with T1D in the United States before (baseline) and 90 days after (follow-up) initiation of the tubeless Omnipod DASH® Insulin Management System from October 2018 until February 2021.

Materials and methods: This was a retrospective analysis of an existing dataset of patient information regularly collected by Clinical Service Managers (CSMs) across the US as part of standard Omnipod DASH initiation procedures. CSMs include certified diabetes educators, registered dieticians, and registered nurses who work with patients, caregivers, and HCPs to support users of the Omnipod DASH therapy. Clinical data were collected directly from the patient's medical record or self-reported if medical records were unavailable. The primary outcome was change in HbA1c levels from baseline to follow-up. Secondary outcomes were change in total daily dose (TDD) of insulin and frequency of hypoglycemic events (HE) per week (#/week <70 mg/dL). Outcomes were assessed by prior treatment modality (MDI or CSII), and by age (children: <18y, adults: ≥18y).

Results: The cohort of Omnipod DASH users included 3,436 patients. Children (n=1,020) were aged (mean±SD) 10±4y and 48% female, with diabetes duration 2.4±2.8y. Adults (n=2,416) were aged 44±17y and 60% female, with diabetes duration 17±14y. Both cohorts consisted primarily of prior MDI users (80% of children, 63% of adults), with some prior CSII users (10% of children, 23% of adults). At follow-up, significant and clinically relevant reductions in HbA1c levels were demonstrated for the study population, regardless of age group. The overall change in HbA1c was -0.8±1.9% for children and -0.9±1.6% for adults (both p<0.0001) (Table 1). The change in HbA1c for prior MDI users was -0.9±2.0% in children and -1.0±1.7% in adults (both p<0.0001). In prior CSII users, the change in HbA1c was -0.3 ±1.2% in children (p>0.05) and -0.6±1.1% in adults (p<0.0001) (Table 1). For children, TDD remained roughly the same after initiating the system (change: -0.9±11.3U/d, p>0.05), while adults had a significant reduction in the amount of insulin used (change: -12.5±30.2U/d, p<0.0001). A reduction of HE frequency was seen regardless of the age group The self-reported HE frequency decreased significantly by -1.4±2.7 and -1.6±3.2 episodes per week in children and adults, respectively (both p<0.0001).

Conclusion: These results show that in this large cohort of patients with T1D initiating the Omnipod DASH System, there were significant reductions in HbA1c, TDD, and number of HE after 90 days of use across age groups and/or compared to prior treatments.

Table 1: Outcomes of patients with T1D before and 90 days after initiating Omnipod DASH® Insulin Management System

	HbA1c (%)			TDD of Insulin (U/d)			HE (#/week)		
	Baseline	Follow-up	Change	Baseline	Follow-up	Change	Baseline	Follow-up	Change
Pediatric Patients									
All <18 (n=1,020)	8.6 ± 2.1	7.7 ± 1.3	-0.8 ± 1.9*	32.4 ± 24.4	31.5 ± 22.2	-0.9 ± 11.3	2.8 ± 2.8	1.4 ± 1.8	-1.4 ± 2.7*
Prior CSII (n=102)	8.5 ± 1.6	8.2 ± 1.5	-0.3 ± 1.2	40.8 ± 23.1	38.4 ± 21.5	-2.4 ± 8.2	2.3 ± 1.8	1.3 ± 1.6	-0.9 ± 2.1
Prior MDI (n=814)	8.6 ± 2.1	7.7 ± 1.3	-0.9 ± 2.0*	31.0 ± 23.4	30.2 ± 21.0	-0.8 ± 11.6	2.9 ± 2.9	1.4 ± 1.8	-1.5 ± 2.7*
Adult Patients									
All ≥18 (n=2,416)	8.5 ± 2.0	7.6 ± 1.3	-0.9 ± 1.6*	63.0 ± 41.9	50.5 ± 31.9	-12.5 ± 30.2*	3.0 ± 3.4	1.4 ± 1.9	-1.6 ± 3.2*
Prior CSII (n=560)	8.0 ± 1.5	7.4 ± 1.1	-0.6 ± 1.1*	53.9 ± 28.8	49.1 ± 41.0	-4.9 ± 36.6	3.4 ± 4.1	1.7 ± 2.5	-1.8 ± 3.7*
Prior MDI (n=1,527)	8.6 ± 2.1	7.6 ± 1.4	-1.0 ± 1.7*	64.1 ± 43.6	49.5 ± 27.9	-14.6 ± 27.3*	2.8 ± 3.1	1.3 ± 1.6	-1.6 ± 2.9*

Follow up after 90 days post-Omnipod initiation.
*P<0.0001 for changes from baseline to follow-up (Paired t-test)

Supported by: Insulet

Disclosure: **G. Aleppo:** None.

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Pharmacodynamics, pharmacokinetics, safety, and tolerability of INS068 vs insulin degludec in type 1 diabetes at steady state: a phase I, randomised, double-blind, cross-over-trial

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Background and aims: To assess steady state (SS) pharmacokinetics (PK) and pharmacodynamics (PD), safety and tolerability of INS068, a novel long-acting basal insulin analog, vs insulin degludec (IDeg) in patients with type 1 diabetes mellitus (T1DM).

Materials and methods: In this single-center, randomized, double-blind, cross-over study, patients with T1DM were randomized (1:1:1:1:1:1) to a sequence of 2 treatment periods with once-daily subcutaneous INS068 or IDeg for 9 days at one of three doses (0.4, 0.6 or 0.8 U/kg), separated by a 7 to 21-day washout period. Patients at all doses underwent a 42-h euglycemic clamp after the last dose of each treatment period. The primary endpoint was molar dose ratio with INS068 vs IDeg, as assessed by the area under the glucose infusion rate (GIR) time curve during a dosing interval (tau) at SS ($AUC_{GIR,tau,ss}$).

Results: 98 (57.1% male, age 34.8 ± 11.7 yrs, BMI 25.3 ± 2.9 kg/m^2, HbA1c 7.6 ± 1.2% [mean ± SD]) of 99 randomized patients received treatment. The molar dose ratio with INS068 vs IDeg based on $AUC_{GIR,tau,ss}$ was 0.85 (95% CI: 0.75-0.93) using a linear mixed effects model. $AUC_{GIR,tau,SS}$ and $GIR_{max,SS}$ were similar for INS068 and IDeg (with a time to $GIR_{max,SS}$ of

12-13 h post-dose) over the dose range of 0.4-0.8 U/kg (Fig 1A). Duration of action of INS068 was in line with IDeg, lasting for > 42 h at all doses (Fig 1B). Fluctuation of GIR at SS were comparable with INS068 vs IDeg across all doses based on peak-to-trough fluctuation ($PTF_{GIR,tau,SS}$; range of least-squares geometric mean, 0.54-0.86 vs 0.46-0.77) and average fluctuation ($AUCF_{GIR,tau,SS}$; range of geometric mean, 0.12-0.17 vs 0.10-0.17). The glucose lowering effect of INS068 was even between the 1st and 2nd half of the dosing interval at SS ($AUC_{GIR,12\text{-}24h,SS}/AUC_{GIR,tau,SS}$; range of geometric mean, 0.46-0.50 for INS068 vs 0.49-0.50 for IDeg). PK parameters ($AUC_{tau,SS}$ and $C_{max,SS}$) at SS were proportional to dose within 0.4-0.8 U/kg for INS068 and IDeg. The mean half-life of elimination ($t_{1/2,SS}$) was estimated as 21 h for INS068 and 26 h for IDeg across all doses. The accumulation ratio for INS068 as compared with IDeg was 1.7 vs 1.6 for AUC and 1.6 vs 1.4 for C_{max}. Adverse events (AEs) were mostly mild, with no deaths and only one case of serious AE reported (hypoglycemia [IDeg], reported after patient withdrawal). Treatment-related AEs occurred in 17.2% of patients with INS068 and 13.3% with IDeg, with the most common (incidence ≥2%) being hyperglycemia (6.9% vs 2.2%), injection site pain (3.4% vs 4.4%) and headache (3.4% vs 4.4%). Hypoglycemic episodes were reported in 85.1% of patients with INS068 vs 86.7% with IDeg.

Conclusion: INS068 was well-tolerated and demonstrated generally similar PD and PK profiles to IDeg at SS. The glucose-lowering effect and exposure of INS068 were dose proportional at doses of 0.4-0.8 U/kg.

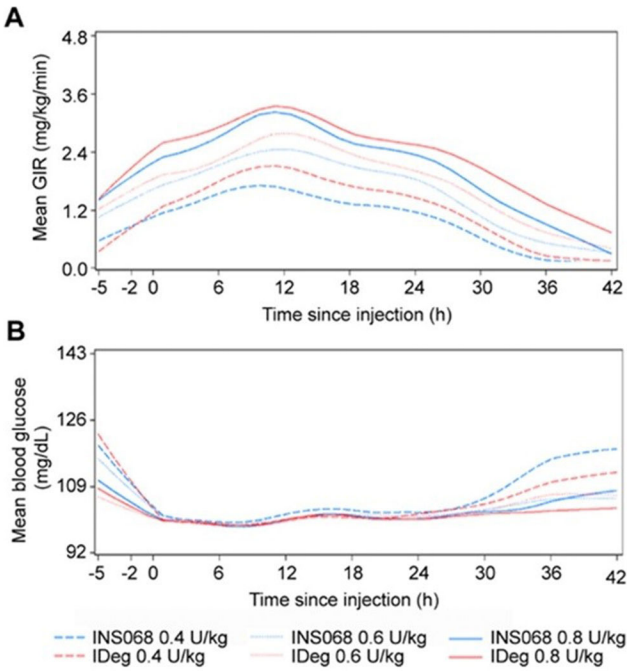

Figure 1. Mean GIR-time (A) and blood glucose-time (B) curves post last dose of INS068 or IDeg at SS.

Disclosure: **M. Hernandez:** Employment/Consultancy; ProSciento.

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Glycaemic control with once weekly basal insulin Fc in persons with type 2 diabetes using continuous glucose monitoring in a phase 2 study

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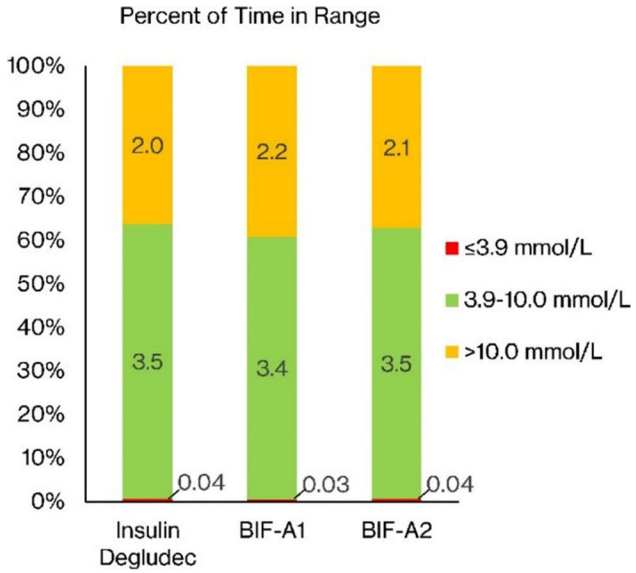
Background and aims: Basal insulin Fc (BIF; LY3209590) is a novel, once-weekly, long-acting IgG Fc-fusion protein assessed for the treatment of diabetes mellitus. A 32-week study evaluating the safety and efficacy of BIF vs degludec in persons with type 2 diabetes mellitus previously treated with a basal insulin showed HbA_{1c} non-inferiority of BIF vs degludec with significantly fewer hypoglycaemic events (≤3.9 mmol/L (70 mg/dL)). Here we present continuous glucose monitoring (CGM) data derived by Dexcom G6, allowing a more detailed assessment of glycaemic control of BIF vs degludec.

Materials and methods: The study included 2 dosing algorithms for BIF with different fasting glucose (FG) targets: ≤7.8 mmol/L (140 mg/dL) (BIF-A1) and ≤6.7 mmol/L (120 mg/dL) (BIF-A2). Degludec was titrated to FG ≤5.6 mmol/L (100 mg/dL). Subjects were randomized to 1 of the 3 arms.

Results: Subject (N=399) mean age was 60.2 years and baseline HbA_{1c} was 65.2 mmol/mol (8.1%). For the entire 32 weeks, the percent of 24 hours in range, hyperglycaemia and hypoglycaemia was similar for the 3 arms (Figure). At Week 32, total duration of hypoglycaemia was similar across 7 days post-injection for BIF-A1 and A2, showing that duration of hypoglycaemia is independent of day post-injection.

Conclusion: CGM data confirm that BIF showed similar glycaemic control vs degludec despite higher FG targets and numerically lower time in hypoglycaemia. The flat pharmacokinetic profile enables near peak less insulin concentrations without an increase in hypoglycaemia risk at highest exposure.

Clinical Trial Registration Number: NCT03736785



Data presented are the mean percentage of a 24-hour period spent ≤3.89 mmol/L (70 mg/dL), 3.94-9.99 mmol/L (71-180 mg/dL), and >9.99 mmol/L (180 mg/dL).

Abbreviations: BIF, basal insulin Fc; BIF-A1, BIF dosing algorithm with different fasting glucose targets of ≤7.8 mmol/L (140 mg/dL); BIF-A2, BIF dosing algorithm with different fasting glucose targets of ≤6.7 mmol/L (120 mg/dL).

Supported by: Eli Lilly and Company

Disclosure: **C. Kazda:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

OP 37 Cytokine storm in type 1 diabetes: from signalling to interventions

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Specific alterations in the STAT1/STAT6 axis may contribute to beta cell loss in type 1 diabetes

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Background and aims: Loss of the anti-inflammatory transcription factor, Signal Transducer and Activator of Transcription (STAT)-6, coupled with sustained up-regulation of the pro-inflammatory transcription factor, STAT1, have been reported in the islets of subjects with type 1 diabetes (T1D). These concerted events could play an important role in promoting β-cell loss during the autoimmune attack, by exacerbation of pro-apoptotic signalling. However, the possibility that the pathways regulated by STAT1 and STAT6 may interact coordinately to regulate β-cell viability, has not been investigated. This scenario has been studied in the present work.
Materials and methods: Cultured INS-1E rodent and human EndoC βH1 β-cells were employed, with sections of pancreas from control subjects and those with recent-onset T1D. Knockdown of target proteins was achieved using interference RNA techniques while qRT-PCR and western blotting were employed to monitor gene and protein expression, respectively. Interferon-responsive promoter activity was studied using a luciferase reporter assay in β-cells. Target proteins were detected in human pancreas sections by immunohistochemistry.
Results: Treatment of β-cells with the anti-inflammatory cytokine, interleukin-13 (IL-13) resulted in the activation of STAT6 (via increased phosphorylation) and this was accompanied by a marked loss of STAT1 protein (control: 1AU; STAT1: 0.5, $p<0.05$). By contrast, targeted knockdown of STAT6 was associated with a 2-fold increase STAT1 expression ($p<0.05$) and this correlated with a large increase in STAT1 activity as judged by reporter assays (6 fold; $p<0.001$). To study these relationships further, we monitored the influence of STAT6 on the expression of SIRPα, a protein previously identified as a regulator of β-cell viability and which is induced by STAT6. Immunoprecipitation of SIRPα from β-cells resulted in significant pulldown of STAT1 suggesting a direct interaction between the two. Conversely, when SIRPα was knocked down selectively, the levels of STAT1 increased by 18-fold ($p<0.001$) implying a second level of regulation, mediated via SIRPα-dependent alterations in STAT1 gene expression. In parallel, we also monitored the expression of a further set of proteins (including SOCS1, PIAS2, SMAD2, SHP1, SHP and RNF2 which act to negatively regulate STAT1 signalling in β-cells and found that exposure to IL-13 resulted in their up-regulation (by up to 7.5-fold; $p<0.001$). The increased production of a series of these molecules (SOCS1, SHP1, SHP2 and SMAD2) were clearly dependent on STAT6 activation since they were no longer up-regulated following STAT6 knockdown. To verify the importance of these responses in T1D, immunohistochemical analysis was undertaken in pancreas sections from matched control subjects with recent-onset T1D. This confirmed that STAT1 was increased in the residual insulin-containing islets in T1D in parallel with a reduction in STAT6 and negative regulators, including PIAS2 (control β-cells: 64.5.4±1.7AU, T1D: 48.01±1.1; $p<0.001$) and SHP2 (control β-cells: 148.4±5.1AU, T1D: 51.9 ±2.3AU; $p<0.001$).
Conclusion: The loss of STAT6, SIRPα and other negative regulators of STAT1 signalling in the β-cells in T1D may exacerbate pro-inflammatory signalling and promote β-cell loss during autoimmune attack. This suggests that selective targeting of the STAT6 pathway might offer a therapeutic opportunity to minimise pro-apoptotic signalling and consequent β-cell loss during the progression of T1D.
Supported by: EFSD Programme Award EFSD/JDRF/Lilly European Programme in Type 1 Diabetes Research 2020
Disclosure: **K. Afi Leslie:** None.

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Type III interferons are expressed in human pancreas at type 1 diabetes onset and induce immunostimulatory and antiviral activities in human beta cells

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Background and aims: Type I and II interferons (IFNs) have been proposed to contribute to beta cell destruction in human type 1 diabetes. Type III IFNs (IFN-λ 1-4) constitute a group of more recently described IFNs that are produced by both immune and parenchymal cells during inflammation and infection. Type III IFNs are known to induce a gene transcriptional signature similar to that of type I IFNs and they have been demonstrated to contribute to antiviral defense. We have previously shown that type III IFNs are expressed by human islets infected with Coxsackievirus B (CVB) in vitro and that type III IFNs in parallel with the induction of an IFN-induced gene transcriptional signature protects human islets from CVB infection. If type III IFNs play a role in type 1 diabetes has previously not been investigated. Moreover, the direct effect of type III IFNs on beta cells remains unexplored. In the present study, we aimed to investigate if IFNλ are expressed in the human pancreas at diabetes onset and to describe the effects that IFNλ have on beta cells by in-depth proteome, islet transcriptome and immune marker analysis. We also investigated the function of IFNλ induced pathways in β-cells with regards to immune status and antiviral defence.
Materials and methods: EndoC-βH1 cells were exposed to IFNs, poly I:C or infected with CVB serotypes. Proteome analysis was performed with HiRIEF LC-MS, and selected genes were quantified by qPCR. IFNλ expression, viral infection, immune activation markers and HLA-ABC expression was analyzed by FACS. Laser-captured islets of biopsies from 5 newly diagnosed T1D patients and 18 control pancreas donors were analyzed by Affymetrix Gene Arrays
Results: The proteome of IFN- λ1 or IFN- λ2 exposed EndoC-βH1 cells revealed 93 and 119 differentially expressed proteins compared to mock exposed cells. The abundance of a large set of proteins corresponding to known interferon stimulated genes (ISGs) were similarly increased by the two cytokines. The cell surface expression of MHC class I on EndoC-βH1 cells was induced by IFN-λ1 and IFN-λ2, as were key antiviral proteins as RIG-I and MxA. EndoC-βH1 cells were productively infected by all six CVB serotypes. The Coxsackie and Adenovirus Receptor (CAR) was expressed on the beta cells and blocking antibodies to the receptor attenuated CVB infection. IFN-λ1 or IFN-λ2 treatment strongly reduced cellular permissiveness to CVB infection. Finally, we discovered that the genes encoding IFN-λ1/2 showed increased expression in islets from diabetic individuals compared to healthy controls.
Conclusion: Type III IFNs are expressed in the human pancreas at type 1 diabetes onset. Type III IFNs increase the expression of MHC class I and activate antiviral defense in human beta cells. Collectively, our studies show that type III IFNs are expressed in the islets at disease onset and that this family of IFNs induce the expression of MHC I and ISGs, markers of human type I diabetes. We also show that type III IFNs have antiviral activity. In summary, these results highlight a potentially important immunomodulatory function of type III IFNs during development of type 1 diabetes.
Supported by: Novo Nordisk Foundation, The Swedish Research Council, The Strategic Diabetes Research Program at Karolinska Institutet
Disclosure: **E.E. Ringqvist:** None.

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Using the HALO image analysis platform to study pancreas pathology and insulitis in young people with recent-onset type 1 diabetes

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Background and aims: Worldwide, <600 type 1 diabetes (T1D) pancreata are available for research and, of these, fewer than 80 are from individuals who were <10y at onset with <1y disease duration. We have accessed the majority of these samples and used the digital pathology platform, HALO, to study insulitis and β-cell loss in multiple islets across each individual. The aim was to gain a more complete understanding of the progression of the disease in young children.
Materials and methods: Serial sections from 56 T1D cases (from the Exeter Archival Diabetes Biobank [EADB] & Seattle Children's Hospital [SCH] collection) and 8 non-diabetic controls (EADB) were stained for insulin/glucagon/CD45, CD8/CD20/glucagon and CD4/CD20/glucagon. Pancreas samples from 55 non-diabetic donors collected by the Network for Pancreatic Organ Donors with Diabetes (nPOD) were used as additional controls. Islets with ≥15 CD45+ cells were defined as inflamed. The average number of islets/mm^2 tissue and total numbers of CD45+, CD8+ T-, CD4+ T- and CD20+ B-cells in the islet and acinar tissue were calculated. We also examined the intensity of islet insulitis by calculating immune cell densities (CD45+ cells/mm^2 islet).
Results: 7646 islets from the 56 T1D cases and 3508 islets from 8 EADB control subjects, respectively were analysed initially. Of these, 34.6% (T1D) and 99.9% (controls) contained insulin and 14% and 0.03% respectively, were inflamed. CD20+ B-cell, CD4+ and CD8+ T-cell numbers were assessed in 35 cases (including all 6 from SCH) and in all 8 EADB controls. Cases with an average of <3 B cells/insulin-containing islet (ICI) (defined as type 1 diabetes endotype 2 [T1DE2], n=15) had a median age at diagnosis of 15.5y and a median 32.9% residual ICIs, 10.8% of which were inflamed. Mean CD45+ cell and CD20+ B-cell numbers/ICI were 8.48±2.0 and 0.20 ±0.0.4, respectively. Cases with ≥3 B cells/ICI (T1DE1, n=20) had a median age at diagnosis of 6y, a median of 10.3% residual ICIs, 77.9% of which were inflamed. Mean CD45+ cell and B-cell numbers/ICI were 63.7±9.3 and 28.1 ±7, respectively, differing significantly from T1DE2 in all criteria ($p<0.05$). Islet inflammation was more intense in T1DE1 donors than in those with T1DE2 as the median number of CD45+ cells/mm^2 ICI was 496.2 (IQR 98.1, 1191) vs 0 (IQR 0, 179.2; $p<0.0001$). The average number of islets/mm^{2}was reduced in all 56 T1D donors (irrespective of endotype) compared with the 63 age-matched controls ($p<0.0001$).
Conclusion: Individuals with young-onset T1D (<7y) display a markedly different profile of insulitis from those diagnosed ≥13y, consistent with the existence of disease endotypes. The youngest children (T1DE1) have fewer residual ICIs at diagnosis and are their islets infiltrated with large numbers of T- and B-cells than those who are older at diagnosis (T1DE2). Type 1 diabetes pancreata also have fewer islets/mm^2 of tissue compared with controls, which could contribute to early loss of glucose tolerance.
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Disclosure: **R.C. Wyatt:** None.

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Ag019 ActoBiotics as monotherapy or in association with teplizumab in recent-onset type 1 diabetes was safe and demonstrated encouraging metabolic and immunological effects

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Background and aims: AG019, a genetically modified *Lactococcus lactis* secreting human proinsulin and interleukin-10, is a novel, antigen-specific immune-modulating therapy for Type 1 Diabetes Mellitus (T1D). A Phase 1b/2a study is ongoing to assess the safety and tolerability, and potential treatment effects of AG019 alone and in association with anti-CD3 monoclonal antibody (teplizumab).
Materials and methods: Individuals with recent-onset T1D were treated with AG019 monotherapy (Phase 1b; 2 or 6 capsules BID for 8 weeks, n=19) or AG019 /teplizumab combination therapy or double-placebo (Phase 2a; 6 capsules BID for 8 weeks and 12 days teplizumab infusions, n=17). Safety was assessed as the incidence of treatment emergent adverse events (TEAEs). C-peptide area under the curve (AUC) levels were calculated after a mixed-meal tolerance test and preproinsulin (PPI)- and islet-specific CD4+ and CD8+ T-cell responses were measured using a Class II islet- and PPI-peptides activation assay and a Class I pooled tetramer assay, respectively.
Results: An interim analysis was performed on all Phase 1b monotherapy participants (10 adults and 9 adolescents; up to 12 months after treatment start) and on 14 Phase 2a combination therapy participants (12 adults (4:1) and 2 adolescents (open label); up to 6 months after treatment start). AG019 was well tolerated and safe when administered for 8 weeks as monotherapy or in association with teplizumab. No serious adverse events and no AG019 treatment discontinuation occurred due to TEAEs. Most TEAEs reported were mild (72.3%) and sometimes moderate (24.3%). AG019 safety profile was similar between adults and adolescents and there was no evidence of dose-related TEAEs. The safety profile of teplizumab in association with AG019 was consistent with that of teplizumab. C-peptide (mean AUC versus baseline) stabilized (coefficient of variation on at least 3 visits ≤ 9.7%) or increased at month 6 in 58% (7/12) of monotherapy participants aged 17 or older. In a subset analysis, AG019 monotherapy was associated with an antigen-specific immune response at 3 months, including a trend towards increased frequency of PPI-specific Tr1-cells and islet-reactive Treg-cells (4/4 adults), and a decreased mean frequency of PPI-specific CD8+ T-cells ($p=0.08$, 5 adults / 5 adolescents). C-peptide stabilized or increased at month 6 in 70% (7/10) of combination therapy participants aged 17 or older. In combination therapy, trends towards an increased frequency of PPI- and islet-reactive Tr1 cells (2/5 and 3/5 adults, respectively), and decreased mean frequency of PPI-specific CD8+ T-cells ($p=0.2$, 7 adults / 1 adolescent) was seen at 3 months.
Conclusion: AG019 was safe and well tolerated, and may favorably modulate autoimmune T-cell responses. The metabolic and immunological data suggest that AG019, alone or associated with teplizumab, may have the potential to be effective in preserving insulin-production in recent-onset T1D. AG019 has the convenience of oral administration and data suggests an opportunity for an improved effect with prolonged treatment duration.
Clinical Trial Registration Number: NCT03751007
Disclosure: **C. Mathieu:** Employment/Consultancy; Consultant for Precigen Actobio.

OP 38 Novel agents

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The novel GIP, GLP-1, and glucagon triple receptor agonist LY3437943 exhibits robust efficacy in preclinical models of obesity and diabetes

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Background and aims: The ever-growing prevalence of obesity and its associated comorbidities (type 2 diabetes, NASH/NAFLD) is driving the need to discover new therapies for improving metabolic health. Recently, multi-receptor agonists have offered promise for meeting this need. Here, we characterize LY3437943, a novel single agent tri-agonist at the glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1), and glucagon (Gcg) receptors (R).
Materials and methods: All in vitro assays in HEK293 or endogenous cells were performed in the absence of albumin to allow direct comparison to native peptides without the confounding influence of albumin binding. Intravenous glucose tolerance tests (IVGTT) were performed in anesthetized Wistar rats. The effects of LY3437943 on gastric emptying, body weight, food intake, and energy expenditure were determined in diet-induced obese (DIO) C57/Bl6 mice.
Results: Pharmacologic analysis of LY3437943 in cAMP assays using recombinant cell lines expressing the individual receptors indicated a potency balance favouring GIPR agonism (1.7- and 2.5-fold less potent at the GLP-1R and GcgR, respectively, but 7-fold more potent at the GIPR; all potencies in relation to the native ligands). In endogenous cells, LY3437943 regulated adipocyte lipolysis and hepatocyte glucose output. In vivo studies demonstrated regulation of multiple metabolic endpoints. Acute treatment with LY3437943 dose-dependently inhibited semi-liquid gastric emptying in mice and enhanced glucose dependent insulin secretion in rat IVGTT experiments. Chronic studies in DIO mice reduced food intake and body weight (45% weight loss primarily via reduced fat mass) superior to other GIPR and GLP-1R agonists. In these experiments, LY3437943 lowered blood glucose and plasma insulin, indicating improved insulin sensitivity. Additionally, chronic administration improved biomarkers of liver health, decreasing both plasma alanine aminotransferase and liver triglycerides. Rodent and cynomolgus monkey PK modeling also suggested the potential for weekly dosing in humans.
Conclusion: Taking these findings together, LY3437943 is a novel tri-agonist at the GIPR, GLP-1R, and GcgR, producing superior weight loss and glycaemic control compared with other incretin receptor-targeting molecules and offers additional benefit for liver health. These findings prompt evaluation of the potential clinical benefit of LY3437943 in individuals with obesity and metabolic diseases.
Supported by: Eli Lilly and Company
Disclosure: **T. Coskun:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

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Novel GIP/GLP-1/glucagon receptor agonist LY3437943: a first in human dose study in healthy subjects

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Background and aims: Multifunctional incretins are in clinical development for several metabolic conditions. Novel LY3437943 has potent agonist activity on glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1) and glucagon receptors. The primary objective of this randomised, double-blind, placebo-controlled, Phase 1, first in human study was to assess safety and tolerability of single-ascending doses of LY3437943.
Materials and methods: Forty-five healthy participants were randomised (6:2) to subcutaneous LY3437943 (6 rising dose levels) or placebo. Vital signs, ECGs, laboratory data and adverse events (AEs) were monitored to assess safety and tolerability. LY3437943 pharmacokinetics (PK) as well as change from baseline in fasting insulin, C-peptide and weight were measured. Appetite was assessed using a visual analog scale (VAS).
Results: The most common treatment-emergent AEs were gastrointestinal, including vomiting (with higher doses), abdominal distention and nausea, which were dose-dependent, mostly mild in severity, occurred within 4 days of dosing and resolved within a week of onset. Dose-dependent increases in heart rate and decreases in systolic blood pressure were observed, which returned to near baseline by Day 29. Mean terminal half-life of LY3437943 ranged from 134-165 h (~7 days) across the 6 doses, supporting once-weekly dosing. Dose-dependent increases in mean fasting insulin and C-peptide, with maximum levels observed at 24 and 48 h, returned to near baseline by Day 15. Dose-dependent weight loss was statistically significant with the 3 highest doses vs placebo (up to 3.5 kg at the highest dose). Weight loss was maintained up to Day 43 following single administration of the two highest doses. Overall VAS score significantly increased with higher doses compared with placebo, reflecting decreased appetite.
Conclusion: Triple-agonist peptide LY3437943 had a safety and tolerability profile similar to other incretins in Phase 1 trials with PK and pharmacodynamic outcomes that support further clinical evaluation.
Clinical Trial Registration Number: NCT03841630
Supported by: Eli Lilly and Company
Disclosure: **C.T. Benson:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

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Safety and pharmacokinetic study of CPL207280, a novel GPR40 receptor agonist, after a multiple-dose in healthy volunteers

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Background and aims: G-protein-coupled receptor 40 (GPR40) is a free fatty acid receptor mainly expressed in pancreatic β-cells and mediates signalling from free fatty acid (FFA) to modulate glucose-stimulated insulin secretion (GSIS). GPR40 emerged as an anti-diabetic target and was proposed as a novel treatment modality for type 2 diabetic (T2D) patients. First in class developed and evaluated in clinical trials GPR40 agonist - fasiglifam, was great hope for diabetic society - both patients and clinicians - as it might have offered a new modality for the treatment of diabetes. It was supposed to supersede a popular sulfonylurea as it showed limited hypoglycemia risk and it appeared as a potential competitor to insulinogenic GLP-1 analogues. However, it caused liver toxicity in phase 3 of clinical trials in T2D patients thereby its development was stopped. This example suggested that toxicity might be a hallmark of the entire class and hence quenched both the research field and hopes for a new secretagogue to be launched to the market in the nearest future. However, later some papers appeared, suggesting hepatotoxicity related more closely to the molecule than to the target. The aim of this Phase 1 clinical study is to determine the safety, tolerability and pharmacokinetic (PK) profile of CPL207280 a new GPR40 agonist - in healthy subjects in fasting condition.
Materials and methods: This was a multiple-dose, randomized, double-blind, placebo-controlled, ascending dose study in healthy volunteers with administrations once daily over 2 week. CPL207280 was administrated orally as a prolonged-release tablet at four doses: 60, 120, 240, 480 mg among 32 subjects (n = 8 per cohort, with randomization 3:1)

who met all the inclusion and none of the exclusion criteria. Blood samples for pharmacokinetic analysis were collected on days 1, 8, 14. On the other days, samples were collected just before and 1 hour after CPL207280 administration and up to 48 hours following the last dosing. CPL207280 concentration in human plasma was measured using HPLC/MS/MS. Safety assessment included adverse events (AE) reporting, clinical laboratory tests, vital signs, physical examination, electrocardiography (ECG).

Results: The administration of CPL207280 was generally safe and well-tolerated with no serious AEs. All adverse events were classified as not related to the study product. No increase in the tested hepatic parameters (ALT, AST, ALP, bilirubin, creatinine) was observed. There were no hypoglycaemia episodes during the study. CPL207280 plasma concentration-time profile of all subjects was determined during the study. CPL207280 mean Cmax values during all dosing days were in the range of 106 - 500 ng/mL for cohort 1 and 4, respectively, and were observed at 0.33-4 hours after administration. The CPL207280 exposure increased in a dose-dependent (between the cohorts) and in a time-dependent (inside the cohorts, between day 1 and 14) manner.

Conclusion: The administration of CPL207280 for 14 days was safe and well-tolerated. The pharmacokinetic profile of CPL207280 confirms findings from the single administration study in healthy volunteers, supports once-daily administration and justify further development of the therapy for patients suffering from T2D.

Clinical Trial Registration Number: NCT04622111

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Disclosure: **K. Bazydlo-Guzenda:** Employment/Consultancy; Celon Pharma employee.

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DA-1241 a novel GPR119 agonist: Safety, tolerability, pharmacokinetics, and pharmacodynamics: Part 2 of multiple ascending dose study in type 2 diabetes patients

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Background and aims: DA-1241 is a novel small molecule selective GPR119 agonist. In preclinical studies DA-1241 enhanced metabolic hormones, and improved metabolic control characteristics. The primary objective of Part 2 was to assess safety and tolerability of multiple once daily oral doses of DA-1241 versus placebo and Sitagliptin (SG) in Type 2 Diabetes Mellitus (T2DM). Secondary objectives were to establish pharmacokinetic (PK) and pharmacodynamic (PD) characteristics.

Materials and methods: Part 2 was a double blind placebo and SG controlled study with three sequential cohorts of T2DM (n=25/cohort) blinded and randomized (3:1:1) to receive DA-1241: 25, 50 or 100 milligram (mg; n=15/cohort), placebo (n=5/cohort) or SG (n=5/cohort) single daily oral doses for 56 days.

Results: 84 subjects were enrolled in Part 2, 3 subjects were withdrawn due to Treatment Emergent Adverse Events (TEAEs; 1 active, 2 placebo), 72 subjects completed the study. There were no relevant demographic imbalances (age 56.8 ± 7.3, BMI 29.4 ± 3.3). Doses tested were generally safe and well tolerated. The most frequent TEAEs were mild GI side effects (nausea, diarrhea, abdominal pain), all resolved spontaneously. Day 56 PK C_{max} and $AUC_{0\text{-}tau}$ parameters were dose proportional. Fasting plasma glucose trended towards improvement for all DA-1241 doses, as did incremental glucose exposure after Mixed Meal Tolerance Tests (MMTTs; Change from Baseline to Day 56 $iAUC_{0\text{-}4h}$: 25mg: 18.5 ±89.2, 50mg: -5.1±86.8, 100mg: -38.6±58.7, Placebo: 34.4±145.9, SG: -23.9±132.8) and time spent at blood glucose < 180 mg/dL. HbA1c trended towards improvement across dosing groups, as did body weight. In pooled analyses of the data on Day 56, glucose $AUC_{0\text{-}4h}$ values during MMTT were well correlated with HbA1c (%) and glucose $AUC_{0\text{-}24h}$ values assessed using a continuous glucose monitoring system (r = 0.626 and 0.685, respectively for 72 subjects; $p<0.001$). Total GIP, GLP-1 and PYY were increased at day 56, consistent with the mechanism of action of DA-1241. Among them, after single administration of DA-1241 tablet on Day 1, plasma excursion ($AUC_{0\text{-}4h}$) of total GLP-1 during MMTT showed a distinctive positive correlation with both the $AUC_{0\text{-}24h}$ and C_{max} of DA-1241 (r = 0.4999 and 0.3834, respectively for total 46 patients; $p<0.05$).

Conclusion: This phase 1b study in T2DM showed favorable safety, tolerability and PK profiles of DA-1241. Biomarkers and PD data confirmed the mechanism of action and showed favorable efficacy data trends.

Clinical Trial Registration Number: NCT03646721

Disclosure: **M. Hompesch:** Employment/Consultancy; ProSciento. Stock/Shareholding; ProSciento.

OP 39 Glucagon metabolism in humans

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Different patterns of glucose- and glucagon-stimulated insulin secretion in new diabetes subphenotypes

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Background and aims: Glucagon-like peptide-1 (GLP-1) improves insulin secretion in people with type 2 diabetes by affecting GLP-1 receptors on beta-cells. Recent data indicate that glucagon mostly exerts its insulinotropic effects via these GLP-1 receptors. We hypothesized (i) that the rise of insulin secretion after glucagon administration reflects a GLP-1 effect on beta-cells and (ii) that the GLP-1 effect differs between the novel clusters (subgroups) of diabetes.
Materials and methods: We compared insulin response during intravenous Glucose Tolerance Test (IVGTT) and Glucagon Stimulation Test (GST) in 562 people with recent-onset diabetes and 190 controls from the German Diabetes Study (GDS), and used the known genetically mediated incretin resistance by the variant rs7903146 in *TCF7L2* as a benchmark. Furthermore, we compared GST and IVGTT data between the diabetes subgroups. Linear mixed models were used to compare the effects of age, BMI, insulin resistance, and fasting glucose on insulin response, given as fold change C-peptide, in IVGTT and GST. Multivariable linear regressions were performed to assess the effect of the *TCF7L2* genotype, diabetes status, and the interaction of risk-genotype and glucose dynamics on insulin response.
Results: Intravenous glucagon bolus elicited a higher insulin response despite less increase in blood glucose, compared to glucose injection during IVGTT (fold change C-peptide 2.4±0.9 vs. 1.8±1.3, p<0.001; blood glucose 129.8± 23.9 vs. 245.9± 40.6 mg/dl, p<0.001). Glucagon-enhanced insulin secretion was less impacted by glycemia, aging, obesity and insulin resistance than glucose-stimulated insulin secretion ($p_{interaction} \leq 0.002$). Incretin resistance was evident in GST by reduced glucose-dependent insulin release in rs7903146 risk allele carriers (p=0.038). The risk allele was negatively associated with insulin release during both tests in severe insulin resistant diabetes (SIRD) (all p<0.05), but positively in mild age-related diabetes (MARD) (β=0.07, p=0.033).
Conclusion: These results from large comprehensively phenotyped human cohort are consistent with previous preclinical data that glucagon acts as a partial agonist of the GLP-1 receptors in beta-cells. We show that diabetes subgroups have distinct impairments in insulin secretion and that the TCF7L2 risk genotype exhibits different effects on insulin secretion in these diabetes subgroups.
Clinical Trial Registration Number: NCT01055093
Disclosure: **K. Prystupa:** None.

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Increased glucagon sensitivity in totally pancreatectomised patients

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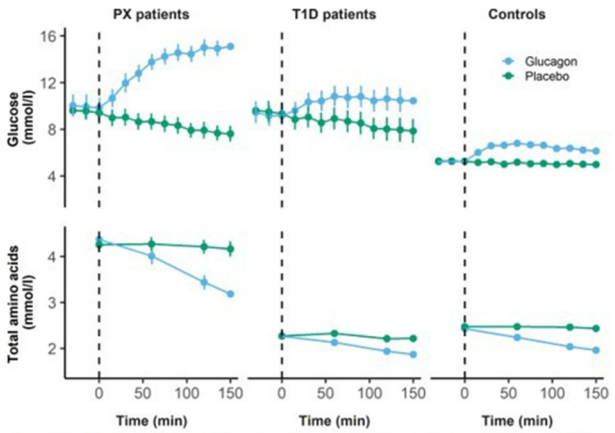
Background and aims: Glucagon secretion and signalling are affected in several metabolic disorders including different types of diabetes. The aim of this study was to investigate the effect of exogenous glucagon on glucose, amino acids, and lipid metabolism in participants deprived of endogenous glucagon and/or insulin.
Materials and methods: We included nine patients who had undergone total pancreatectomy (PX, age 64.2 [mean] years; BMI 22 kg/m^2), eight patients with C-peptide negative type 1 diabetes (T1D, age 61.6 years; BMI 23.9 kg/m^2) and nine healthy controls (age 62.0 years; BMI 23 kg/m^2) in a randomised, double-blinded, placebo-controlled, crossover study. Participants received a 2.5-hour i.v. infusion of glucagon (4 ng/kg/min) and placebo (saline), respectively, under fasting conditions with usual basal insulin administration.
Results: Glucagon infusion increased plasma glucagon to high physiological levels in all groups (PX 67.6±3.8 [mean±SEM] pmol/l; T1D 64.1 ±3.0 pmol/l; and controls 68.7±3.7 pmol/l) with no difference between groups (*p*=0.655). Compared with placebo, glucagon infusion increased insulin levels significantly in the controls (*p*<0.001). Baseline-subtracted glucose levels increased in all groups compared to placebo (PX 7.1±0.4 mmol/l, *p*<0.001; T1D 2.8±1.2 mmol/l, p=0.060; and controls 1.2±0.2 mmol/l, p<0.001; Figure). In the PX group, baseline-subtracted AUC (bsAUC) for glucose was 2.2 and 3.5-fold higher compared to the T1D group (*p*=0.015) and the control group (*p*<0.001), respectively. PX patients were characterised by 1.9 and 1.8-fold elevated fasting plasma levels of amino acids compared to the T1D group (*p*<0.001) and control group (*p*<0.001), respectively (Figure). In the PX group, glucagon reduced bsAUC for total amino acids by 2.8 and 2.3-fold compared to the T1D group (*p*=0.003) and the control group (*p*=0.008), respectively. In the healthy controls, glucagon infusion reduced bsAUC for glycerol (*p*<0.001) and palmitate (*p*=0.002), whereas no changes were observed in the diabetic groups. Glucagon did not change plasma levels of LDL, HDL or total cholesterols, or triglycerides in any of the groups.
Conclusion: We conclude that PX patients in terms of amino acid turnover and endogenous glucose production are hypersensitive to exogenous glucagon; most likely because of upregulation of glucagon receptive mechanisms in response to their lack of pancreatic glucagon secretion.



Time courses of plasma glucose (upper panel) and total amino acid levels (lower panel) during 2.5-hour intravenous infusions of glucagon (4 ng/kg/min; black curves/symbols) and placebo (saline; grey curves/symbols), respectively, initiated at time 0 min (broken lines) in totally pancreatectomised (PX) patients (n=9), patients with type 1 diabetes (T1D) (n=8) and healthy controls (n=9).

Clinical Trial Registration Number: NCT03526445

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Disclosure: **I. Rix:** Employment/Consultancy; Zealand Pharma A/S.

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Splanchnic and leg glucagon metabolism in healthy adults

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Background and aims: Regional and systemic glucagon metabolism is poorly understood in humans. To study post absorptive glucagon turnover, we applied isotope dilution technique combined with organ catheterization to estimate splanchnic and leg glucagon metabolism in healthy nondiabetic adults.
Materials and methods: After IRB approval and informed consent, subjects admitted to the Interventional Radiology Unit after an overnight fast, had catheters placed in the right femoral artery and vein under aseptic precautions. Under fluoroscopic guidance, a hepatic venous catheter was positioned through the femoral vein. $^{13}C^{15}N$ Glucagon tracer was infused intravenously to measure glucagon turnover, and Indocyanine Green infused via the femoral artery sheath to measure splanchnic and femoral plasma flows. After a basal sampling period, exogenous glucagon was infused @ 0.65 ng/kg/min (medium) for 60 minutes followed by 1.5 ng/kg/min (high) for another 60 minutes to determine the effects of plasma glucagon concentrations spanning the physiological range on regional glucagon extraction and turnover. Samples were collected periodically from the femoral artery, femoral vein, and hepatic vein for $^{13}C^{15}N$ Glucagon (tandem MS/MS), glucagon (Mercodia), glucose (YSI) and indocyanine green (UVA-Vis) concentrations. Splanchnic and leg glucagon extraction and glucagon turnover was calculated using regional plasma flow, $^{13}C^{15}N$ Glucagon concentrations and steady state equations.
Results: Data from 8 healthy (age 23.1 ± 2.9 yrs., 4 females, BMI 26.6 ± 3.5 kg/m^2, fasting plasma glucose 4.7 ± 0.5 mM; HbA1c 5.0 ± 0.2 %) subjects analyzed thus far is shown. There was substantial fractional glucagon extraction across the splanchnic (23.5 ± 12.4 vs. 30.4 ± 16.3 vs. 23.4 ± 13.1 %: basal vs. medium vs. high) and leg (35.9 ± 17.9 vs. 28.5 ± 8.0 vs. 22.4 ± 7.9 %: basal vs. medium vs. high) regions during arterial glucagon concentrations spanning the physiological range (26.8 vs. 39.9 vs. 66.4 pg/ml: basal vs. medium vs. high; Fig 1: upper panel). Interestingly, systemic rate of appearance of glucagon rapidly dropped to undetectable levels with exogenous glucagon administration (Fig 1: lower panel).
Conclusion: These initial data in healthy nondiabetic adults demonstrate substantial fractional glucagon extraction across the splanchnic and leg regions and that exogenous glucagon administration inhibits endogenous glucagon production.

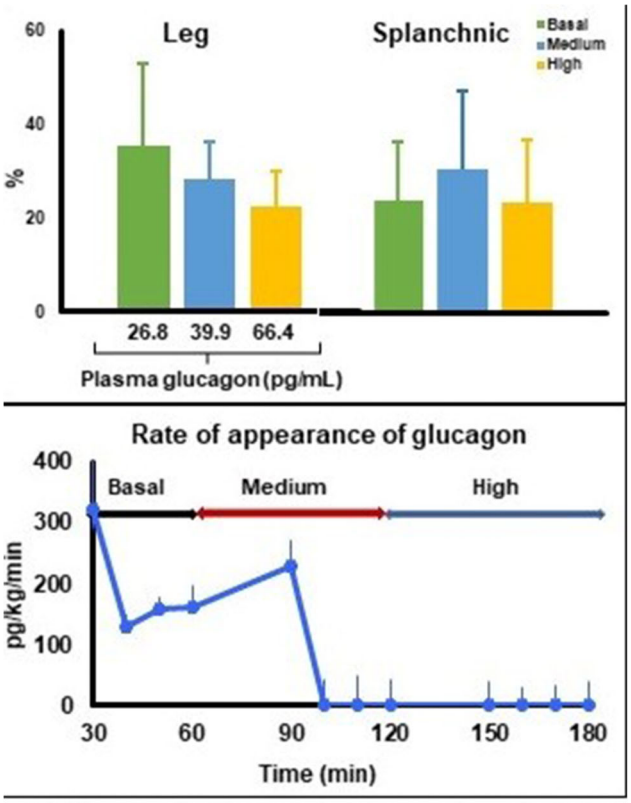

Fig 1: Upper panel - % glucagon extraction across leg and splanchnic tissues; Lower panel – Systemic Ra of endogenous glucagon

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A 2-year follow-up of RYGB surgery in obesity and type 2 diabetes: enhanced responses of multiple hormones to oral glucose but not i.v. arginine challenge

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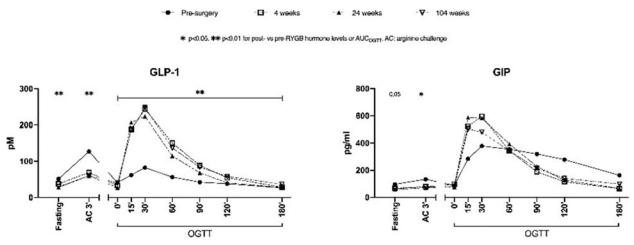
Background and aims: Bariatric surgery, in particular roux-en-Y gastric bypass (RYGB), is known to greatly improve glycemic control in patients with type 2 diabetes, even to its remission. This effect is largely independent of the magnitude of weight loss, and endocrine and neural responses are likely to be involved. We aimed at shedding light on the effects of RYGB on the dynamic endocrine crosstalk contributing to this metabolic shift. A systematic assessment of insulin, glucagon, gut hormones, growth hormone (GH) and the hypothalamus-pituatary-adrenal (HPA) axis following fasting and nutrient challenges, respectively, was performed repeatedly up to 2 years post-RYGB.
Materials and methods: Our cohort includes 13 patients with T2DM (up to ten years of disease duration, age 18 to 65 y/o, BMI 30-45 kg/m^2) undergoing RYGB who were compared to 6 patients with standard of care but no RYGB (randomized design, but controls not included in the present analyses). We analyzed plasma samples for insulin, glucagon, GLP-1, GIP, ACTH and cortisol (PYY and GH ongoing) on four occasions: 1) before surgery; 2) 4 weeks; 3) 24 weeks; 4) and 104 weeks post-RYGB; and in three conditions assessed sequentially on the same day: 1) in the morning after an overnight fast; 2) during i.v. arginine challenge, 20 g; 3) during OGTT 75 g, 3h.

Results: Following RYGB there was a reduction of fasting levels of insulin, glucagon, GLP-1 and GIP ($p<0.001$, $p<0.02$, $p<0.001$, $p=0.05$, respectively; figure) as well as during arginine challenge ($p<0.001$, $p=0.22$, $p<0.001$, $p=0.03$). The relative stimulating effect of arginine at 3 minutes was not altered by RYGB and remained intact throughout all visits. During OGTT, we identified a more rapid rise for GIP levels following RYGB but with essentially unchanged AUC. There was also a marked increase for glucagon, GLP-1 (see figure) and, surprisingly, for ACTH and cortisol (all $p<0.01$). All post-RYGB effects were sustained throughout the 2-year follow-up.

Conclusion: There was a stimulating effect of i.v. arginine on insulin, glucagon, GLP-1, and GIP, and this effect was maintained following RYGB, albeit with overall lower absolute levels. Following RYGB, we found raised OGTT-stimulated secretion of glucagon, GLP-1, ACTH, and cortisol and a more rapid GIP secretion pattern, and this was sustained over 2 years. Thus, oral glucose stimulation of multiple hormones, with pituitary, adrenal, pancreatic islet and gut origin, were similarly amplified following RYGB, and this was sustained over at least 2 years. in contrast, the fold effect following arginine stimulation remained unchanged after RYGB. Dynamic regulation of multiple hormones may contribute to the beneficial effects of RYGB on glycemic control and energy balance in obesity and type 2 diabetes.



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OP 40 Protecting the kidney in diabetes

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Protective impact and potential mechanisms of Elabela on DKD via ß-arrestins

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Background and aims: ELABELA (ELA), an endogenous ligand for apelin receptor (APJ), is decreased in plasma of DKD patients. The effects of ELA on relieving diabetic renal lesion have not been reported. This study was performed to explore the protective impact and potential mechanisms of ELA on DKD via β-arrestins.

Materials and methods: Sixteen eight-week-old male db/db mice were randomly divided into diabetic nephropathy group (db/db group) and intervention group (db/db+ELA group) with administration of ELA ($5mg \cdot kg^{-1} \cdot day^{-1}$) for 8 weeks. 8 age-matched male db/m mice were recognized as normal control group (db/m group). Both db/db and db/m group received equivalent normal saline injection for 8 weeks. Body weights and blood glucose levels were measured once a week. After 8 weeks, blood and urine samples were collected. Serum creatinine and urinary albumin to creatinine ration (UACR) were examined. The kidneys were taken after sacrificing mice. The morphology change of kidney tissues were assessed by HE and PAS staining. The expression of β-Arrestin1/2 was detected by immunohistochemistry. The levels of fibronectin (FN), collagen type IV (Col-IV) and β-arrestin1/2 in kidney tissues were examined by western blotting.

Results: (1) With the intervention of ELA, UACR, serum creatinine and the ratio of kidney weight to body weight of db/db mice were significantly decreased ($P<0.05$). (2) Compared with db/m mice, db/db mice exhibited renal tubular epithelial cells edema, decreased ratio of non-lumen area to total area, thickened basement membrane and increased glycogen accumulation, however, those pathological abnormality were mitigated by ELA. (3) The levels of FN, Col-IV and β-Arrestin-1/2 in db/db mice were higher than db/m mice, while ELA treatment suppressed the expression of FN, COL-IV and β-Arrestin-1/2 ($P<0.05$).

Conclusion: ELA may play a role in protection from diabetic kidney damage via down-regulating the expression of β-Arrestin-1/2 and inhibiting the secretion of extracellular matrix.

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Disclosure: **M. Shi:** None.

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Chop-ASO ameliorates glomerular and tubular damage on top of ace-inhibition in diabetic nephropathy

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Background and aims: Diabetic nephropathy (dNP) is the leading cause of end-stage renal disease globally. Despite promising therapeutic developments dNP still lacks an efficient therapy halting or even reversing the disease. Maladaptive endoplasmatic reticulum (ER)-stress signaling has been linked to dNP and is mediated by the transcription factor C/EBP homologous protein (Chop) that is hard to target by conventional modalities like small molecule inhibitors or antibodies.

Materials and methods: We identified locked nucleic acid (LNA)-modified antisense oligonucleotides (ASOs) specifically targeting Chop (Chop ASO) and investigated therapeutic efficacy in vivo and in vitro. Renal functional parameters, morphological changes and differentially regulated genes and pathways were assessed in db/db mice treated with Chop ASO alone or on top of ACE-inhibition (ACEi).

Results: Chop ASO efficiently reduced Chop protein levels in diabetic mice in early (8 weeks aged db/db mice) and late stages (16 weeks aged db/db) of experimental dNP. Therapy with Chop ASO in early stage dNP led to a reduction of albuminurea, glomerular damage and interstitial fibrosis to a similar extent than ACEi. Combined intervention significantly reduced interstitial fibrosis as compared to each intervention alone. Intervention in late stage dNP with a combination of Chop ASO + ACEi resulted in superior therapeutic efficacy with regard to reduction of mesangial matrix accumulation and glomerular basement membrane thickness compared to the respective monotherapies. Strikingly, in contrast to ACEi, Chop ASO alone or in combination with ACEi showed therapeutic effects in the tubular compartment as measured by tubular diameter, apoptosis and expression of the damage marker KIM-1. Gene set enrichment analysis after gene expression analysis revealed the disease gene sets *Hyperglycemia, Tubulointerstitial Fibrosis and Hypertensive Disease* being the top three pathways regulated by Chop ASO therapy. Slc5a2 (SGLT2), the target of the increasingly used SGLT2 inhibitors was one of the genes downregulated under Chop ASO therapy. In line with the in vivo findings in mice, we observed a normalization of Chop expression and a reduction of apoptosis in glucose-stressed, Chop-ASO-treated human renal cells.
Conclusion: LNA-modified ASOs targeting the transcription factor Chop efficiently reduced renal Chop expression in a mouse model of dNP. The therapeutic intervention with Chop ASO especially at late stages of dNP provide an added benefit on top of the current standard of care (ACEi). Our studies demonstrate that ASO-based therapies that efficiently reduce maladaptive Chop expression in kindey diseases have the potential to provide a novel treatment option for patients in need.
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SGLT2 inhibition prevents acute kidney injury by inhibiting glucose transport and mTORC1 activity in proximal tubule cells

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Background and aims: Patients with diabetes are prone to develop acute kidney injury (AKI), which markedly increases the risk for end-stage kidney disease. SGLT2 inhibitors (SGLT2i) exert robust kidney protection in patients with diabetic kidney disease (DKD). Previous reports suggested that SGLT2i treatment might increase the risk for AKI, with subsequent publication of a "black box" warning by the FDA. However, randomized-controlled clinical trials showed that treatment with SGLT2i is associated with decreased, rather than increased incidence of AKI. We have recently reported that SGLT2i prevent DKD by inhibiting glucose transport and mTORC1 activity in renal proximal tubule cells (RPTCs). Herein, we studied whether a similar mechanism operates in the context of drug-induced AKI.
Materials and methods: Eight-week old wildtype mice were injected intraperitoneally with a single dose of folic acid (FA; 250 mg/kg) and were pretreated with or without dapagliflozin for 5 days. Mice were sacrificed 2 or 14 days following FA injection and analyzed for the expression of markers of tubular injury, fibrosis and inflammation, and glycolytic enzymes. mTORC1 activity was analyzed by Western blotting and immunofluorescence for phospho-S6. Cre-lox system was used to generate RPTC-specific *Raptor* knockout mice.
Results: AKI developed 2 days after FA administration, evidenced by increased plasma creatinine and urea nitrogen levels and increased gene and protein expression of tubular injury, fibrosis and inflammation markers, along with increased expression of glycolytic enzymes, DNA damage repair (phosphorylated-p53) and autophagy. FA-induced tubular injury resulted in tubular atrophy and dilatation, and interstitial fibrosis by Masson's trichrome staining at 14 days. SGLT2i prevented these alterations and the late development of tubular atrophy and fibrosis. AKI was associated with marked activation of RPTC mTORC1 activity, which was prevented by pre-treatment with dapagliflozin. Heterozygous *Raptor* KO in RPTCs attenuated the activation of mTORC1 by FA and inhibited mTORC1-TGFβ-mediated fibrosis.
Conclusion: SGLT2i are highly effective in protecting the kidney from drug-induced kidney injury, which is more prevalent in patients with diabetes. The protective effects of SGLT2i are mediated via inhibition of glucose transport and prevention of mTORC1-TGFβ induced kidney fibrosis.
Disclosure: **A. Kogot-Levin:** None.

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Acute effects of dapagliflozin on renal oxygenation and perfusion in type 1 diabetes with albuminuria: a randomised, double-blind, placebo-controlled crossover trial

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Background and aims: Inhibitors of the sodium-glucose cotransporter 2 (SGLT2) slow the progression of diabetic kidney disease, possibly by reducing the proximal tubule transport workload with subsequent improvement of renal oxygenation. We aimed to test this hypothesis in individuals with type 1 diabetes and albuminuria.
Materials and methods: A randomised, double-blind, placebo-controlled, crossover trial with a single dose of 50 mg of the SGLT2 inhibitor dapagliflozin and placebo in random order, separated by a two-week washout period. Magnetic resonance imaging (MRI) was used to assess renal R_2* (a low value corresponds to a high tissue oxygenation), renal perfusion (arterial spin labelling) and renal artery flow (phase contrast imaging) at baseline, three- and six hours from tablet ingestion. Exploratory outcomes, including baroreflex sensitivity, peripheral blood oxygen saturation and peripheral blood mononuclear cell mitochondrial oxygen consumption rate, were evaluated at baseline and 12 hours from medication.
Results: Between February 3, 2020 and October 23, 2020, 31 individuals were screened, and 19 eligible individuals were randomised. Three dropped out before receiving any of the interventions and one dropped out after receiving only placebo. We included 15 individuals (33% female) in the per-protocol analysis with a mean age of 58 (SD 14) years, median urinary albumin creatinine ratio of 46 [IQR 21-58] mg/g and an eGFR of 73 (32) ml/min/1·73m^2. The mean changes in renal cortical R_2* from baseline to six hours were for dapagliflozin -1·1 (SD 0·7) s^{-1} and for placebo +1·3 (0·7) s^{-1}, resulting in a difference between interventions of -2·3 s^{-1} [95% CI -4·0 to -0·6]; p=0·012. No between-intervention differences were found in any other MRI outcomes, physiological parameters or exploratory outcomes. There were no adverse events.
Conclusion: Dapagliflozin improved renal cortical R_2* without changing renal perfusion or blood flow. This suggests improved renal cortical oxygenation due to a reduced tubular transport workload in the proximal tubules. Such improved oxygenation may in part explain the long-term beneficial renal effects seen with SGLT2 inhibitors.
Clinical Trial Registration Number: The study is registered in the EU Clinical Trials Register (EudraCT 2019-004557-92), on ClinicalTrials.gov (NCT04193566), and is completed.
Supported by: The study was funded by the Novo Nordisk Foundation grant PROTON personalising treatment of diabetic nephropathy (NNF14OC0013659).
Disclosure: **J.C. Laursen:** None.

OP 41 Building the evidence base for new devices

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Cambridge hybrid closed-loop in very young children with type 1 diabetes: a multi-national 4-month randomised trial

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Background and aims: We assessed safety and efficacy of Cambridge hybrid closed-loop compared with sensor-augmented pump therapy over 16 weeks in very young children with type 1 diabetes (T1D).
Materials and methods: In a multi-national, multi-centre, randomised crossover trial, children aged 1 to 7 years with T1D on insulin pump therapy were recruited from seven centres across the UK, Austria, Luxembourg and Germany. Participants underwent two 16-week periods comparing the CamAPS FX hybrid closed-loop system (CL) with sensor-augmented pump therapy (control) in random order. The primary endpoint was the between group difference in time in target glucose range (3.9 to 10.0mmol/L) measured by sensor glucose levels. Key secondary endpoints were analysed hierarchically and included time in hyperglycaemia (>10.0mmol/L), glycated haemoglobin, mean sensor glucose, and time in hypoglycaemia (<3.9mmol/L). Analysis was by intention-to-treat.
Results: We randomised 74 participants: mean (±SD) age 5±2 years, 58% (n=43) male and baseline HbA1c 7.3±0.7%. The proportion of time glucose was in the target range was 8.7 percentage points (95% CI 7.4 to 9.9) higher during CL compared to control period (p<0.001). Time with glucose >10.0mmol/L was 8.5 percentage points lower (95% CI 7.1 to 9.9; p<0.001) during CL than during control period. Mean glucose was 0.7mmol/L lower (95% CI 0.5 to 0.8; p<0.001), and glycated haemoglobin 0.4% lower (95% CI 0.3 to 0.5; p<0.001) with CL than with control therapy. Time in hypoglycaemia (<3.9mmol/L) was similar between CL and control periods (p=0.74). Mean closed-loop usage was 93±8% over 16 weeks. One severe hypoglycaemia event occurred in CL and none in the control period. There were no other serious adverse events.
Conclusion: The Cambridge hybrid closed-loop system is safe and significantly improves glycaemic control in very young children with T1D, without increasing time in hypoglycaemia.

Hierarchical Endpoints	Closed-loop (n=73)	Sensor-augmented pump (n=74)	Mean adjusted difference (95% CI)[d]	P value[d]
Time glucose 3.9-10.0mmol/L (%)[a]	72 ± 6	63 ± 9	8.7 (7.4, 9.9)	<0.001
Time glucose >10.0mmol/L (%)[b]	23 (19, 27)	32 (23, 40)	-8.5 (-9.9, -7.1)	<0.001
HbA1c (mmol/mol)[b] [HbA1c %]	49.0 ± 5.9 [6.6 ± 0.5]	52.8 ± 7.2 [7.0 ± 0.7]	-3.9 (-4.9, -2.9) [-0.4 (-0.5, -0.3)]	<0.001
Mean glucose (mmol/L)[b]	8.1 ± 0.7	8.8 ± 1.0	-0.7 (-0.8, -0.5)	<0.001
Time glucose <3.9mmol/L (%)[b]	4.9 (3.3, 6.7)	4.5 (2.9, 7.3)	0.07 (-0.4, 0.5)	0.74
Secondary Endpoints[c]				
Time glucose <3.0mmol/L (%)	1.0 (0.6, 1.4)	0.9 (0.4, 1.6)	0.02 (-0.1, 0.1)	0.63
Time glucose >16.7mmol/L (%)	2.0 (1.2, 3.1)	3.1 (1.3, 5.7)	-1.0 (-1.6, -0.6)	<0.001
Glucose SD (mmol/L)	3.3 (3.0, 3.6)	3.6 (3.2, 4.0)	-0.3 (-0.4, -0.3)	<0.001
Glucose CV (%)	41 (39, 43)	41 (38, 44)	-0.7 (-1.5, 0.05)	0.07

Table legend: Outcomes during hybrid closed-loop insulin delivery and sensor-augmented pump therapy over 16 weeks.

Table footnote:
Data are mean±SD, median (IQR).
[a]Primary endpoint.
[b]Tested in hierarchy as listed to control the type 1 error using the fixed-sequence method.
[c]Adjusted for multiple comparisons using Benjamini-Hochberg procedure to control false discovery rate.
[d]Based on linear mixed model adjusting for repeated participant measures, baseline value, period as fixed effects, site as random effect.

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Cambridge hybrid closed-loop in children and adolescents with type 1 diabetes: a multicentre 6-month randomised trial

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Background and aims: We compared safety and efficacy of Cambridge hybrid closed-loop algorithm with usual care over 6 months in children and adolescents with type 1 diabetes.
Materials and methods: In an open-label multicentre, multinational, single-period, parallel randomised controlled trial, participants aged 6-18 years using insulin pump therapy were assigned to closed-loop insulin delivery or usual care (control) for 6 months. We used the Cambridge algorithm in two consecutive hardware iterations, FlorenceM (modified Medtronic 640G pump, Medtronic Guardian 3 sensor, algorithm on locked-down smartphone) or CamAPS FX (Sooil Dana RS pump, Dexcom G6 sensor, algorithm on unlocked smartphone). Primary endpoint was HbA1c at 6 months.
Results: We randomised 133 participants, 65 to closed-loop and 68 to control (mean±SD baseline HbA1c 8.2±0.7% vs 8.3±0.8%). At 6 months mean HbA1c was 0.32% lower with closed-loop compared to control (95%CI 0.04 to 0.59; p=0.02). Closed-loop usage was low and variable (40% [26, 53]; median [IQR]) with FlorenceM due to unreliable hardware, and consistently high (93% [88, 96]) with CamAPS FX. In the cohort using CamAPS FX (n=21), HbA1c was 1.05% lower (95%CI 0.67 to 1.43; p<0.0001) and time in target range 3.9-10.0mmol/L was 15.0 percentage points higher (95%CI 8.0 to 22.1; p=0.0001) compared to control (n=25), without an increase in hypoglycaemia (p=0.15). Severe hypoglycaemia and DKA events were similar between closed-loop and control.
Conclusion: Cambridge hybrid closed-loop algorithm is safe and significantly improves glycaemic control in children and adolescents with type 1 diabetes. Efficacy relies on consistently high usage of closed-loop as demonstrated with CamAPS FX.

	Baseline		6 months			
	Closed-loop (n=21)	Control (n=25)	Closed-loop (n=21)	Control (n=24)	Adjusted difference at 6 months (95% CI)[†]	P value[‡]
HbA1c (mmol/mol)* [HbA1c %]	63 ± 10 [7.9 ± 0.9]	64 ± 6 [8.0 ± 0.6]	51 ± 6 [6.8 ± 0.5]	63 ± 8 [7.9 ± 0.8]	-11.5 (-15.7, -7.3) [-1.05 (-1.43, -0.67)]	<.0001
	Baseline		6 months			
CGM-based metrics**	Closed-loop (n=21)	Control (n=24)	Closed-loop (n=19)	Control (n=24)	Adjusted difference at 6 months (95% CI)[†]	P value[‡]
Time spent at glucose level (%)						
3.9 to 10 mmol/L*	50 ± 11	51 ± 9	63 ± 9	49 ± 13	15.0 (8.0, 22.1)	0.0001
>10 mmol/L	41 ± 14	39 ± 11	24 ± 8	42 ± 17	-18.4 (-26.9, -9.8)	0.0001
>16.7 mmol/L	6.7 (3.7, 9.7)	5.4 (3.0, 9.7)	2.8 (1.8, 5.4)	5.7 (3.1, 11.3)	-3.23 (-8.37, -0.41)	0.03
<3.9 mmol/L	8.6 (6.1, 11.2)	8.7 (4.8, 14.5)	10.8 (5.7, 20.7)	6.3 (1.7, 16.5)	3.13 (-1.25, 7.51)	0.15
<3.0 mmol/L	3.4 (0.9, 4.9)	3.5 (0.6, 6.9)	2.9 (1.6, 6.1)	1.4 (0.2, 6.2)	0.91 (-0.96, 2.49)	0.16
Mean glucose (mmol/L)	9.6 ± 1.7	9.3 ± 1.3	7.8 ± 1.0	9.8 ± 2.1	-1.98 (-3.08, -0.88)	0.0009
Glucose SD (mmol/L)	4.5 ± 0.9	4.3 ± 0.8	3.9 ± 0.8	4.3 ± 1.0	-0.60 (-1.11, -0.09)	0.04
Glucose CV (%)	47 ± 7	46 ± 7	49 ± 8	45 ± 9	2.5 (-1.4, 6.4)	0.21

Table legend: Comparison of outcomes in CamAPS FX closed-loop group and control group

Table footnote:
Data are mean ± SD, median (IQR).
*Primary endpoint
**CGM-based outcomes obtained from 14 days of masked data from Abbott Libre Pro. Minimum of 120 hours of CGM data required to calculate outcomes
[†]A negative difference denotes lower values in the closed-loop group
[‡]Adjusted for baseline value of the metric, baseline HbA1c, age, and site as a random effect.

Clinical Trial Registration Number: NCT02925299
Supported by: National Institutes of Health and National Institutes of Diabetes, Digestive and Kidney Diseases (UC4 DK108520)
Disclosure: **C.K. Boughton:** None.

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Effect of intermittent-scanning CGM to glycaemic control including hypoglycaemia and quality of life of patients with type 1 diabetes (ISCHIA study)

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Background and aims: To investigate the effect of intermittent-scanning CGM (isCGM) combined with structured education focused on the frequent scanning of the sensor (10 times/day or more) and the utilization of the trend arrow to prevent hypoglycemia in patients with type 1 diabetes mellitus, we conducted a crossover study comparing isCGM (Intervention) and the self-monitoring of blood glucose (SMBG) accompanied by masked retrospective CGM (Control).
Materials and methods: The ISCHIA study was a randomized crossover study of type 1 diabetes mellitus patients who used multiple daily injections. The study included a 28-day run-in period, an 84-day sequence 1 (Intervention or Control), a 28-day washout period, and an 84-day sequence 2 (Control or Intervention). Participants continued SMBG three times per day or more throughout the study period in order to comply with the approved labeling of isCGM in Japan that demands adjunctive use of isCGM to SMBG. The primary endpoint was the decrease in the time spent in hypoglycemia (<70 mg/dL) per day.
Results: Out of 104 participants, 102 participants were randomized and 93 participants completed the study. The mean age of the participants was 51.4±15.3 years, 52.7% of the participants were female, the mean HbA1c was 7.3±0.7%, and the rate of isCGM-naïve participants was 46.2%. The time spent in hypoglycemia per day was significantly reduced in the Intervention arm (2.42±1.68 hour/day) compared to the Control arm (3.10±2.28 hour/day) ($P = 0.012$). Time-in-range (TIR [70-180 mg/dl]) was not significantly different between the two arms (14.54±2.66 hour/day vs. 13.75±2.45 hour/day). Time-above-range (>180 mg/dl) was 7.03±3.12 hour/day vs. 7.15±3.50 hour/day, respectively. The change of glycoalbumin (GA) was not significantly different between the two arms (0.3±2.3% vs. 0.1±2.3%). Frequency of severe hypoglycemia (SH) was not significantly different between the two arms (2.1% vs. 6.5%). No severe adverse events related to the study excluding SH occurred. Frequency of non-severe adverse events related to the study was not significantly different between the two arms (17.0% vs. 17.4%).
Conclusion: The usage of isCGM combined with structured education significantly reduced the time spent in hypoglycemia without the deterioration of TIR or GA.
Clinical Trial Registration Number: jRCT1052180075
Supported by: AMED, Japan IDDM Network
Disclosure: **T. Murata:** None.

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Evaluation of accuracy and safety of the next generation 180-day long-term implantable Eversense CGM system: the PROMISE Study

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Background and aims: Use of Continuous Glucose Monitoring Systems (CGM) is being rapidly adopted as standard of care for insulin-requiring patients with diabetes. The PROMISE study was designed to evaluate the accuracy and safety of the next generation implantable Eversense CGM System for up to 180 days.
Materials and methods: The study was prospective, multi-center, unblinded, involving 181 subjects with T1D or T2D at 8 sites in the United States. Accuracy was evaluated by comparing CGM to reference values from a YSI 2300 glucose analyzer (YSI) during 10 clinic visits from day 1-180, which included hyperglycemia and hypoglycemia challenges. Eighty-five (85) subjects had 1 sensor and ninety-six (96) subjects had 2 sensors inserted in the arm. Counting 2 replaced sensors, there were a total of 279 sensors inserted (558 insertions/removals). The CGM prompts 2 calibrations/day until day 21, after it prompts 1 calibration/day. The incidences of device-related and sensor insertion/removal procedure-related serious adverse events up to 180 days post-insertion were evaluated. Precision between two implanted sensors was measured by paired ARD (PARD) and percent coefficient of variation (PCV).
Results: The accuracy analyses were based on 49,613 matched pairs over 180 days. Percent CGM readings within 15/15% and 20/20% of YSI values were 85.6% and 92.9%, respectively, and the overall MARD was 9.1%. MARDs across different glucose ranges from 40-400 mg/dL (2.2-22.2 mmol/L) were ≤9.4% (Table). The MARD across individual visit days between days 1-180 were ≤11.0% with highest MARD of 11.0% (day 1) and lower MARDs of 8.4% (day 30), 7.7% (day 60) and 8.2% (day 90). The CGM System readings within 20/20% by day were 89% (day 1), 91% (day 7), 92% (day 14), 94% (day 22), 95% (day 30), 96% (day 60), 94% (day 90), 93% (day 120), 93% (day 150), and 90% (day 180). The evaluation for precision yielded a PARD of 10.1% and PCV of 7.1%, demonstrating high precision between the two sensors in an individual patient. The confirmed hypoglycemia detection rates at 70 mg/dL (3.8 mmol/L) and 60 mg/dL (3.3 mmol/L) were 93% and 87%, respectively. Hyperglycemia detection rate at 180 mg/dL (10 mmol/L) was 99%. There were no device or insertion/removal procedure-related

serious adverse events. Two mild skin infections occurred. All sensors were removed during the initial removal procedure.
Conclusion: We conclude that next generation Eversense long-term implantable CGM System has sustained accuracy and safety up to 180 days with primarily one calibration/day.

YSI Glucose Ranges mg/dL (mmol/L)	Percent 15/15% of Reference	Percent 20/20% of Reference	Mean Absolute Relative Difference (%)	Median Absolute Relative Difference (%)
Overall	85.6	92.9	**9.1 (8.7, 9.5)**	**6.7**
40-60* (2.2-3.3)	83.2	89.4	9.4 (8.4, 10.5)	7.0
61-80* (3.4-4.4)	84.1	92.2	8.8 (8.2, 9.4)	7.0
81-180 (4.5-10)	82.7	90.9	9.0 (8.6, 9.4)	6.7
181-300 (10.1-16.6)	87.9	94.7	7.7 (7.3, 8.1)	5.9
301-350 (16.7-19.4)	90.6	96.5	7.1 (6.7, 7.6)	5.9
351-400 (19.5-22.2)	87.8	93.9	8.0 (6.7, 9.3)	6.3
*The absolute difference from the YSI reading is measured in mg/dL if the YSI reading is ≤80 mg/dL				

Table: Sensor vs YSI Agreement by Glucose Range During the Entire 180 Day Period

Clinical Trial Registration Number: NCT03808376
Supported by: The study was funded by Senseonics, Inc
Disclosure: **S. Garg:** None.

OP 42 Cardiovascular disease: predictors and outcomes

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Respective predictive value of glycation gap and silent myocardial ischaemia for cardio-vascular events in asymptomatic patients with type 2 diabetes

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Background and aims: Protein glycation is involved in diabetic complications. It can be evaluated by the glycation gap (GG), difference between glycation in the erythrocyte (HbA1c) and the plasma compartments (fructosamine). In a diabetic population, an association has been reported between GG and history of cardio-vascular events (present in 30% of the population) and mortality. The present study aimed to analyse the predictive value of GG for major cardiac (MCE) or cardio-vascular (MCVE) events in patients with type 2 diabetes (T2D) free of cardio-vascular history or symptom who had been assessed for silent myocardial ischemia (SMI).
Materials and methods: We included 741 T2D patients who had concomitant HbA1c and fructosamine measurements. Using the strong correlation between HbA1c and fructosamine we calculated predicted HbA1c and GG (= measured HbA1c - predicted HbA1c). The patients had a stress myocardial scintigraphy to detect SMI, with a coronary angiography in those with SMI.
Results: SMI was present in 221 patients, 83 of them had significant coronary stenoses (CS) on angiography. Among the 695 patients who could be followed (during 5.4±3.6 years), a MCE (acute coronary syndrome, congestive heart failure, secondary coronary revascularization or cardiac death) occurred in 65 patients, and a MCVE (MCE or amputation or peripheral revascularization) in 107 patients. The patients were separated in 2 groups: GG ≥1% (n=154) or <1% (n=587). The prevalence of SMI and CS, and the incidence of MCE (12.6% and 8.5% respectively; p=0.147) and MCVE (18.9% and 14.5%, p=0.195) did not differ significantly between the 2 groups. The incidence of MCE and MCVE was higher in the patients with SMI compared to those without (16.7% *vs* 6.2% and 26.2% *vs* 10.7%; p<0.0001 for both comparisons). In Cox logistic regression, SMI (OR 2.3[1.4-3.8], p<0.001) but not GG predicted MCE and MCVE.
Conclusion: These results do not support the predictive value of GG for cardio-vascular events in T2D patients free of cardio-vascular history but confirm the good predictive value of SMI.
Disclosure: **P. Valensi:** None.

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Validation study for coronary heart disease (CHD) among diabetes patients based on automatic retinal image analysis (ARIA) method

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Background and aims: Coronary heart disease (CHD) is common age-related disease that constitute a growing health and social burden, especially for long-term management of diabetes patients. Although the existing diagnostic tools for this disease is reliable, their complexity and high cost hinder their use for screening the general population, while risk engines based on traditional risk factors have shown poor performance. Thus, there is an urgent need for new, simple and inexpensive high-accuracy techniques for use as complementary screening tools. In this

study, we employed data from Hong Kong for training a risk assessment model for coronary heart disease, and evaluate if retinal image analysis can be used as risk assessment tool for diabetes complications management in UK.

Materials and methods: Patients in the training data were enrolled from the Cardiology Department of a Traditional Chinese Medicine Hospital (TCM) from December 2017 to September 2019. Baseline demographic and medical information were collected from participants' hospital medical records. Retinal images of both eyes of each participant were taken within six months of admission. A total of 388 subjects were included in this study. Among them, 272 subjects were used for training of the classification model. They included 132 CHD patients obtained from TCM, and 140 controls obtained from a lifestyle study with healthy individuals. A set of 116 subjects with 56 CHD and 60 controls were used as testing data. All subjects have their retinal images taken by a non-mydriatic fundus camera (Canon CR2). A fully automatic retinal image analysis for CHD risk (ARIA-CHD) was developed to estimate retinal microvascular characteristics and incorporate machine-learning technique to derive an overall estimation of CHD risk. An external testing data include 43 CHD patients from UK and 61 controls from the lifestyle study.

Results: The training analysis has very high accuracy with sensitivity and specificity of 92.9% and 91.7% respectively. The 10-fold cross validation using support vector machine (SVM) approach has sensitivity of 91.5% and specificity of 88.0%. A validation analysis using 61 control subjects from CCRB, 56 CHD data from TCM, and 43 CHD from UK achieved high accuracy in classification with mean CHD probabilities of 0.28 (95% CI of 0.24, 0.32), 0.73 (95% CI of 0.69, 0.77), and 0.73 (95% CI of 0.69, 0.76) in the control group and CHD groups of the lifestyle study and UK respectively. The 95% confidence intervals for two CHD groups were overlapping but significantly different from control group, indicating good performance in classification.

Conclusion: Method to estimate CHD risk based on retinal images has been developed and validated. ARIA-CHD is a fast, convenient and non-invasive alternative for the community pre-screening program for CHD. The retinal images in UK have similar accuracy for CHD detection using the model developed by retinal images from Hong Kong and TCM.

Disclosure: **D.R. Owens:** None.

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Cardiovascular and renal diseases in type 1 compared with type 2 diabetes: a nationwide observational study

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Background and aims: Type 1 diabetes (T1D) and type 2 diabetes (T2D) increase risks of cardiovascular (CV) and renal disease compared with diabetes-free populations. There are only few studies comparing T1D and T2D for the risk of these clinical events. We examined these issues in a nationwide analysis in France.

Materials and methods: All patients aged >=18 seen in French hospitals in 2013 with at least 5 years of follow-up were identified and categorized by their diabetes status. A total of 50,623 patients with T1D (age 61.4 ±18.6, 53% male) and 425,207 patients with T2D (age 68.6±14.3, 55% male) were followed over a mean period of 4.3±2.1 years (median 5.3, interquartile 2.8-5.8 years). Prevalence and event rates of myocardial infarction (MI), heart failure (HF), ischemic stroke, chronic kidney disease (CKD), all-cause death and CV death were assessed with age stratification of 10-year intervals. Cox regression analyses were used to estimate risk with adjustment on sex and age.

Results: The age and sex-adjusted prevalence of CV diseases was higher in T2D for ages above 40 years whereas the adjusted prevalence of CKD was more common in T1D between ages 18 and 69 years and higher in T2D for ages above 80 years. During 2,033,239 person-years of follow-up, there were 27,497 patients with MIs (yearly rate 1.4%), 24,892 with ischemic strokes (yearly rate 1.2%), 100,769 with incident HF (yearly rate 5.4%), 65,928 with incident CKD (yearly rate 3.4%) and 197,858 deaths (yearly rate 9.7%) including 49,026 CV deaths (yearly rate 2.4%) were recorded. Age and sex-adjusted event rates comparing T1D versus T2D showed that MI risk was increased for ages above 60 (1.2-fold for T1D versus T2D) and HF between ages 18-29 and above 60 years (1.1-1.4-fold). Adjusted risk of ischemic stroke did not markedly differ between T1D and T2D. Risk of incident CKD was 1.1-2.4-fold higher in T1D between ages 18-49 and above 60 years. The all-cause death risk was 1.1-fold higher in T1D at age >=60 years, the cardiovascular death risk being 1.1-fold higher in T1D between 60 and 69 years.

Conclusion: The adjusted prevalent burden and risk of incident renal disease are greater among patients with T1D compared with T2D patients across most ages. Although the prevalent burden of cardiovascular diseases may be lower in T1D than in T2D, patients with T1D may have a higher risk of incident MI, HF, all-cause death and cardiovascular death at middle-older ages, highlighting their need for improved prevention.

Disclosure: **D. Angoulvant:** None.

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A longitudinal study on the incidence of cardiovascular events in a population of Northern Italy

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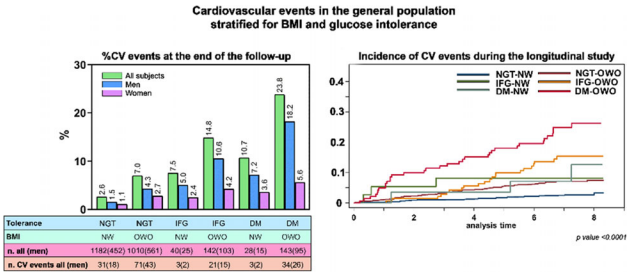
Background and aims: The CA.ME.LI.A (CArdiovascular risks, MEtabolic syndrome, Liver, and Autoimmune disease) study is a project promoted by University of Milan in 2009-2011, aimed to identify risk factors for cardiovascular (CV), metabolic and liver diseases in a population representative of the Northern Italy. Here we aim to assess whether impaired glucose metabolism (IGM) and/or overweight/obesity (OWO) are independently associated to an increased CV risk.

Materials and methods: Time 0 was the enrolling in the study; the observation ended on August 30th, 2017, or at the first CV event (recovery or death), or with the loss of the subject to the follow-up. The study of the incidence of events was conducted through the Kaplan-Meier method using log rank test. A univariate analysis of CV events and continuous variables was conducted with Cox time-dependent models to a single variable. The variables that, to the univariate analyses carried out by means of the log rank test or of a time-dependent model of Cox to a single variable (continuous variables), were significantly associated with the incidence of CV events, were introduced in a multivariate time-dependent Cox model to identify those that had an independent prognostic value. The population (n=2545, 1257 men) was stratified into 6 categories: 1. Normal glucose tolerance (NGT) (FG<110 mg/dL)/ BMI≤24.9 kg/m^2 (Normal weight, NW); 2. NGT/ BMI ≥25 kg/m^2 (overweight or obese, OWO); 3. Impaired fasting glucose (IFG) (FG 110-125 mg/dL)/ NW; 4. IFG/OWO; 5. Diabetes (DM) (FG≥126 mg/dL)/NW; 6. DM/ OWO.

Results: During the follow-up, 163 CV events occurred with an incidence rate of about 1.5 cases per 100 year-patient; men had a higher number of events as compared to women (106 vs 57; 4.2% vs 2.2%, p=0.001, Fig.1). DM had a grater incidence of events compared both to IFG and NGT (IFG 13% vs DM 21%, p=0.02; NGT 5%, p<0.0001). Even OWO was associated with higher CV risk (NW 3% vs OWO 10%, p<0.0001). When a IGM coexisted with OWO, the incidence of CV events further increased, with DM/OWO being higher than in IFG/OWO (24% vs 15% p=0,0001); in NGT/NW the incident was 3%. Considering the association between IGM and OWO, we found that there wasn't difference in the number of events between only DM and DM/OWO (p=0.09). Instead, there was a difference in

CV events between OWO and DM/OWO (p<0.0001) and between OWO and IFG/OWO (p=0.0016).

Conclusion: Survival curves shows that in both sexes OWO, IFG and DM show higher mortality than NW and NGT. Among OWO patients, the concomitant presence of diabetes leads to a higher risk of events or death: DM/OWO have a higher incidence of CV events than only OWO. Also IFG increases mortality compared to NGT subjects as well as IFG/OWO have significantly more CV events than OWO only. These data suggest that not only DM but also IFG subjects need to be treated to prevent CV.



Supported by: Regione Lombardia, MIUR, Dipartimento di Scienze della Salute dell'Università degli Studi di Milano

Disclosure: **E. Bianco:** None.

OP 43 Genes, epigenes and telomeres in type 1 diabetes

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Birth weight, BMI in adulthood and latent autoimmune diabetes in adults: a Mendelian randomisation study

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Background and aims: Latent autoimmune diabetes in adults (LADA) is a hybrid form of diabetes with adult onset and a pathogenesis that includes both type 1-like and type 2-like features. Observational studies found that both lower birth weight and higher adult BMI were associated with an increased risk of LADA. The present study aimed to revisit these findings using two-sample Mendelian randomization (MR).

Materials and methods: Forty-four independent SNPs of genome-wide significance were selected as instruments for birth weight from a genome-wide association (GWAS) study including individuals of European descent from UK Biobank and 30 other studies. 820 independent SNPs were identified as instruments for BMI in adulthood from a GWAS study including individuals of European ancestry from UK Biobank and the GIANT (Genetic Investigation of ANthropometric Traits) study. The selected instruments explained 1.62% variance in birth weight and 7.43% variance in adult BMI. Summary statistics for the SNP-LADA associations were extracted from the GWAS study conducted in 2634 LADA cases and 5947 population controls of European ancestry. Inverse-variance weighted (IVW) method was used to examine the causal association, supplemented by several sensitivity analyses, namely robust IVW, weighted median, MR-Egger, MR-PRESSO (Mendelian randomization pleiotropy residual sum and outlier test), and a conservative analysis excluding hypothetically pleiotropic SNPs.

Results: One SD decrease in genetically predicted birth weight was associated with a 69% higher risk of LADA (OR: 1.69, 95% CI: 1.13-2.53). All sensitivity analyses showed the same direction of association as found in the main analysis. MR-Egger (P for directional pleiotropy: 0.119) and MR-PRESSO (P for global pleiotropy: 0.173) indicated no directional pleiotropy. One SD increase in genetically predicted BMI in adulthood was associated with a 40% (OR: 1.40, 95% CI: 1.14-1.71) higher risk of LADA, and sensitivity analyses also showed similar risk estimates. MR-PRESSO detected two outliers for adulthood BMI but detected no distortion in risk estimates by the outliers.

Conclusion: These findings provide genetic support for a causal link between low birth weight and adult overweight in relation to LADA.

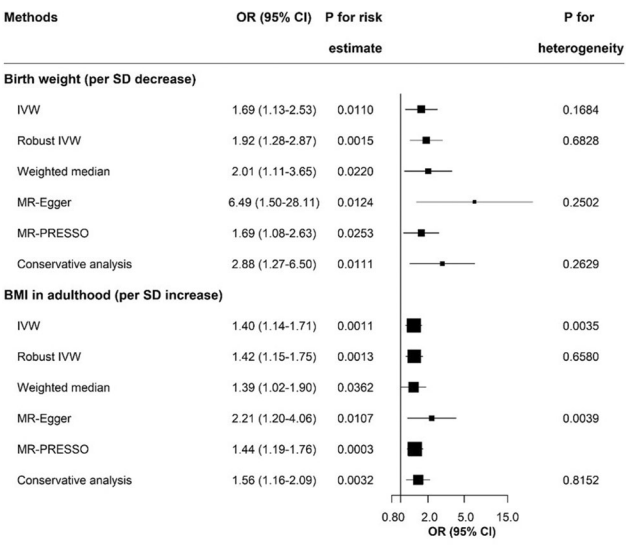

Figure 1. Risk of LADA in relation to genetically determined birth weight and BMI in adulthood

Supported by: CSC, VR, FORTE, and Novo Nordisk Foundation
Disclosure: **Y. Wei:** None.

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The influence of HLA haplotype, number of injections and administration route on the effect of GAD-specific immunotherapy in type 1 diabetes

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Background and aims: Genetic risk for T1D is largely associated with variations in HLA genes as is individual disease pathology. In line with this, we have previously shown that immunomodulatory treatment with GAD-alum has a positive effect specifically in individuals carrying the high-risk HLA haplotype DR3-DQ2. In this study we sought to further investigate the influence of HLA, number of injections and administration route on the clinical effect of GAD-alum treatment in preserving endogenous insulin production in individuals with recent-onset T1D.
Materials and methods: We combined individual-level data (n=627) from four placebo controlled randomized GAD-alum clinical trials in individuals recently diagnosed with type 1 diabetes. We estimated the treatment effect at 15 months from baseline on C-peptide retention and HbA1c, using mixed model repeated measures including terms for subgroups defined by HLA haplotypes DR3-DQ2 and DR4-DQ8 as well as number of doses. The effect of administration route (subcutaneous vs intralymphatic) was evaluated using a Bayes method.
Results: Analysis showed a treatment effect ratio compared to placebo of 1.18 in the full population (P=0.001), 1.36 in individuals carrying HLA DR3-DQ2 (n=313; 95% CI 1.18, 1.57; P<0.0001), and 1.57 in individuals carrying HLA DR3-DQ2 but not DR4-DQ8 (n=149; 95% CI 1.30, 1.89; P<0.0001). The effect was highest in subjects receiving a higher number of injections, three or four, with an estimated treatment effect ratio of 1.48 (95% CI 1.26,1.74; P<0.0001) in individuals carrying DR3-DQ2, and 1.77 in individuals carrying DR3-DQ2 but not DR4-DQ8 (95% CI 1.43, 2.19; P<0.0001). GAD-alum treatment also had a significant effect on lowering HbA1c compared to placebo, again highest in subjects receiving a higher number of doses where an effect of -4.79 mmol/mol was seen in individuals carrying the DR3-DQ2 haplotype (95% CI -8.08, -1.50; P<0.01) and -6.84 mmol/mol individuals carrying DR3-DQ2 but not DR4-DQ8 (95% CI -11.71, - 1.97, P<0.01). There was a 98% and 99% probability that three intralymphatic injections were superior to three subcutaneous injections regarding effects on C-peptide retention and HbA1crespectively.
Conclusion: These analyses highlight the importance of genetics, dosing regimen and route in immunotherapeutic treatment of type 1 diabetes and a clinically relevant potential for using intralymphatic GAD-alum.

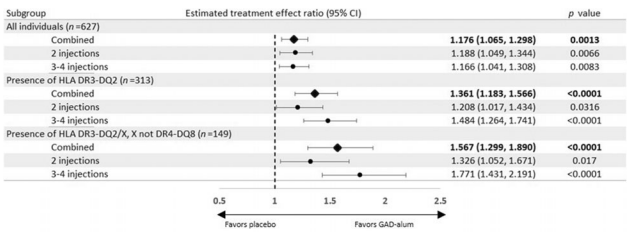

Figure 1 - Estimated treatment effect ratio (C-peptide retention; active vs placebo) at 15 months post baseline. Nominal p values are reported along with two-sided 95% CI.

Disclosure: **U. Hannelius:** Employment/Consultancy; I am employed by Diamyd Medical that develops GAD-alum. Stock/Shareholding; I own shares in Diamyd Medical that develops GAD-alum.

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Epigenome-wide association study identifies differentially methylated DNA sites for diabetic kidney disease in type 1 diabetes

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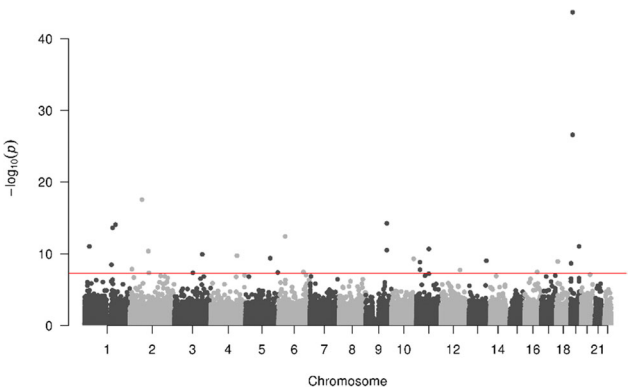
Background and aims: Epigenetic modification such as DNA methylation impact the transcription of genes and it can have long-term effects on disease. The aim of this study was to identify differentially methylated CpG sites (dmCpGs) in whole-blood DNA, associated with diabetic kidney disease (DKD) in individuals with type 1 diabetes.
Materials and methods: This study included 651 individuals with DKD and 651 individuals with long-duration type 1 diabetes and no evidence of DKD matched for age, sex and diabetes duration from the Finnish Diabetic Nephropathy study (FinnDiane, n=800) and the United Kingdom-Republic of Ireland (UK-ROI, n=502). An epigenome-wide analysis of 862,927 CpGs sites assessed with the Illumina MethylationEPIC BeadChip was performed using the RnBeads and Bioconductor R packages for each cohort separately. Results from both cohorts were meta-analysed based on p-values and weighted by sample-size using the METAL software.
Results: Altogether six hypermethylated and 25 hypomethylated sites were associated ($P_{FDRadj} \leq 1.0x10^{-8}$) with DKD after adjusting for age, sex, and cell-type heterogeneity (Figure 1). Of these, cg17944885 ($P_{FDRadj} = 2.0x10^{-44}$) and cg25544931 ($P_{FDRadj} = 2.3x10^{-37}$), located between *ZNF788* and *ZNF20* on chromosome 19, were the most significant hypermethylated sites across both cohorts. This site has previously been associated with kidney disease, also in diabetes. Strongest hypomethylation was identified for dmCpGs in *LOC101927438 (*cg05710777*,* $P_{FDRadj} = 3.0x10^{-18}$), *ROD1* (cg00008629, $P_{FDRadj} = 5.4x10^{-15}$) and *NME7* (cg08150816, $P_{FDRadj} = 8.2x10^{-15}$), of which none have previously been associated with kidney disease. Further adjustment for smoking status reduced the number of epigenome-wide significant dmCpGs to 18, but the top-ranked dmCpGs remained. After additional adjustment for diabetes duration, BMI, HbA_{1c}, HDL cholesterol and triglycerides, seven dmCpGs remained ($FDRadjp \leq 1.0x10^{-8}$) across both cohorts. The top-ranked dmCpGs located in genes including *ROD1*, *NME7*, *SLC27A3*, *SLC1A5* and *LOC101927438* remained consistently associated across each of the analyses.
Conclusion: We find that whole blood DNA methylation in individuals with type 1 diabetes are significantly associated with DKD at several dmCpGs, even after adjustment for major confounders, with the majority demonstrating a hypomethylation profile.



Supported by: NIH (1R01DK105154-01A1) GENIE II
Disclosure: **E.H. Dahlstrom:** None.

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Telomeres do not always shorten over time in people with type 1 diabetes: a FinnDiane substudy

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Background and aims: Telomeres are repetitive sequences at chromosome ends that maintain chromosome integrity. Shorter telomeres in white blood cells (WBC) are associated with, and predictive of, poor health outcomes, including in relatively small studies in people with type 1 diabetes (T1D). Both genetic and acquired factors modulate telomere length (TL) and their shortening rate. There are cellular mechanisms to maintain and even increase TL. Our aim was to characterise changes in telomere length in adults with T1D over time, and the associated clinical variables.

Materials and methods: Adults with T1D in the Finnish Diabetic Nephropathy (FinnDiane) cohort (n=618) with WBC DNA from two time-points (median [range] follow-up 6.8 [2.8-17.7] years were studied. Relative TL (rTL) was measured by quantitative PCR, with intra- and inter-assay CVs of 1.8% and 4.0%, respectively. Both samples (in triplicate) from a subject were on the same plate. rTL change was calculated as % change per annum (p.a.) and a total change of <4% (assay CV) was considered stable. Central obesity was defined as waist-height-ratio ≥0.5. Albuminuria was defined as AER ≥30 mg/24 h in two out of three consecutive urine collections.

Results: At baseline, mean (SD) age and T1D duration was 36.7 (12.1) and 22.2 (11.6) years, 49.2% were male, and 23.7% had albuminuria. Baseline rTL correlated inversely with age (r= -0.093, p=0.021) and was shorter in men vs. women (-0.38 vs. -0.094, p=0.0011, age-adjusted). Comparing the 1st and 3rd baseline rTL tertile, the 1st (shortest rTL) tertile was older (+3.5 years), had more men (+19.9%), and individuals with albuminuria (+9.2%). In adjusted models (sex, age), the shortest rTL group was prescribed more antihypertensive medication (AHT, 38.5 vs. 23.8%, p=0.019), had higher BMI (25.8 vs. 24.6 kg/m^2, p=0.003), more central obesity (55.8 vs. 33.0%, p=2.8×10^{-4}), lower eGFR (81.4 vs. 90.4 mL/min/1.73 m^2, p=0.004), but no difference in HbA_{1c} or frequency of albuminuria. Baseline rTL was a strong determinant of rTL change (r=-0.66, p=5.4×10^{-78}). Over time, telomeres shortened in 342 (55.3%) subjects, lengthened in 247 (40.0%) individuals and remained stable in 4.7%. In the 1st vs. 2nd plus 3rd baseline rTL tertiles, rTL %-change was median (IQR) +5.3 (-0.9, 15.6) % p.a. vs. -3.3 (-7.3, 0.6) % p.a., p=3.0×10^{-21}. Higher baseline BMI and central obesity were associated (linear) with increasing rTL after adjustment for sex and age. Association with BMI and rTL change remained significant after adjusting for baseline rTL (Beta=0.24, p=0.030). Those in whom rTL increased (vs. those whom rTL decreased) were more likely to start AHT during follow-up (24.5 vs. 17.4%, p=0.047, adjusted for sex, age, and baseline AHT). HbA_{1c} and change in albuminuria were not associated with TL change.

Conclusion: In a T1D longitudinal study, rTL inversely correlated with age, and over median 6.8 years, did not shorten in 44.7%. Associates of change in TL over time were higher baseline BMI, central obesity, rTL and the start of AHT during follow-up. The biology of these relationships and clinical significance of TL change over time in people with T1D merit further investigation.

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Disclosure: **A. Syreeni:** None.

OP 44 Diabetes around the clock!

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The influence of residual beta cell function upon free-living postprandial and nocturnal glycaemic control in individuals with type 1 diabetes

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Background and aims: Residual β-cell function can persist many years after diagnosis of type 1 diabetes (T1D), indicated by low concentrations of stimulated endogenous insulin and C-peptide. Residual β-cell function is associated with improved free-living glycaemic control, measured by continuous glucose monitoring (CGM). However, it is unclear which glucose excursions throughout the day are improved by retaining low levels of functional β-cells. We explored whether β-cell function affects nocturnal and postprandial glycaemic control.

Materials and methods: Individuals with T1D >1 year (n=66, M/F: 35/31, age: 41 ± 12, diabetes duration: 21 ± 13 years, HbA1c: 58 ± 10 mmol/mol, BMI: 25 ± 3 kg/m^2) completed a postprandial urine C-peptide creatinine ratio test (UCPCR), wore a CGM, and kept a food and insulin diary for 7 days in free-living conditions. Participants were split by UCPCR into groups: undetectable ($Cpep_{und}$ [UCPCR < 0.001 nmol/mmol], $n = 36$), low ($Cpep_{low}$ [0.001 - 0.19], $n = 13$), and high ($Cpep_{high}$ [≥ 0.2], $n = 19$). CGM outcomes were compared between groups using one-way ANOVA for 24hrs (00:00-00:00 hrs), nocturnal (00:00-06:00 hrs), and postprandial (0-300 mins) periods.

Results: Data are presented in Table 1 as means ± SD (significance accepted at $p \leq 0.05$). **24hrs:** $Cpep_{high}$ spent more time in euglycaemia (3.9-10mmol/L) (72 ± 17%) than $Cpep_{low}$ (62 ± 13%, $p = 0.2$), and significantly more time than $Cpep_{und}$ (58± 20%, $p = 0.028$) overall. $Cpep_{high}$ spent significantly less time in hyperglycaemia (>13.9mmol/l) than $Cpep_{und}$ ($p < 0.001$). SD glucose was significantly lower in $Cpep_{high}$ than $Cpep_{und}$ ($p < 0.001$). **Nocturnal:** $Cpep_{high}$ spent significantly more time in euglycaemia (76 ± 20%) than $Cpep_{low}$ (60 ± 20%, $p = 0.025$) and $Cpep_{und}$ (58 ± 20%, $p = 0.002$) overnight. $Cpep_{high}$ spent significantly less time in hypoglycaemia (<3.9mmol/l) ($p = 0.023$) and hyperglycaemia (10-13.9mmol/l) ($p = 0.036$) and had lower mean and SD glucose than $Cpep_{und}$. **Postprandial:** $Cpep_{high}$ spent more time in euglycaemia (68 ± 22%) than $Cpep_{low}$ (53 ± 23%, $p = 0.2$), and significantly more time than $Cpep_{und}$ (51± 22%, $p = 0.045$) postprandially. There were no group differences in mean, SD, or CV glucose ($p > 0.05$).

Conclusion: T1D with higher residual β-cell function demonstrate improved free-living glycaemic control, specifically with greater protection from nocturnal and postprandial dysglycaemia. Further research, patient education, and clinical management should focus on improving nocturnal and postprandial glycaemia. This is of particular importance in those with no residual β-cell function.

Glycaemic outcome	Overall (00:00-00:00hrs)			Nocturnal (00:00-06:00hrs)			Postprandial (0-300mins)		
	$Cpep_{und}$	$Cpep_{low}$	$Cpep_{high}$	$Cpep_{und}$	$Cpep_{low}$	$Cpep_{high}$	$Cpep_{und}$	$Cpep_{low}$	$Cpep_{high}$
Mean Glucose	8.90 ± 1.48	8.43 ± 1.62	8.06 ± 1.59	8.72 ± 2.21	8.28 ± 2.17	7.21 ± 1.73*	9.58 ± 2.29	9.59 ± 1.92	8.97 ± 1.69
SD Glucose	3.42 ± 0.67	3.08 ± 0.72	2.79 ± 0.94*	1.52 ± 0.43	1.29 ± 0.52	1.12 ± 0.41*	2.28 ± 0.85	1.91 ± 0.58	1.81 ± 0.59
CV Glucose	0.38 ± 0.07	0.37 ± 0.08	0.34 ± 0.07	0.19 ± 0.05	0.16 ± 0.06	0.16 ± 0.06	0.24 ± 0.06	0.20 ± 0.05	0.20 ± 0.05
% Time > 13.9 mmol/L	10.46 ± 7.99	6.84 ± 5.69	5.74 ± 9.39*	11.47 ± 11.90	4.08 ± 6.21	3.26 ± 6.28	15.24 ± 13.35	12.19 ± 8.48	6.92 ± 11.27
% Time 10-13.9 mmol/L	23.38 ± 10.60	23.41 ± 12.13	17.28 ± 11.20	21.82 ± 16.13	25.77 ± 22.73	13.47 ± 16.46*	26.84 ± 18.43	32.35 ± 16.46	23.71 ± 15.86
% Time > 10 mmol/L	33.84 ± 16.51	30.24 ± 16.67	23.02 ± 17.18	33.38 ± 23.81	29.85 ± 24.53	14.79 ± 18.30	42.09 ± 26.39	44.54 ± 22.30	30.62 ± 21.75
% Time 3.9- 10 mmol/L	60.53 ± 14.59	62.25 ± 13.17	71.78 ± 16.99*	57.74 ± 20.34	59.77 ± 20.24	76.37 ± 19.89*†	51.41 ± 21.68	53.36 ± 23.33	68.05 ± 21.85*
% Time <3.9 mmol/L	5.63 ± 6.15	7.51 ± 8.52	5.19 ± 4.45	9.03 ± 12.11	10.38 ± 12.98	8.95 ± 11.92*	6.50 ± 10.55	2.09 ± 3.64	1.32 ± 2.22
% Time 3.0-3.9 mmol/L	3.70 ± 3.41	5.95 ± 7.10	4.23 ± 3.64	5.29 ± 7.11	2.09 ± 3.64	1.15 ± 2.13	4.47 ± 7.05	2.09 ± 3.64	1.15 ± 2.13
% Time < 3mmol/l	1.93 ± 3.46	1.56 ± 1.94	0.96 ± 1.31	2.03 ± 5.10	0.00 ± 0.00	0.18 ± 0.40	2.03 ± 5.10	0.00 ± 0.00	0.18 ± 0.40

Table 1: Overall, nocturnal, and postprandial glycaemic outcomes (means ± SD) as measured by CGM amongst UCPCR groups: $Cpep_{und}$ (*UCPCR < 0.001 nmol/mmol, n = 36*), $Cpep_{low}$ (*0.001 - 0.19, n = 13*), $Cpep_{high}$ (*≥ 0.2, n = 19*). SD, Standard deviation of interstitial glucose; CV, coefficient of variation of interstitial glucose. * Significantly different to $Cpep_{und}$, † significantly different to $Cpep_{low}$.

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Diurnal rhythms in the human skeletal muscle metabolome are altered in insulin resistant individuals

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Background and aims: Up to ~10-20% of the human muscle transcriptome, lipidome and metabolome have been shown to display 24h rhythmicity. These diurnal changes in muscle metabolism likely facilitate transitions between glucose and fat metabolism in the fed and fasted state, respectively. As insulin resistant (i.e. pre-diabetes) older individuals are characterized by glucose intolerance and metabolic inflexibility, we hypothesized that diurnal rhythms in muscle metabolism are disturbed in this population. Therefore, we here compared diurnal rhythms of the skeletal muscle metabolome between insulin resistant older individuals and young, healthy volunteers, measured under controlled 24h conditions.
Materials and methods: Five sequential skeletal muscle biopsies were obtained from 12 older insulin resistant men (65±9 years, BMI: 30±3 kg/m²) and 12 young healthy men (22±2 years, BMI: 22±2 kg/m²) over the course of 24h, i.e. at 8AM, 1PM, 6PM, 11PM and 4AM. Volunteers were provided with three standardized meals, and biopsies were taken directly before meals to prevent direct postprandial effects. Muscle metabolites were determined in these muscle biopsies using UPLC/HRMS-based semi-targeted metabolomics. Individual metabolites were classified as rhythmic according to 24h cosinor analysis. If metabolites were classified as rhythmic in both groups, the *Circacompare* mixed model was used to detect differences in mean levels and amplitude and to detect phase shifts. For non-rhythmic metabolites and metabolites that were only rhythmic in one group, a 2-way repeated measures mixed-effects model was used to detect differences over time between groups.
Results: Out of 115 metabolites that could be detected in both groups, 48 were rhythmic in the young healthy men and 51 in the older insulin resistant adults. 35 were rhythmic in both groups. The 13 metabolites that were only rhythmic in young men included e.g. coenzyme A, adenosine, asparagine and kynurenic acid. The 16 metabolites that were only rhythmic in insulin resistant men included e.g. ADP, glucose, glycerate, citric acid, histidine, lactate and uric acid. From the 35 shared rhythmic metabolites, most metabolites displayed higher mean levels in the young healthy men, such as hydroxybutyrate. In contrast, ADP-ribose, flavin adenine dinucleotide (FAD), malate and methionine had higher mean levels in insulin resistant men. FAD and uridine diphosphate were phase delayed, i.e. peaked at a later time of the day, in insulin resistant men. Interestingly, differences in metabolite levels between groups were most pronounced during the night at 4AM, with 38 metabolites being significantly different between groups.
Conclusion: Diurnal rhythms in the muscle metabolome were altered in insulin resistant men with some metabolites losing but others gaining rhythmicity. Differences in the muscle metabolome were especially apparent during the night, which might be linked to the reduced switch from glucose to fat oxidation observed upon insulin resistance. Future studies should explore how diurnal rhythms in the muscle metabolome can be modified and which metabolites, that are affected during the night, can be mechanistically linked to the metabolic inflexibility.

Clinical Trial Registration Number: NCT03733743
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Disclosure: **J. Harmsen:** None.

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Diurnal pattern of meal tolerance and insulin sensitivity in type 2 diabetes

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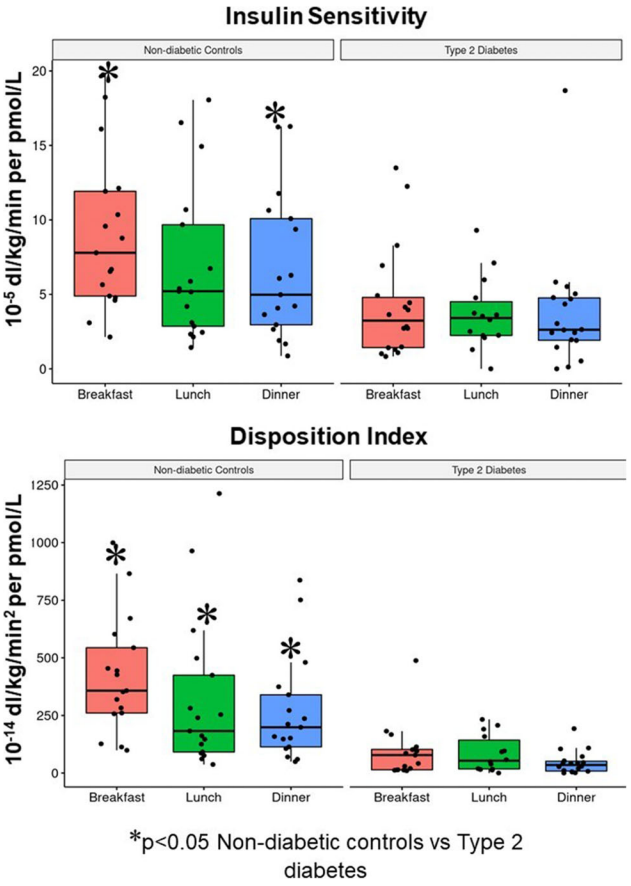
Background and aims: We have previously shown that individuals with type 2 diabetes (T2DM) have nocturnal hyperglycemia due to increased endogenous glucose production (EGP). The objective of this study was to assess the diurnal regulation of carbohydrate metabolism and insulin sensitivity in individuals with and without T2DM.
Materials and methods: 19 T2DM [8F; Age: 60±11 yrs; BMI:32 ±5 kg/m^2] and 19 anthropometrically matched non-diabetic (ND) [11 F; Age: 53±12 yrs; BMI:29±5 kg/m^2] subjects were studied during breakfast (B), lunch(L) and dinner (D) with identical mixed meals (75 gm carbs) on three consecutive days in randomized Latin square design. The triple tracer technique was used to estimate meal rate of appearance (MRa), endogenous glucose production (EGP), and rate of glucose disappearance (Rd). Indices of Insulin sensitivity (SI), insulin secretion (Φ) and β-cell responsivity (Disposition Index: DI) were estimated using the oral glucose minimal model and the C-peptide model, respectively.
Results: Fasting and post meal glucose concentrations (iAUC) were lower ($p< 0.01$) in ND than T2DM subjects after all three meals. In contrast, insulin iAUC was higher ($p<0.05$) in ND vs. T2DM subjects after L and D but not after breakfast. Of note insulin concentrations achieved following D was significantly lower in T2D than what was observed at B, L. MRa did not differ between ND and T2DM subjects for all meals. However, fasting EGP was higher ($p<0.02$) and post prandial EGP was less suppressed ($p<0.05$) in T2DM than ND subjects during all three meals. Rd was significantly higher ($p<0.01$) in ND than T2DM subjects after all three meals. SI was significantly higher ($p<0.05$) in ND than T2DM subjects during B and D but though numerically higher did not reach statistical significance during L ($p=0.07$). Φ and DI were significantly higher ($p<0.01$) in ND than T2DM subjects for all three meals.
Conclusion: These data demonstrate 1) the presence of a diurnal pattern of carbohydrate tolerance in T2DM which differs from what is observed in ND subjects and 2) impairment of insulin sensitivity and beta cell function in T2D responsible for impaired glucose tolerance throughout the day. We conclude that the lower DI in T2D coupled with lower Rd and higher EGP following dinner may be partly responsible for the nocturnal hyperglycemia previously observed.



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Disclosure: **Y. Yadav:** None.

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The effect of melatonin on incretin hormones: results from experimental and randomised clinical studies

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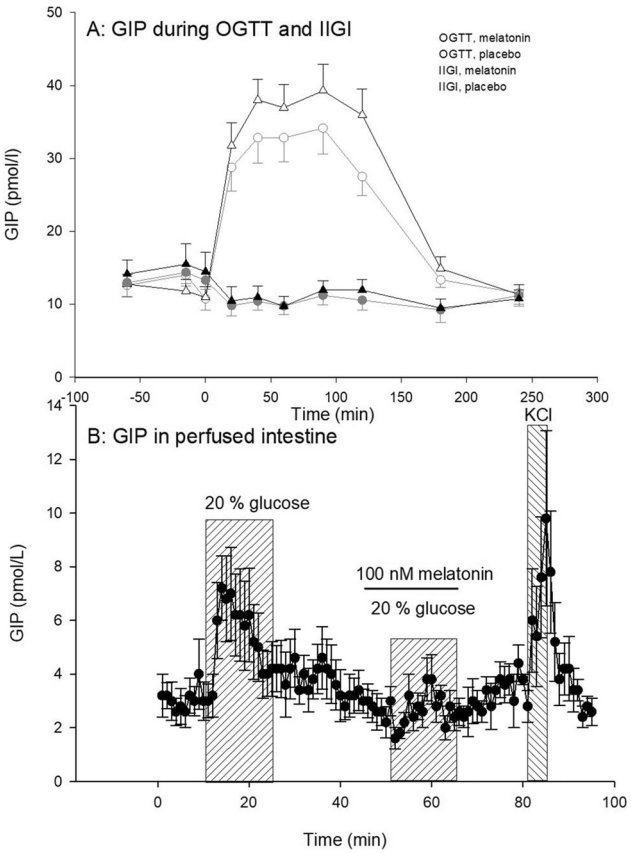
Background and aims: Glucose homeostasis is under circadian control through both endocrine and intracellular mechanisms with several lines of evidence suggesting that melatonin affects glucose homeostasis. We aimed to evaluate the acute *in-vivo* and *in-situ* effects of melatonin on secretion of the incretin hormones, GLP-1 and GIP, and their impact on β-cell insulin secretion.
Materials and methods: A human randomized, double-blinded, placebo-controlled crossover study combined with confirmatory *in-situ*-studies of perfused rat intestines and pancreases were performed. Fifteen healthy male participants were examined 2 x 2 times: An oral glucose tolerance test (OGTT) was performed on day one and an isoglycemic intravenous glucose infusion (IIGI) replicating the blood glucose profile of the OGTT day was performed on day two. This pair of study days were completed two times during either melatonin (10 mg capsules administered orally each hour for four hours, M) or placebo (identical placebo capsules, P) treatment. For the *in-situ* studies, six rat intestines and four rat pancreases were perfused arterially with perfusion buffer ± melatonin. The intestines were concomitantly perfused with glucose through the luminal compartment.
Results: In humans, GIP levels were significantly reduced during an OGTT after melatonin treatment compared with placebo (iAUC difference 0.74±0.3 μM x min, p=0.047) (Panel A). There was a significant time x treatment interaction (ANOVA p=0.003), and *post-hoc* analysis revealed a difference at t = 40, 90, and 120 minutes (M: 33±3, 34±4, and 28±3 vs P: 39±3, 39±4, and 36±4 pM, P=0.02, 0.02, and <0.001). This effect of melatonin on GIP secretion was also observed in the perfused rat intestines (ANOVA time x treatment p=0.003) (Panel B) in which GLP-1 secretion was also impaired by arterial melatonin infusion (ANOVA time x treatment p<0.001). Despite a decrease in GIP levels, the *in-vivo*-glucose-stimulated insulin secretion was unaffected by melatonin (p=0.78). Likewise, melatonin perfusion of the rat pancreas did not change glucose-stimulated insulin secretion (p=0.77).
Conclusion: Melatonin reduced GIP secretion during an oral glucose challenge in healthy young men but did not affect the insulin secretion. Reduced GIP secretion was confirmed in an *in-situ* model of the rat intestine.



Clinical Trial Registration Number: NCT03204877
Supported by: The Novo Nordisk Foundation (NNF16OC0022280) and The Danish Diabetes Academy (DDA1022 8201)
Disclosure: **E. Stistrup Lauritzen:** None.

OP 45 What surgery can do for you

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Sleeve gastrectomy suppresses hepatic glucose production and increases hepatic insulin clearance independent of weight loss

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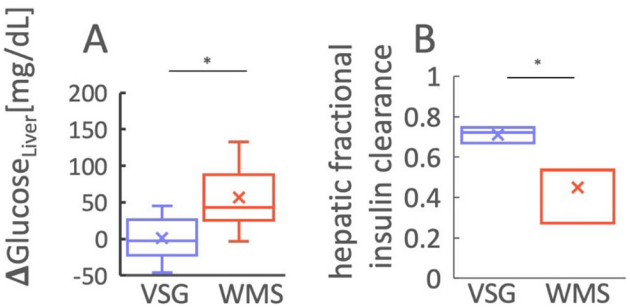
Background and aims: Bariatric surgeries induce weight-loss which is associated with an improvement in hepatic steatosis and reduction in hepatic glucose production. It is not clear whether these outcomes are entirely due to weight-loss, or whether the new anatomy imposed by the surgery contributes to the improvement in the metabolic function of the liver.
Materials and methods: We performed vertical sleeve gastrectomy (VSG) on obese mice provided with a high-fat high-sucrose diet and compared them to diet and weight-matched sham-operated mice (WMS).
Results: 40 days after surgery, VSG-operated mice displayed lesser hepatic steatosis compared to WMS. By measuring the fasting glucose and insulin levels in the blood vessels feeding and draining the liver we showed directly that hepatic glucose production was suppressed after VSG in fasting animals. Insulin levels were elevated in the portal vein of VSG-operated mice but not in venous or arterial blood. indeed, over 70% of the insulin was cleared in the liver of VSG-operated mice, while less than 50% was cleared in WMS-operated mice. The hepatic expression of genes associated with insulin clearance was upregulated as well. We repeated the experiment in lean mice and observed that portal insulin and glucagon are elevated, but only insulin clearance is increased in VSG-operated mice. Therefore the liver of VSG-operated mice experiences hyperinsulinemia and hyperglucagonemia.
Conclusion: Direct measurement of glucose and insulin in the blood entering and leaving the liver shows that VSG affects glucose and insulin metabolism through weight-loss-dependent and independent mechanisms.



A. The increase in glucose level in blood leaving the liver compared to entering the liver.
B. Fraction of insulin cleared by the liver in VSG and WMS-operated mice.

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Disclosure: **D. Ben-Zvi:** Grants; ERC StG H2020.

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Beta cell mass in type 2 diabetes before and after gastric bypass surgery, measured as pancreatic uptake of radiolabeled exendin in human

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Background and aims: An approach for in vivo beta cell imaging is targeting the glucagon-like peptide-1 (GLP-1) receptor by radiolabeled exendin-4. Currently, the role of beta cells in the onset, course and remission type 2 diabetes mellitus (T2DM) is not clear. Beta cell mass (BCM) and function (BCF) might be related to T2DM remission after gastric bypass surgery (RYGB). The aim of this study is examining BCF and pancreatic uptake of ^{68}Ga-exendin-4 (EX) before and after RYGB in patients with T2DM.
Materials and methods: Thirteen patients were included between December 2017 and September 2019. Arginine stimulation test (AST), oral glucose tolerance test (OGTT) and EX PET/CT were performed pre- and one year post-RYGB. Total pancreatic uptake of EX per injected activity (kBq/MBq) was measured quantitatively on PET/CT as marker for BCM.
Results: Preliminary analysis in nine patients with complete follow-up was performed. Six were female, mean age of 54 years and mean duration of T2DM was 12 years. Preoperatively, six patients were on insulin therapy (104±53IU/day) and three on metformin (1-2g/day). Postoperatively, average BMI and HbA1c decreased from 39±4.7 to 27±3.7 kg/m2 and from 63±10 to 47±17 mmol/mol, respectively. Preoperatively, pancreatic uptake of EX was lower in the insulin than the metformin group: 1.55 [range: 0.53-2.66] vs 2.95 [range: 2.47-3.20] kBq/MBq (p=0.017). In addition, the c-peptide response during OGTT was smaller in the insulin as compared to the metformin group (1.1 vs 2.9 nmol/l, p=0.015). Postoperatively, in the insulin group, one patient had complete remission (i.e. no antidiabetics and normal HbA1c), two patients had little improvement (insulin or sulfonylurea treatment and unchanged HbA1c) and three patients had improvements in between. The mean pancreatic uptake increased to 2.29 [1.86-3.06] kBq/MBq (p=0.025) in the insulin group. This seems to be related to the degree of improvement; relative increase was 56-340% in three patients with most improvement, and 8-30% in patients with least improvement. In the metformin group all patients had complete remission. The mean pancreatic uptake remained stable or decreased, with relative changes ranges from -47% to +13%.
Conclusion: As could be expected, patients with insulin dependent T2DM have lower beta cell mass and function as compared to patients with non-insulin dependent T2DM. In the metformin group, average pancreatic uptake of EX decreased after RYGB, probably reflecting reduction of beta cell hyperplasia. Insulin-dependent patients with large improvement in glucose regulation, showed increased pancreatic uptake. This may indicate towards recovered beta cell mass and has not been observed in patients so far. Mechanisms behind recovering of BCM and increasing pancreatic EX uptake need further investigation.
Clinical Trial Registration Number: NCT03182231
Disclosure: **L.N. Deden:** None.

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Identification of myokines potentially involved in the improvement of glucose homeostasis induced by bariatric surgery

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Background and aims: The contribution of myokines to the improvement of glucose homeostasis induced by bariatric surgery is unknown. Our study aims to assess the putative contribution of changes in myokines to the improvement of glucose homeostasis induced by bariatric surgery.
Materials and methods: Obese candidates to bariatric surgery were evaluated clinically and biologically before and 3 months after surgery.

Insulin sensitivity was modeled via the HOMA test. Muscle biopsies were taken from vastus lateralis to study the changes in muscle transcriptome by mRNA-Sequencing (RNA-Seq, Illumina, NovaSeq6000). Differential transcripts expression was assessed with DESeq2 package for R using a paired design. P-values were corrected for multiple testing using a false discovery rate (Benjamini-Hochberg, FDR). The identification of transcripts encoding for secreted proteins (myokines) was based on the prediction of a signal peptide and annotations from well-known databases. Significant expression changes identified by RNA-Seq were confirmed by RT-qPCR.

Results: Muscle biopsies were obtained before and after surgery in 38 patients. Their age and BMI were 44 (±12) years and 43.5 (±5.0) kg/m², 19 were women, 27 had undergone sleeve gastrectomy and 11 gastric bypass. All the clinical and biological parameters showed significant improvement after surgery except for HDL cholesterol. In particular, bariatric surgery was significantly associated with reduced body weight (-24.0 kg, $p < 0.0001$) and increased insulin sensitivity (HOMA-S +45%, $p < 0.0001$). Twelve patients were included in the RNA-Seq study (39 (±12) years, BMI 42.8 (±3.9) kg/m², 3 women, 10 sleeve gastrectomies). The analysis of muscle transcriptome identified 1363 transcripts whose expression was significantly changed by surgery (FDR ≤0.10). Among those, 41 up-regulated (fold-change ≥1.3) and 56 down-regulated (fold-change ≤0.7) transcripts encoded putative myokines. Increased expression of *CX3CL1* (fractalkine, +71%, $p<0.0001$), *ADAMTS9* (+34%, $p<0.0001$), *BDNF* (+32%, $p=0.007$) and *ANG* (angiogenin +31%, $p<0.0001$) as well as decreased expression of *MSTN* (myostatin, -45%, $p<0.0001$) and *FNDC5* (irisin, -26%, $p<0.0001$), expressing myokines known to regulate glucose homeostasis, were confirmed by RT-qPCR in the whole cohort. We also showed a significantly reduced expression of *MYH1* (-38%, $p=0.003$) and a significant increased expression of *MYH2* (+19%, $p=0.037$) suggestive of a less fast muscle phenotype.

Conclusion: Our study shows that bariatric surgery changes muscle transcriptome and more specifically the expression of myokine genes known to regulate glucose homeostasis (fractalkine, ADAMTS9, BNDF, angiogenin, myostatin, irisin). The analysis of the other identified transcripts and involved pathways are ongoing to identify new myokines and mechanisms engaged in the improvement of glucose homeostasis induced by bariatric surgery.

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Disclosure: **L. Orioli:** Grants; Yes.

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Bariatric surgery, type 2 diabetes remission and proteomic changes

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Background and aims: Bariatric surgery (BS) results in metabolic pathway recalibration. When major metabolic change occurs, blood protein components have a key role and can be altered significantly. The study of proteins and their functions has been important in helping investigators to decipher cellular mechanisms. We set out to identify potential biomarkers of change in plasma following BS in people achieving remission of type 2 diabetes mellitus (T2DM).

Materials and methods: Longitudinal analysis was performed on serum samples from 10 individuals who achieved remission of T2DM following Roux-en-Y gastric bypass (n=7) or Sleeve gastrectomy (n=3). Sequential window acquisition of all theoretical fragment ion spectra Mass Spectrometry (SWATH-MS) was on serum samples taking at 4 months before and 6+12 months after BS.

Results: 467 proteins were quantified by SWATH-MS. Principal component analysis resolved samples from distinct time points after selection of key discriminatory proteins: Twenty-five proteins were differentially expressed between pre-surgery/6 months post-surgery; thirty-nine proteins between baseline/12 months. Relative to pre-surgery samples, 8 proteins were significantly different at both 6- and 12-months post-surgery (Figure). Of those 8 proteins, 3 showed increased expression after BS (FC: Fold change Baseline vs 12 months), p Value); SHBG (1.95, $p<0.01$), leucine-rich alpha-2-glycoprotein (LRG1) (0.59, $p < 0.05$) and N-acetylmuramoyl-L-alanine amidase (PGLYRP2) (0.43, $p < 0.05$), whilst the remaining 5 showed decreased expression; TF (-0.78, $p < 0.01$), proteoglycan 4 (PRG4) (-0.78, $p < 0.05$), APOA4 (-1.38, $p < 0.05$), HSPA4 (-0.38, $p < 0.05$), bifunctional epoxide hydrolase 2 (EPHX2) (-0.47, $p < 0.05$). The greatest fold change was seen for SHBG approaching a 2-fold elevation after BS.

Conclusion: We found significant changes in the proteome for 8 proteins at both 6+12 months post-BS. Several of these are key components in metabolic/inflammatory pathways. These protein changes may act as potential marker signatures of remission of T2DM following bariatric surgery.

Disclosure: **Z. Iqbal:** None.

OP 46 (Path)ways to develop human beta cells

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Generation of stem cell derived islets with adult-like cytoarchitecture and function in vitro

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Background and aims: Generation of functional stem cell derived islets (SC-islets) *in vitro* represents one of the most promising avenues for curing diabetes. However, most current protocols yield SC-islets with partial *in vitro* function or rely on cell-specific purification and reaggregation strategies for acquisition of SC-islet function. We aimed to improve upon pre-existing protocols in creating mature beta-cells *in vitro*- and characterize the progress of their maturation.
Materials and methods: We optimized published protocols and patents to devise a novel SC-islet differentiation protocol, which did not require sorting or reaggregation steps. This protocol included an extended maturation stage (S7) in CMRL1066 medium supplemented with an anti-proliferative aurora kinase inhibitor, n-acetyl cysteine and T3. We characterized the timeline of the acquired maturation events with morphometric analyses and assays of dynamic insulin secretory function from the beginning (S7w0) until the 6th week of in vitro maturation (S7w6).
Results: The S7w0 SC-islets contained c. 40% beta cells, 15% polyhormonal cells and 5% alpha cells. By S7w3 the rate of polyhormonality reduced to <5% and the number of alpha cells increased to 45%, while the number of beta cells remained stable. Markers of beta cell proliferation reduced by 80% from S7w0 to S7w3 (2% to 0.4%). The SC-islets underwent cytoarchitectural changes mimicking fetal development and culminating in an adult-like, intermingled organization by S7w6. Concurrently, the SC-islets increased their insulin content 6-fold and exhibited mature insulin granule morphology. At S7w0, the SC-islets were glucose-unresponsive, but responded to GLP1-R agonism and membrane depolarization triggered by tolbutamide and KCl. However, by S7w2 the SC-islets acquired biphasic glucose-stimulated insulin secretion, the magnitude of which increased steadily until S7w6 to a 9-fold first phase secretion and a 7-fold 2nd phase secretion, which could be maintained for >70 minutes. This transition from non-responsive to glucose-responsive SC-islets from S7w0 to S7w2, was marked by a 65% reduction in the fractional insulin release in low glucose. The threshold glucose concentration for triggering of insulin secretion was unphysiologically low (3 mM) in S7w2 SC-islets whereas at w3 and w6 the setpoint was physiological (5 mM). The S7w3 SC-islets showed a glucose response even under a tolbutamide clamp, indicating activation of the metabolic amplifying pathways Both the reduction of proliferation and acquisition of glucose-stimulated insulin secretion were dependent on the S7 additives.
Conclusion: We describe a protocol generating SC-islets with adult-like cytoarchitecture and glucose sensitivity, engaging the triggering pathway and the neurohormonal and metabolic amplifying pathways of insulin secretion. We also describe the sequence in which these aspects of mature function are acquired. The acquisition of function in SC-islets was associated with cytoarchitectural remodeling, reduced beta cell proliferation and increased alpha cell fraction.
Supported by: Academy of Finland, Novo Nordisk Fonden, Sigrid Jusélius F.
Disclosure: **V. Lithovius:** None.

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Mapping glucose metabolism and function in human pluripotent stem cell derived islets

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Background and aims: The complex metabolic network mediating the coupling of glucose to insulin release in pancreatic beta cells, is still not fully understood. The use of pluripotent stem cell-derived models of islet differentiation and maturation forms a reproducible tool by which to probe the underlying biology of endocrine cell function. Here we generate pluripotent stem cell-derived islet clusters (SC-islets), using an optimized differentiation protocol, and use this model to interrogate the active pathways of glucose metabolism through metabolite tracing analyses. Through these assays we are able to map the flow of nutrient-derived metabolites and correlate the shifts in these pathways to the functional maturation of SC-islets and primary islet tissue.
Materials and methods: SC-islets were differentiated in vitro using an optimised 7-stage protocol. Acute exposure of radiolabeled [U-13C6] glucose at basal and stimulatory concentrations were used prior to LC-MS metabolite flux analyses. Metabolite tracing was undertaken over 6 weeks of SC-islet maturation and compared to human primary adult islets. Small molecule inhibition of specific enzymatic steps within the pathways of glucose metabolism were also assayed for metabolic and functional outcomes.
Results: SC-islets develop glucose-sensitive insulin release profiles comparable to primary islet tissue following 2 weeks of in vitro maturation. However, divergent patterns of glucose-derived metabolite production were seen between SC-islets and primary islet tissue. SC-islets displayed overall higher degrees of lactate production and lower flux of TCA metabolites than primary islets. This was also shown in respirometric assays of glucose stimulation. However, during in vitro maturation, overall lactate production was reduced, which also coincided with heightened control of the hexokinase step of glycolysis. SC-islets also demonstrated a more restricted production of late-glycolytic intermediates, and higher levels of de novo serine/glycine biosynthesis, than was seen in primary islets. Conversely, primary islets displayed enhanced patterns of labelling within purine synthesis pathways, glutathione production and other TCA-derived amino acids. Differences in temporal patterns of metabolite production (such as for citrate) also offer clues to the shifts in glucose metabolism over time.
Conclusion: SC-islets develop comparable levels of glucose-dependent insulin secretion to primary islets, following extended in vitro maturation, despite differences in overall patterns of glucose metabolism. SC-islets show lower mitochondrial metabolite flux and respirometric coupling to glucose than primary islets, but over time display lowered anaerobic metabolite flux and tighter control of the hexokinase-driven glycolytic step. Other TCA-derived metabolites are generated from glucose in primary islets that are reduced in SC-islets, such as aspartate, glutamate and glutathione. Such metabolites may be necessary for prolonged glucose-sensitive islet function and aid acute glucose responsiveness. These data offer clues to understanding in greater detail the glucose-driven metabolic shifts within islet tissue and applies this understanding to the acquisition of SC-islet functional maturity.
Disclosure: **T. Barsby:** None.

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Single cell transcriptomic profiling of the developing human pancreas reveals unique features of maturing islet cells

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Background and aims: Islet transplantation can improve glucose homeostasis in diabetes but there are insufficient donor islets to make this a widespread therapy. Strategies are therefore being developed to generate unlimited amounts of β-cells from stem cells for transplantation therapy. However, current differentiation protocols, based on gene cascades in

mouse pancreas development, have not been successful in reproducibly generating functional human β-cells in vitro. To understand the unique developmental landscapes of human β-cells, we have now performed transcriptomic analysis of the developing human pancreas.

Materials and methods: Human fetal pancreases at 13-, 14-, 15-, 18-, 19- and 20-weeks post conception (wpc) obtained from the MRC/Wellcome Trust Human Developmental Biology Resource, were dissociated into single cells on the day of retrieval, multiplexed with DNA-barcoded antibodies, live-sorted then sequenced following the 10x Genomics scRNA-seq Chromium protocol. Cells were reassigned to their original developmental timepoint by genetic demultiplexing, which agreed 100% with their DNA-barcodes. Cells were batch-corrected by regularized negative binomial regression normalization and visualized by UMAP embedding. Differentiation trajectories of the cell types were determined with Monocle3 and Slingshot pseudotime analysis. The influence of the pancreas microenvironment on endocrine progenitor differentiation was analysed by a connectome-based network analysis.

Results: 14,154 cells were sequenced, with 1,826 median genes per cell. After quality control, 11,273 cells (13wpc: 1,993 cells; 14wpc: 1,991 cells; 15wpc: 1,530 cells; 18wpc: 2,066 cells; 19wpc: 2,196 cells; 20wpc: 1,497 cells) were analysed. Unsupervised clustering revealed 15 distinct cell populations, of which the most abundant were mesenchymal, endocrine progenitor, endocrine, ductal and acinar. Each cell population was verified by differential expression of established markers and novel cluster-specific genes were identified. For example, GNG8, PLIN5, AC020909.2, RBP1 and CD24 were highly expressed in the endocrine progenitor cell population (n=101 genes, log fold change >0.25 and $p<2.23 \times 10^{-24}$). In addition, progenitor cells were identified in both acinar and endocrine clusters as well as within multiple sub-populations of other cell types. Expression of key genes (INS, GCG, PDX1, NEUROG3 and SOX9) was validated by immunohistochemistry. Trajectory analysis identified branch-specific genes for pancreatic progenitors (PDX1+, SOX9+ cells) differentiating towards acinar or ductal lineages and NEUROG3+ endocrine progenitors differentiating towards β-, α- or δ-cells, indicating that transcriptional maturation occurred over the developmental timeframe. Cell-cell connectivity analysis showed that endocrine progenitors received extensive paracrine and autocrine signalling from endocrine and non-endocrine cell types (n=819 unique cell-type-ligand-receptor-cell type interactions) via ApoE, BMP, SLIT and ephrin family members.

Conclusion: We have uncovered multiple established and novel pancreatic cell populations, and also gene candidates that regulate progenitor differentiation to acinar, ductal, and endocrine lineages within the developing human pancreas at different timepoints. These data provide the basis for optimizing differentiation of human endocrine progenitor cells into β-cells in vitro.

Supported by: Novo Nordisk UK Research Foundation, Diabetes UK

Disclosure: **O. Olaniru:** None.

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tRNA derived small RNAs regulate the maturation of neonatal beta cell

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Background and aims: tRNA-derived fragments (tRFs) are an emerging class of small non-coding RNAs with distinct cellular functions. Here, we aimed to identify the role of tRFs in the regulation of postnatal β-cell maturation, a critical process that may lead to diabetes susceptibility in adulthood.

Materials and methods: A small RNA-sequencing was performed on neonatal and adult rat islets. Candidate tRFs were inhibited in neonatal islets, and β-cell function was analyzed. Moreover, the global impact of tRF inhibition on β-cells transcriptome and proteome was assessed.

Results: We identified three highly abundant tRFs in neonatal pancreatic islets originating from the 5'-halves of histidine (tiRNA-5^{HisGTG}) and glutamate tRNAs (tiRNA-5^{GluCTC} and tiRNA-5^{GluTTC}). Pooled inhibition of these fragments reduced the proliferative and secretory capacities of neonatal β-cells. Furthermore, two of these nuclear-encoded tRFs, tiRNA-5^{HisGTG} and tiRNA-5^{GluCTC}, were enriched in mitochondria. Accordingly, their inhibition in neonatal islets reduced the level of the mitochondrial pyruvate carrier, MPC1, and perturbed mitochondrial respiration. Finally, we illustrated that the tiRNA-5 levels are dysregulated in the islets of diabetic and diabetes-prone animals.

Conclusion: tRFs represent a new class of regulators of β-cell maturation and the deregulation of tiRNA-5s, a subclass of tRFs, in neonatal islets may lead to diabetes susceptibility in adulthood.

Supported by: SNSF

Disclosure: **B. Bayazit:** None.

OP 47 Hypoglycaemia consequences at system level

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Associations of hypoglycaemia and glycaemic variability with cardiac arrhythmias using a long-term monitoring approach in insulin-treated patients with type 2 diabetes

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Background and aims: Insulin-treated patients with type 2 diabetes (T2D) are at risk of hypoglycemia, which is associated with an increased risk of cardiovascular disease and mortality. Using a long-term monitoring approach, we investigated the association between episodes of hypoglycemia, glycemic variability and cardiac arrhythmias in a real-life setting.
Materials and methods: Insulin-treated patients with T2D (N=21) and microvascular complications ([mean±SD] age 66.8±9.6 years, BMI 30.1 ±4.5 kg/m^2, HbA1c 6.8±0.4% [51.0±4.8 mmol/mol]) were included for a one-year observational study employing continuous glucose monitoring (118±6 days) and implantable cardiac monitors (ICMs), which provide continuous electrocardiographic monitoring with reporting of arrhythmic events according to an inbuild algorithm.
Results: Time spend in hypoglycemia was higher during nighttime than during daytime ([median and interquartile range] 0.7% [0.7-2.7] vs. 0.4% [0.2-0.8]). The ICMs detected 724 episodes of clinically relevant arrhythmias in 12 (57%) patients, with atrial fibrillation and pauses accounting for 99% of the episodes. No association between hypoglycaemia and cardiac arrhythmia was found during daytime. During nighttime, subject-specific hourly incidence of cardiac arrhythmias tended to increase with the occurrence of hypoglycaemia but only slightly with increasing time in hypoglycaemia (Figure). Subject-specific incidence of cardiac arrhythmias during nighttime increased with increasing glycaemic variability as estimated by coefficient of variation and standard deviation.
Conclusion: Cardiac arrhythmias were common in insulin-treated patients with T2D and were associated with glycemic variability with distinctive diurnal differences, whereas arrhythmias were not strongly associated with hypoglycemia.

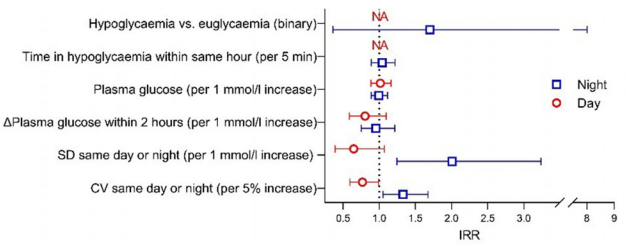

Figure. Incident rate ratio (95% CI) for cardiac arrhythmia (as defined by the study protocol) according to within-subject change in glycaemic summaries (generalized linear mixed model). Note that no arrhythmias were detected during daytime hypoglycaemia. Abbreviations: CV, coefficient of variation; NA, not applicable; SD, standard deviation.

Clinical Trial Registration Number: NCT03150030
Supported by: Novo Nordisk Foundation and the Capital Region of Denmark
Disclosure: **A. Andersen:** None.

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The impact of acute fluctuations in plasma glucose on echocardiographic derived measures of systolic function in patients with type 1 diabetes

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Background and aims: Patients with type 1 diabetes (T1D) and poor glycaemic control have an increased risk of cardiovascular disease, including heart failure. Rapid fluctuations in plasma glucose (PG) are an inevitable consequence of insulin-treatment in T1D, but the effects of fluctuations in PG on cardiac function remains uncertain. Here evaluated cardiac function change in patients with T1D during acute hypoglycaemia followed by rebound hyperglycaemia or euglycaemia.
Materials and methods: Patients with T1D (n=23, [mean±SD] age 52.8 ±11.4 years, HbA1c 7.5±0.8% [57.6±8.9 mmol/mol], BMI 25.7±3.1 kg/m²) underwent two clamp days in random order in a cross-over design. Both clamp days included three steady-state phases, with the two first phases being identical on both clamp days: 1) a hyperinsulinemic-euglycemic phase (PG=6 mmol/l) for 45 min; 2) a hyperinsulinemic-hypoglycaemic phase (PG=2.5 mmol/l) for 60 min and 3) a recovery phase (Clamp A: PG: 20 mmol/l; Clamp B: PG:6 mmol/l) for 60 min. Cardiac function was evaluated with echocardiography during steady-state at each phase.
Results: Acute hypoglycaemia significantly increased left ventricular ejection fraction (LVEF) and global longitudinal strain (GLS) from baseline (Table 1). LVEF and GLS were significantly increased during acute hyperglycaemia compared with baseline euglycemia. During the recovery phase, LVEF was significantly greater during hyperglycaemia compared with euglycaemia, although LVEF during euglycaemia was significantly increased compared with baseline.
Conclusion: Our findings suggest that acute fluctuations in PG affect echocardiographic derived measures of systolic function in patients with T1D. Hypoglycaemia, recent hypoglycaemia, or subsequent hyperglycaemia may result in overestimation of left ventricular contractile function and thereby be of importance when evaluating LVEF in patients with T1D.

		First phase (Euglycaemia)		Second phase (Hypoglycaemia)			Last phase (Recovery)		
		Mean	95% CI	ΔMean	95% CI	*p**	ΔMean	95% CI	*p**
LVEF (%)	Clamp A	58.5	[56.7;60.3]	6.5	[5.0;8.1]	<0.0001	5.0	[3.2;6.8]	<0.0001
	Clamp B	59.2	[57.6;61.2]	6.2	[4.6;7.9]	<0.0001	2.1	[0.5;3.8]	0.0116
	ΔClamp day						2.9	[1.0;4.7]	0.0046
GLS (%)	Clamp A	-17.6	[-19.1;-16.2]	-3.1	[-4.5;-1.6]	0.0006	-2.1	[-3.7;-0.6]	0.0097
	Clamp B	-17.8	[-18.6;-16.9]	-3.5	[-4.6;-2.3]	<0.0001	-1.3	[-2.8;-0.3]	0.0092
	ΔClamp day						0.9	[-0.8;2.6]	0.3007

Table 1: Changes in left ventricular systolic function during acute hypoglycaemia and recovery phase (Clamp A: acute hyperglycaemia. Clamp B: euglycaemia) compared with first phase euglycaemia. Abbreviations: CI, confidence interval; GLS, global longitudinal strain; LVEF, left ventricular ejection fraction.

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Disclosure: **C.R. Andreasen:** None.

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Investigating the blood flow responses of different thalamic nuclei to hypoglycaemia in intact and impaired awareness of hypoglycaemia

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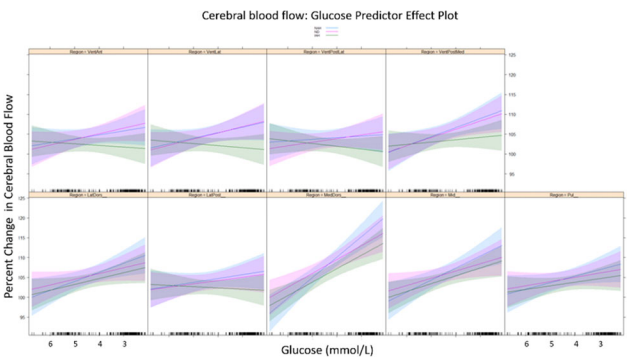
Background and aims: The brain is critical in the recognition and physiological responses to hypoglycaemia. Differences in regional cerebral blood flow have been observed in people with no diabetes (ND), type 1 diabetes and normal awareness of hypoglycaemia (NAH) and type 1 diabetes and impaired awareness of hypoglycaemia (IAH) with the thalamus shown to have an important role in symptomatic awareness. The thalamus has numerous distinct nuclei each with separate roles involved in the relay and filtering of sensory or motor signals. We wanted to evaluate which of the functionally distinct nuclei within the thalamus played a dominant role in driving the known blood flow responses to hypoglycaemia.
Materials and methods: Fifty-one participants (15 ND, 15 NAH and 21 IAH) underwent a two-step hyperinsulinaemic hypoglycaemic clamp. Thalamic and global CBF was estimated using pcASL MRI at 6 timepoints: two euglycemic, two intermediate and two hypoglycaemic. Mixed linear models were performed in R using the lme4 package in which global cerebral blood flow was controlled for as well as an interaction term allowing estimation of the effect of each region by group.
Results: The mean glucose at the six timepoints were 5.32, 5.30, 3.06, 2.82, 2.54 and 2.55 mmol/L. Symptom responses to hypoglycaemia were brisk and similar in ND and NAH, however they were absent in IAH. After adjusting for the global increase in cerebral blood flow, the mixed linear models demonstrated significantly different regional cerebral blood flow responses between the distinct nuclei. The region with the largest effect was the medial dorsal nucleus which has been shown to have a crucial role in attention and planning ($p < 2*10^{-16}$). The region by group interaction, showed that the medial dorsal nucleus underwent a significantly reduced increment in CBF in the IAH group compared to NAH ($p = 0.015$).
Conclusion: In this first analysis of distinct thalamic nuclei responses, we show that regions involved attention and planning show the greatest blood flow response to hypoglycaemia. The specific nuclei involved indicate the critical role of the thalamus in attention towards key sensory signals during hypoglycaemia. These responses are reduced in IAH, further highlighting the importance of normal thalamic responses in the generation of hypoglycaemic symptom generation.



Supported by: JDRF, DUK
Disclosure: **P. Jacob:** None.

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Differential symptoms and hormonal counter-regulation during hypoglycaemia in people with type 1 diabetes and post bariatric hypoglycaemia

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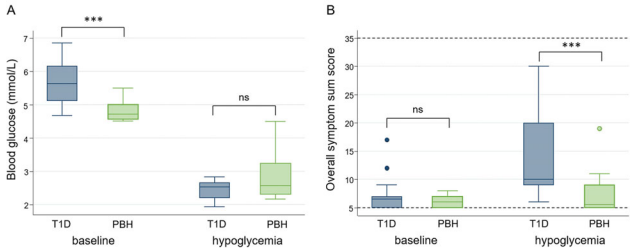
Background and aims: Hypoglycemia is one of the most cumbersome acute complications of diabetes. However, hypoglycemia is not restricted to diabetes but is increasingly recognized also after bariatric surgery (postbariatric hypoglycemia, PBH). There is currently little evidence on mechanisms and symptom perception in PBH. We compared symptoms during hypoglycemia in people with type 1 diabetes (T1D) and PBH, and assessed counter-regulatory response.
Materials and methods: This analysis comprised 18 subjects with T1D (age 30 [26;40] yrs, 12 males, HbA_{1c} 7.1[6.6;7.4] %), and 10 subjects with PBH after gastric bypass (age 34.2 [23.5;48.9] yrs, 2 males, HbA_{1c} 5.2 [5.1;5.4] %). Hypoglycemia was induced in T1D using i.v. insulin, and in PBH by the ingestion of 75g glucose. All individuals rated hypoglycemic symptoms in 5 domains (3 autonomic domains: sweating, palpitations, tremor; and 2 neuroglycopenic domains: incoordination and drowsiness) at baseline (t_{base}) and hypoglycemia (t_{hypo}) on a 7-point scale (1=absent, 7=extreme). Sum scores were calculated for overall, autonomic and neuroglycopenic domains. Epinephrine, norepinephrine, glucagon, cortisol, and growth hormone [GH] were measured at t_{base} and t_{hypo}. Results are shown as median [IQR] and groups were compared using two-sample Wilcoxon test.
Results: Venous blood glucose (BG) at t_{base} in T1D and PBH was 5.6 [5.1;6.2] and 4.7 [4.6;5.0] mmol/L ($p<0.001$), respectively. BG values at t_{hypo} were 2.5 [2.2;2.7] and 2.6 [2.3;3.2] mmol/L ($p=0.20$), respectively. Symptom perception at t_{base} was comparable between T1D and PBH (6.5[5;7] vs. 6[5;7], $p=0.32$), and did not change during hypoglycemia in PHB. Conversely, people with T1D reported higher overall, autonomic and neuroglycopenic symptom scores in hypoglycemia: 10 [9;20] vs. 6 [5;9], ($p=0.003$), 8[6;12] vs. 3[3;6], ($p=0.001$), 4[3;7] vs. 2[2;4], ($p=0.05$). In T1D, epinephrine, glucagon, cortisol, and GH increased from t_{base} to t_{hypo}, but decreased in PBH (epinephrine 682.1[326.9;1450.0] % vs. -25.9[-30.8;-16.2] % ($p<0.001$), glucagon 45.3 [2.2;73.3] % vs. -25.9 [-30.8;-16.2] % ($p=0.002$), cortisol 33.1 [-8.4;65] % vs. -21.2 [-56.1;-14.1] % ($p=0.004$), GH 1740.1 [238.7;12067] % vs. -93.5 [-96.;47.1] % ($p<0.001$), respectively). Change in norepinephrine was comparable between the two groups (15.8 [5.5;48.9] % vs. 26.8 [14.4;31.7] % ($p=1.0$)).
Conclusion: Hypoglycemia was associated with significantly more symptoms in well-controlled people with T1D compared to age-matched people with PBH. This difference may be at least partially related to differences in counter-regulatory response. Whether such differential regulation may also indicate hypoglycemia unawareness in PBH (where permanent glucose control is not widely performed) compared with T1D (where continous glucose monitoring is well established) needs to be confirmed.

Figure 1: Glycemia and overall symptom sum scores during hypoglycemia in T1D and PBH. Panel A: Venous blood glucose (mmol/L) values at baseline and in hypoglycemia. Panel B: Overall symptom sum scores at baseline and in hypoglycemia. The dashed, horizontal lines correspond to the absolute minimum and maximum values of the overall symptom sum score. Significance levels are shown as follows: ns= not significant and ***= $p \leq 0.001$. T1D, type 1 diabetes; PBH, postbariatric hypoglycemia.



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Supported by: Swiss National Science Foundation (PCEGP3_186978, CRSII5_183569)
Disclosure: **V. Lehmann:** None.

SO 01 Diabetes epidemiology at scale: registries and large databases

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Disease heterogeneity of adult diabetes patients based on routine clinical parameters at diagnosis: results from the German/Austrian DPV registry

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Background and aims: Clustering newly-diagnosed diabetes patients using parameters available in real-world clinical care at manifestation may help to guide therapy and predict future outcome.
Materials and methods: We included 55,540 adult patients with diabetes manifestation between 1995 and 2019. Patients were clustered hierarchically by Ward´s method using age, sex, BMI, HbA1c, DKA, presence of metabolic syndrome (hypertension/dyslipidemia/hyperuricemia) at onset, and β cell antibody status (positive/negative). The number of clusters was assessed using the Cubic Cluster Criterion, and the Pseudo F/Pseudo t^2 statistics. HbA1c, insulin requirement, and oral antidiabetics were compared after a mean follow-up period of 2(1-3) years using Kruskal-Wallis test. Time until nephropathy (defined as eGFR <60 ml/min/1.73m²) and cardiovascular disease were compared using log-rank test.
Results: We identified five clusters (Table 1). Cluster 1 overlaps with clinical T2D with elevated BMI and negative antibodies. Cluster 2 is characterized by the highest HbA1c at onset and 8.2% DKA. Cluster 3 comprises the highest proportion of clinical T1D patients, highest rate of DKA and positive antibodies, very high HbA1c, and the youngest age. Cluster 4 had low HbA1c and rarely DKA. Patients in cluster 5 were the oldest with low HbA1c and mostly clinical T2D patients. Insulin requirement after 2 years was highest in clusters 2 and 3, while high proportion of patients were treated with "OADs only" in all but cluster 3. Cluster 5 with the oldest patients are rarely treated with insulin. Time until diagnosis of diabetes-related comorbidities was shortest for Cluster 5 and longest for cluster 3.
Conclusion: Adult diabetes is heterogeneous beyond the classical T1D/T2D groups, based on easily available parameters in clinical practice using an automated clustering algorithm which allows both continuous and binary variables. These subgroups are predictive of future antidiabetic therapy and vascular complications.

	Cluster 1 (n=13,038)	Cluster 2 (n=13,624)	Cluster 3 (n=3,786)	Cluster 4 (n=10,311)	Cluster 5 (n=14,781)	p-value
Male sex [%]	55.1	72.4	71.6	63.6	41.4	
Age at diagnosis [y]	49.6±11.5	60.2±12.1	29.4±7.3	53.3±12.9	73.4±8.0	
BMI at onset [kg/m²]	38.3±6.7	27.6±4.4	24.7±4.9	27.9±4.3	29.2±5.1	
HbA1c at onset [%]	8.5±2.2	11.5±2.2	11.4±2.3	6.7±1.1	6.8±1.1	
DKA at onset [%]	0.9	8.2	11.5	1.4	1.2	
Positive β cell AB [%]	2.7	6.5	28.6	4.2	0.1	
Metabolic syndrome [%]	83.3	76.3	63.2	45.4	79.2	
Clinical type 1/2 DM [%]	4.0/96.0	15.3/84.7	75.4/24.6	14.8/85.2	2.2/97.8	<.001
Insulin at 2-yr duration [%]	21.1 (n=3,348)	43.7 (n=2,421)	60.0 (n=911)	17.2 (n=3,209)	15.0 (n=3,375)	<.001
"OAD only" at 2-yr duration [%]	46.3 (n=3,348)	28.8 (n=2,421)	8.5 (n=911)	31.7 (n=3,209)	35.2 (n=3,375)	<.001
Time until CVD [y] (Events)	15.7±0.2 (1,256)	13.5±0.2 (1,749)	15.1±0.2 (88)	13.0±0.1 (1,135)	10.2±0.2 (2,833)	<.001
Time until CKD [y] (Events)	11.4±0.2 (2,493)	8.6±0.2 (3,841)	16.0±0.3 (258)	12.0±0.2 (1,753)	4.7±0.1 (6,230)	<.001

Table 1: Patient characteristics presented as % or mean ± standard deviation. OAD: oral antidiabetics. CKD: chronic kidney disease

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(DDG) and the Robert Koch Institute (RKI) provided further financial support for the DPV registry.
Disclosure: **S.R. Tittel:** None.

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Epidemiology of diabetes in Kazakhstan: data from unified nationwide electronic healthcare system 2014 - 2019

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Background and aims: No published data is available for the epidemiology of Type 1 and Type 2 diabetes mellitus (DM) in Kazakhstan. Therefore, we aimed to estimate prevalence, incidence and all-cause mortality rates of Type 1 and Type 2 DM in Kazakhstan by aggregating and utilizing large-scale administrative healthcare data from the Unified National Electronic Health System (UNEHS) for 2014-2019.
Materials and methods: 475,529 incident Type 1 and Type 2 DM patients were identified through UNEHS, who developed DM between 2014 and 2019. Extracted data included date of birth, sex, ethnicity, date of DM diagnosis and comorbidities. Date of death was obtained through linkage with the National Population Registry. Prevalence, incidence and mortality rates were estimated per 100,000 population. The follow-up period was from the initial date of DM diagnosis until death or end of the follow-up (December 31st, 2019). Cox proportional hazards regression was used to obtain crude and adjusted hazard ratios (aHR) and 95% confidence intervals for associations of all-cause death with age, sex and ethnicity.
Results: Among 475,529 patients there were 31,763 and 443,766 patients with Type 1 and Type 2 DM, respectively. There were 48.1 % and 64.2 % females among patients with Type 1 and 2 DM, respectively. Median age at diagnosis was 26.9 (interquartile range (IQR) 13.4 - 43.5) and 58.5 (IQR 51.3 - 65.8) for Type 1 and 2 DM, respectively. There were 13.0 % deaths among Type 1 and 17.2 % deaths among Type 2 DM patients. The most common comorbidities in Type 1 DM patients were hypertension (22.2 %) and diabetic neuropathy (11.4 %), while hypertension (47.3 %) and coronary artery disease (20.4 %) were the most common complications in Type 2 DM patients. Prevalence of Type 1 and Type 2 DM increased from 88.6 and 1207.2 in 2014 to 152.3 and 2074.9 in 2019, respectively. Mortality of Type 1 and Type 2 DM also increased from 1.06 and 10.4 in 2014 to 4.15 and 68.5 in 2019, respectively. Incidence of Type 1 DM decreased from 25.3 in 2014 to 16.8 in 2019, while incidence of Type 2 DM was similar over the years (242.4 in 2014 to 286.2 in 2019). Median follow up was 5.1 (IQR 2.7 - 8.6) years and 4.9 (IQR 2.4 - 8.2) years for Type 1 and Type 2 DM, respectively. Males with Type 1 and Type 2 DM had a higher risk of all-cause death compared to females: aHR 1.17 (1.08 - 1.28) and 1.20 (1.16 - 1.24), respectively. Other ethnicities than Kazakh had a lower risk of all-cause death in patients with Type 1 and Type 2 DM: aHR 0.88 (0.79 - 0.97) and 0.93 (0.89 - 0.96), respectively. Type 1 DM patients older than 18 had a higher risk of all-cause death compared to patients younger than 18 y.o.: aHR and 95% CI were 2.55 (2.11 - 3.07), 4.71 (3.92 -5.66), 11.96 (9.97 - 14.35), 38.42 (31.37 - 47.07) for 18 - 34 y.o., 35 - 50 y.o., 51 - 70 y.o. and older than 71 y.o., respectively. Type 2 DM patients who were older than 50 had a higher risk of all-cause death compared to patients younger than 18 y.o.: aHR and 95% CI were 2.48 (1.83 - 3.35) and 7.20 (5.31 - 9.77) for 51 - 70 y.o. and older than 71 y.o, respectively.
Conclusion: The prevalence and mortality but not the incidence rate of Type 1 and Type 2 DM increased during the years 2014 - 2019 in Kazakhstan. Male sex, older age and Kazakh ethnicity were associated with a higher risk of all-cause death compared to females, younger age and other nationalities than Kazakh in patients with Type 1 and Type 2 DM.
Supported by: Nazarbayev University Faculty Development Research Grant 240919FD3913
Disclosure: **D. Galiyeva:** Grants; Nazarbayev University Faculty Development Research Grant 240919FD3913.

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Identifying and delineating the Dutch type 2 diabetes population using an all-payer claims database: characteristics, healthcare utilisation, and expenditures

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Background and aims: Studies on healthcare utilization and expenditures of type 2 diabetes populations are often based on data of patient subgroups or self-reported cost data. To obtain a complete overview, we used an all-payer claims database to identify and characterize the Dutch type 2 diabetes population. Additionally, we aimed to delineate the distribution of healthcare utilization and expenditures across the health system from 2016 to 2018.
Materials and methods: This retrospective observational study was based on an all-payer claims database covering 99.9% of the Dutch population from which we identified type 2 diabetes patients based on use of integrated diabetes care and/or glucose-lowering drugs. Comprehensive data on healthcare utilization and expenditures were drawn from the database and analyzed descriptively.
Results: In 2018, 900,522 people (i.e. 6.5% of the adult population) were identified as having type 2 diabetes. The mean age of the population was 68.7 (±12.3) years and 46.7% was female. Commonly identified comorbidities in the type 2 diabetes population were heart disease (12.1%), depression (5.7%), and thyroid disorders (5.0%). Moreover, a considerable share of patients received specialized care for microvascular and macrovascular diabetes-related complications, 16.2 and 5.6% respectively. Almost all type 2 diabetes patients used pharmaceutical care (99.1%) and medical specialist care (97.0%) for either their diabetes or other conditions. Additionally, type 2 diabetes patients used a range of other services: general practitioner consultations, assistive devices, paramedical care, district nursing, and mental health care. In 2018, total healthcare spending on patients with type 2 diabetes was €8,173 million, which was 9.4% of the total national healthcare expenditures. Medical specialist care accounted for the largest share of spending (38.1%). Mean annual per patient expenditures increased by 4.4% from €7,077 in 2016 to €7,386 in 2018.
Conclusion: Analysis of all-payer claims data can help inform health policy and practice, and support better decisions to promote long-term sustainability of healthcare systems. Our findings suggest that the healthcare utilization of the Dutch type 2 diabetes population is widely distributed across the health system. Despite the existence of diabetes care programs in primary care, the utilization of medical specialist care is high. This is likely due to the presence of concurrent conditions and complications. Therefore, a shift from a disease-specific approach to a person-centered, integrated care approach can be beneficial in the treatment of type 2 diabetes.
Supported by: AstraZeneca NL
Disclosure: **R.J. Geurten:** None.

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Association between weight change and incidence of cardiovascular disease events and mortality among adults with type 2 diabetes: a systematic review and meta-analysis

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Background and aims: Weight loss is often recommended in the treatment of type 2 diabetes. While evidence has shown that weight loss ≥15kg may lead to diabetes remission, long-term impacts of weight loss are unclear. We performed a systematic review of studies of weight changes and incidence of cardiovascular disease (CVD) and mortality among people with type 2 diabetes.
Materials and methods: Observational studies of weight changes and CVD events among adults with type 2 diabetes, and trials of behavioural interventions targeting weight loss, were identified through searches of MEDLINE, EMBASE, Web of Science, CINAHL, and The Cochrane Library (CENTRAL). Included studies reported a measurement of change in weight and CVD and/or mortality outcomes among adults with type 2 diabetes, or alternatively reported the effect of a behavioural intervention on CVD events and/or mortality among adults with type 2 diabetes. Risk of bias was assessed using modified ROBINS-I and RoB2 tools. We performed a narrative synthesis of observational studies and meta-analysis of trial data.
Results: Of 13,227 identified articles, 17 (14 observational studies, three trials) met inclusion criteria. Weight gain after diabetes diagnosis (vs no weight change) was associated with higher risks of CVD events (HRs (95%CI) ranged from 1.13(1.00, 1.28) to 1.63(1.11, 2.39) and all-cause mortality (HRs (95%CI) ranged from 1.26(1.12, 1.42) to 1.57 (1.33, 1.85)). Evidence of the effect of weight loss on CVD events was conflicting. Unintentional weight loss was associated with increased all-cause mortality but associations with intentional weight loss were unclear. Trials of behavioural interventions targeting weight loss showed no effect on CVD events [pooled hazard ratio: 0.95 (95% CI: 0.72-1.24)] over a range of 5-10 years (Figure 1). Risk of bias was low to moderate in 14 studies and was high in 3 studies, due to potential uncontrolled confounding and method of weight assessment.
Conclusion: While weight gain among adults with type 2 diabetes is associated with a higher risk of CVD and premature mortality, there is little direct evidence supporting weight loss for CVD prevention. Differences in the duration of weight loss and methods of addressing unintentional weight loss may contribute to heterogeneity in results between studies. Long-term follow up of behavioural intervention trials is needed to understand effects on CVD and mortality and to inform policy concerning weight management support for people with diabetes.

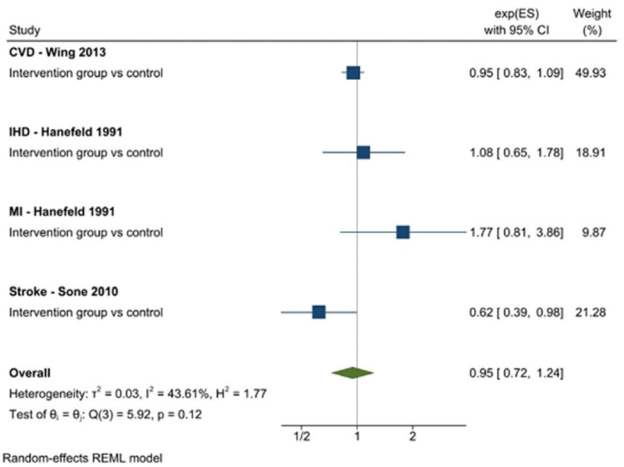

Figure 1. Forest plot and meta-analysis of hazard ratios and 95% confidence intervals from trials of behavioural interventions targeting weight loss and incidence of CVD events, by study and outcome.

CVD: cardiovascular disease; IHD: ischemic heart disease; MI: myocardial infarction

Supported by: MC_UU_12015/4
Disclosure: **J. Strelitz:** None.

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Mortality rates associated with diabetes are now increasing compared to general population: outcomes from a general practice level analysis in England

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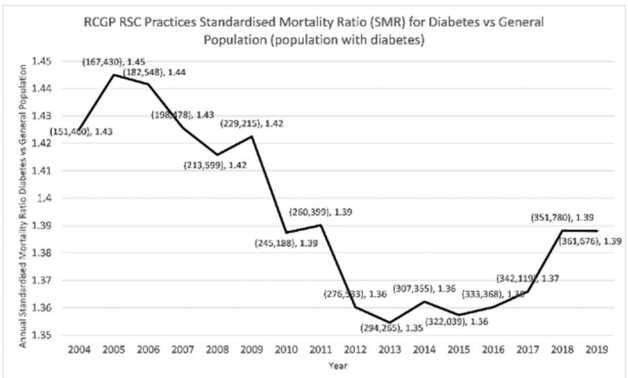
Background and aims: Long-term population mortality rates (MR) have been falling and life expectancy (LE) increasing. The National Diabetes Audit (NDA) mortality report in 2016 gave a standardised mortality ratio (SMR) for diabetes of 1.32 in 2015, a further report gave 1.54 in 2017. The diabetes population has a different age distribution to the total population so SMR was used to compare the relative mortality risk to the standard population, ie ratio between deaths in that population and the expected number of deaths if age-specific mortality rates of the total population were applied to diabetes population age groups. This SMR increase was seen as concerning given the application of new medications and non-pharmacological treatment strategies over recent years. The Royal College of General Practices (RCGP) Research and Surveillance Centre (RSC) provide data on a representative sample of GP practices covering 10% of the English population. Our aim was to confirm this effect and what other factors might be identified.
Materials and methods: Annual data was obtained from RCGP RSC on patient numbers and deaths at general practices for those with diabetes and the total population across 4 age groups (<50, 50-64, 65-80,>80) over 15 years. The population mortality rate in total RCGP RSC practices was compared to the overall national mortality rate given annually by the Office of National Statistics (ONS). The SMR for people with diabetes was calculated for each year and the spread across practices. The investment in diabetes control was measured by the cost of primary care diabetes medication/diabetes patient taken from GP prescribing data for the last 10 years.
Results: During the reporting period 2004 to 2019, data from 552 general practices were analysed. In 2014 they had 3,916,004 population recording 22,820 deaths and within that 154,130 people with diabetes recording 3,332 deaths. In 2019 they had 5,458,786 population recording 44,988 deaths and within that 362,916 people with diabetes recording 11,982 deaths. Comparison with ONS showed between in 2004 the RCGP RSC was capturing 60% of overall deaths. However, since 2013 this has risen from 84% to 94%. The data analysis confirmed that the SMR for diabetes fell from 2004 to 2013. Since 2013 it has grown by 3%. The cost of medication/patient has increased by 20% in the same period. The spread between top and bottom decile practices in SMR was seen to have also fallen from 0.98 to 0.69 between 2013 and 2019; the top decile SMR 1.86 to 1.74 and the bottom decile 0.87 to 1.04.
Conclusion: The relative mortality rate is an important measure of outcome for these patients. However, it reflects differences between those who achieve good and poor control. The source of this increase requires more detailed investigation. The decrease in SMR spread across practices as the overall SMR increased suggests more systematic issues than simply GP practice performance to account for the increase in diabetes SMR.



Disclosure: **M. Whyte:** None.

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The impact of the timing of pregnancies after bariatric surgery on children's health: a retrospective, registry analysis

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Background and aims: Infertility and physical appearance are major reasons in women with severe obesity to undergo bariatric surgery. In the early phase of weight-loss after surgery, pregnancy should be avoided to minimize potential negative impact on the unborn child. The aim of this registry analysis was to analyze the impact of the timing of the pregnancy after bariatric surgery on the children's health.
Materials and methods: The data of the Austrian health insurance were analyzed for women with a history of bariatric surgery in Austria from January 2010 to December 2018. Overall, 14 688 female patients were included. Subsequently, the children's health data of the 1057 (7%) women who gave birth during the observation period were analyzed. The age of the women at the time of bariatric surgery, the time elapsed from surgery to delivery and the days of the offspring in hospital, normalized to the individual observation period, were analyzed.
Results: In 1057 women with pregnancy after bariatric surgery, 1369 births (male: 766, female 633) were registered. The mean time from surgery to birth was 39.4 ± 21.9 months. The age of the mothers at the time of bariatric surgery was on average 27.1 ± 4.9 years. A total of 70 (5%) deliveries were identified within 12 months after bariatric surgery. Within 18 months after the surgery, 252 (18%) births were registered. Overall, 31% of all deliveries occurred within 24 months after bariatric surgery. The children's days hospitalized with birth before/after 12 months of surgery (1.8 ± 4.6 vs. 2.7 ± 11.3 days), before/after 18 months of surgery (2.7 ± 10.2 vs. 2.6 ± 11.3 days) and before/after 24 months of surgery (2.2 ± 8.2 vs. 2.8 ± 12.1 days) were not significant different.
Conclusion: This preliminary data analysis shows that early pregnancies after bariatric surgery are frequently observed. However, the timing of the pregnancy has no significant effect on the children's health based on days hospitalized.
Disclosure: **M. Krebs:** None.

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Childhood bereavement and risk of type 1 diabetes: a Swedish population-based register study

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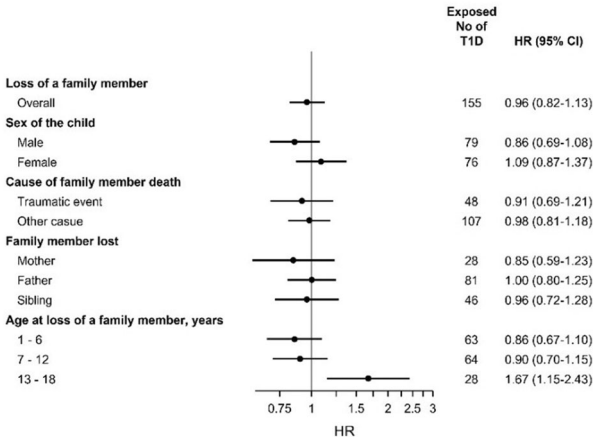
Background and aims: Loss of a first-degree family member in childhood constitutes a major psychological stressor, and is associated both with subsequent psychiatric and somatic morbidity. The potential influence on type 1 diabetes risk has however not yet been fully elucidated. In this study we therefore aimed to investigate the impact of childhood bereavement on type 1 diabetes risk.
Materials and methods: We conducted a population-based study in Sweden, encompassing 2,321,318 children born in Sweden January 1 1990 to December 31 2012. The follow up ended December 31 2013, at death of the child, type 1 diabetes diagnosis, emigration or when the child turned 19 years. All children were followed from the age of one, with exposure defined as death of a mother, father, or sibling. Type 1 diabetes diagnoses were extracted from the National Patient Register. We applied Cox proportional hazards models with attained age as time scale and loss of family member as a time varying variable, adjusting for potential confounders including parental type 1 diabetes, parental country of birth, and region of residence. We further categorized child age at bereavement as pre-school (1-6 years), school age (7-12 years) and teenage (13-18 years).
Results: During follow-up (median 10.8 years), 50,253 (2.2%) children experienced loss of a family member. Median age at loss was 9.6 years, and 32% of all deaths were categorized as traumatic (accident, suicide, violence, or other sudden unnatural deaths). In total 10,965 children were diagnosed with type 1 diabetes during follow-up and median age at diagnosis was 8.5 years. We observed no overall association between childhood bereavement and type 1 diabetes risk (crude HR 1.00, 95% CI 0.86-1.18, adjusted HR 0.96, 95% CI 0.82 -1.13). The risk was not influenced by sex of the child, cause of death of family member, or familial relationship to the deceased. However, we noted an association when the exposure occurred during the teenage years (adjusted HR 1.67, 95% CI 1.15-2.43).
Conclusion: Overall, childhood bereavement was not associated with the risk of type 1 diabetes, but the impact of childhood loss on type 1 diabetes may be modified by age at bereavement.

Figure 1. Childhood bereavement and risk of type 1 diabetes



Supported by: SIMSAM, VR
Disclosure: **M. Wernroth:** None.

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Total costs of care in patients with type 2 diabetes and cardiovascular disease: a comparative cohort study (OFFSET)

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Background and aims: Approximately one third of patients with type 2 diabetes (T2D) have cardiovascular disease (CVD). The 2019 European Society of Cardiology and American Diabetes Association/European

Association for the Study of Diabetes guidelines recommend glucagon-like peptide-1 receptor agonists (GLP-1 RAs) as either first- or second-line antidiabetic medication in these patients. We aimed to investigate the budget implications of treating patients with CVD with GLP-1 RAs rather than standard of care (SoC).

Materials and methods: GLP-1 RA-naïve adults (≥ 18 years old) with T2D in the IBM MarketScan® database who had a claim for an antidiabetic medication (index date) within 6 months after their first hospitalization for CVD were included. Costs of care per month over the 365 days post-index were compared for those who initiated a GLP-1 RA post-hospitalization versus those with a claim for any other antidiabetic medication (SoC).

Results: Prior to hospitalization, total costs were similar for the two groups (GLP-1 RA: $2011; SoC: $1934). After hospitalization and treatment initiation, adjusted mean total costs were lower, although not significantly, for patients receiving a GLP-1 RA compared with SoC ($3584 vs $3638; $p = 0.56$). This was driven by significantly lower inpatient and outpatient costs and significantly higher drug costs (Table).

Conclusion: These findings suggest that the added cost of treating patients with T2D with GLP-1 RAs is offset by significantly lower inpatient and outpatient care costs after CVD hospitalization, resulting in budget neutrality against SoC.

Table. Patient characteristics and costs of care (USD PPPM) for patients receiving GLP-1 RA or SoC*

	GLP-1 RA	SoC
N	1712	122,334
Women, %	40.1	39.2
Age, mean (SD)	58.4 (10.6)	66.5 (12.5)
CCI score, mean (SD)	1.33 (1.67)	1.87 (1.90)
Insurance type (commercial/Medicare), %	77.0/22.8	16.0/52.9
Last treatment before CVD hospitalization, %		
DPP-4i	5.8	5.1
Insulin	33.3	31.5
Metformin	33.1	38.1
Other	19.1	20.8
SGLT-2i	5.8	1.5

	GLP-1 RA	SoC
Unadjusted mean treatment costs before CVD hospitalization†		
Drugs	265	251
Inpatient	1176	1093
Outpatient	570	590
Total	**2011**	**1934**
Unadjusted mean treatment costs after CVD hospitalization		
Drugs	1046	675
Inpatient	1147	1458
Outpatient	1660	2155
Total	**3853**	**4288**

	GLP-1 RA	SoC	
Adjusted mean treatment costs after CVD hospitalization (95% CI)‡			
Drugs	**1201** (1144–1260)	**705** (696–716)	$p < 0.001$
Inpatient	**1070** (944–1213)	**1374** (1308–1444)	$p < 0.001$
Outpatient	**1712** (1615 -1815)	**1970** (1924 -2017)	$p < 0.001$
Total	**3584** (3397–3780)	**3638** (3560–3717)	$p = 0.56$

Study period: 1 September 2012–30 September 2019
Key results are shown in bold text.
*Drug costs included all medications.
†Monthly average of costs over the 12 months post-index (index date was date of initiation of treatment following the first CVD hospitalization), and not including costs for the CVD hospitalization or interim treatment costs between CVD hospitalization and index date. CVD hospitalization was defined as an inpatient admission for any disease of the circulatory system, defined by International Classification of Diseases, Clinical Modification codes, 9th and 10th Revisions.
‡Adjusted means and 95% confidence intervals calculated using a generalized linear model with a log link and gamma-distributed residuals, adjusted for covariates including age, antidiabetic medication at index, sex, CCI score, insurance plan, previous antidiabetic medication, previous CVD medication and baseline cost. *$p < 0.001$ vs GLP-1 RA.
CCI, Charlson Comorbidity Index; CVD, cardiovascular disease; DPP-4i, dipeptidyl peptidase-4 inhibitor; GLP-1 RA, glucagon-like peptide-1 receptor agonist; PPPM, per-patient per-month; SGLT-2i, sodium–glucose cotransporter-2 inhibitor; SoC, standard of care

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Disclosure: **M. Evans:** Honorarium; AstraZeneca, Boehringer Ingelheim and Novo Nordisk.

SO 02 Diabetes across generations

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Paternal ketogenic diet in mice programmes offspring fasting metabolism and physical activity

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Background and aims: Pre-conceptional paternal life experience has been proposed to be an important determinant of offspring metabolic health, including predisposition to metabolic syndrome, cardiovascular disease and diabetes. However, the evolutionary and physiological relevance of such phenomena and the underlying molecular mechanisms remain unclear. In this study we aimed to assess paternal inheritance of metabolic traits following a sustained elevation in circulating ketones, small-chain energy metabolites with epigenetic signalling properties.

Materials and methods: We induced a consistent, sustained 4-fold increase in circulating blood ketones in 4-week old male CD-1 mice, for a 4-week period (the duration of sperm formation in mice) by ad-libitum feeding of a ketogenic diet, with control males fed normal chow. Males fed ketogenic diet did not show significant differences in body-weight or blood glucose levels compared with chow-fed mice. Males were mated for with females fed normal chow and then removed from the cage. Resulting litters were standardized at birth to 8 pups. Male offspring were weaned at 3.5 weeks, weight-matched, monitored throughout life for metabolic differences, and sacrificed at 45 weeks.

Results: Male keto-pups (offspring from ketogenic fathers) showed transient glucose intolerance and a transient reduction in insulin sensitivity that were resolved by 42 weeks, compared with male control-pups. During a 48-hour fasting at 45 weeks, keto-pups were more active (voluntary wheel-running), and had a higher respiratory quotient as measured by metabolic cages. After the 48-hour fast, sacrificed keto-pups show elevated fat content in liver (30% vs. 19%, by Oil Red O staining), and elevated serum HDL, cholesterol and ketones. Transcriptome analysis of keto-pup livers suggested significant differences in circadian rhythm gene expression patterns.

Conclusion: We report here the impact of a paternal ketogenic diet on offspring fasting metabolism and physical activity. Keto-pups were more active during fasting, and showed serum and liver histology changes suggesting better adaptation to prolonged fasting. Transcriptome analysis suggests that the effects involve alteration to the circadian clock, shown previously to be profoundly affected by ketogenic diet (but not shown before in progeny of mice fed a ketogenic diet). We speculate that paternal starvation, via elevated ketone levels, programs the offspring for better coping with life-threatening starvation. The molecular mechanisms underlying paternal transmission in this setting remain to be elucidated, as are the implications for modern humans exercising a ketogenic diet.

Disclosure: **J. Magrill:** None

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Hyperglycaemic memory: role of epigenetic signature in the transmission of gestational diabetes-associated inflammatory and pro-oxidant phenotype

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Background and aims: Chronic hyperglicemia is one of the most potent drivers of atherosclerotic cardiovascular disease (ASCVD). Gestational diabetes (GD) may represent a suitable clinical model to study mechanisms of oxidative stress and inflammation underlying hyperglycemia-induced vascular damage. GD is associated with a range of adverse perinatal and long-term outcomes for both mother and offspring. In this perspective, epigenetic modifications are emerging as key players for the transmission of GD maternal phenotype to the offspring. Recent studies demonstrated a potential involvement of the methyltransferase MLL1, driving H3K4me3, in inflammatory and pro-oxidant pathways. We aimed to investigate whether histone modifications are upstream to the activation of inflammatory and pro-oxidant pathways, along with the potential transmission of epigenetic signature to the offspring.
Materials and methods: We analyzed peripheral blood mononuclear cells (PBMC) from GD and control mothers at third trimester of pregnancy as well as human umbilical vein endothelial cells (HUVEC) and cord blood mononuclear cells (CBMC) isolated from newborn umbilical cords obtained at delivery from both groups. Gene expression was evaluated by real time-qPCR. Protein levels were quantified by western blot and immunocytochemistry. Measurement of reactive oxygen species (ROS) was performed by electron spin resonance spectroscopy. Histone methyltransferase MLL1-dependent trimethylation of histone 3 at lysine 4 amino residue (H3K4me3) on NF-kB p65 subunit promoter region was assessed by chromatin immunoprecipitation and real-time qPCR.
Results: We observed increased gene expression of NF-kB p65 and downstream genes in GD as compared to control cells. For the first time, we demonstrated that MLL1-driven H3K4me3 on NF-kB p65 promoter is upstream to the activation of inflammatory pathway. Indeed, treatment with MLL-1 inhibitor (MM-102) was able to blunt expression of NF-kB p65 as well as VCAM-1, MCP-1 and IL-6 genes. We also found a redox system imbalance in GD cells showed by reduced expression of ROS scavenger aldehyde dehydrogenase 2 (ALDH2), and upregulation of pro-oxidant nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunit NOX4. Interestingly, the increased ROS generation observed in GD leads to the upregulation of MLL1 reverted by the antioxidant effect of vitamin C.
Conclusion: Chronic hyperglycemia-induced oxidative stress and histone modifications may contribute to maternal oxidative and inflammatory phenotypes which could be potentially transmitted to the offspring. This allows a better understanding of epigenetic-induced chromatin remodeling opening towards new pharmacological approaches able to prevent the occurrence of an adverse metabolic phenotypes *leading to ASCVD.*
Disclosure: **N. di Pietrantonio:** None.

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Imprinted genes in beta cell function

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Background and aims: Genomic imprinting is essential for the development and function of the human body. Perturbations in genomic imprinting have been implicated in several diseases including type 2 diabetes. The imprinted genes, the *GRB10* and *H19* have been shown to play key roles in β cell development, and variants at the same loci associated with beta cell function. Here, we aim to identify and characterize novel imprinted genes that can play a role in β cell function.
Materials and methods: A list of 239 imprinted genes in humans was compiled from geneimprint.com and previous studies. Expression data from whole human pancreas(The Genotype-Tissue expression GTEx), pancreatic islets (in-house) and pancreatic cell types (previous publications) were leveraged to elucidate expression of the imprinted genes in these tissues. Expression profile of these genes were examined in 16 fetal pancreatic samples between 5-14 weeks post-conception. Genes were defined as expressed if 75% of the samples had >1 count per million. Subsequently, imprinted genes that were expressed in fetal and adult pancreas, pancreatic islets and were specific or enriched in β cells were selected for further analysis. Genetic regulation of gene expression was assessed in two ways; allele specific expression (ASE) and expressed quantitative trait loci (eQTL) analysis and were conducted on both fetal and adult samples. Variants and their proxies from these analyses were checked for associations with indices of insulin secretion and related phenotypes in cohorts including the Botnia study (n=9210) from Western Finland, the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) as well as Integrated Network for Systematic analysis of Pancreatic Islet RNA Expression Consortium (INSPIRE).
Results: Of the 239 imprinted gene, 173 were expressed in both adult and fetal pancreas, of which 131 was expressed in pancreatic islets. Of these, 7 genes were enriched in β cells compared to other cell types. In pancreatic islets, the expression of *GLIS3* correlated with HbA1c levels. With the exception of *PLAGL1*, the expression of the remaining 6 genes were correlated with that of *INS*. The *GLIS3* and *TSHZ3* genes were expressed in an allele specific manner in pancreatic islets (FDR < 0.05). The rs10758591 SNP in the intron of *GLIS3* was associated with HOMA-B in the MAGIC data ($p = 0.00004$). The rs75438148, rs117246774 and rs56381834 SNPs were associated with the expression of *DKL1*, *TSHZ3* and *PHACTR2* respectively (FDR < 0.05) in the pancreatic islets from the INSPIRE study. These variants and their proxies were assessed for associations with different phenotypes. The s75438148 SNP and its proxies were associated with APOA1 and HDL, whereas the rs117246774 SNP and its proxies were associated with lean body mass, height and LDL in the Botnia study. The *MEG3, TSHZ3* and *SFMBT2* genes were nominally expressed in an allele specific manner in the fetal pancreas (p-value < 0.05).
Conclusion: Our data indicates the possibility of some imprinted genes to have a role in β cell function. The *MEG3, SFMBT2* and *GLIS3* genes showed temporal changes in imprinting; MEG3 and *SFMBT2* showed *ASE* in fetal but not adult tissues whereas *GLIS3* showed ASE in adult islets only. Genetic variants associated with gene expression (eQTL or ASE) of some of these genes also associated with indices of insulin secretion. Studies are ongoing to validate the role of these genes in insulin secretion in β cell models.
Supported by: Crafoord, Åke Wiberg, Heart Lung, Hjelt and Påhlsson foundations.
Disclosure: **G. Hatem:** None.

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Methylomic trajectories in the human pancreas: from fetal development to adulthood

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Background and aims: Despite advances in identifying genes contributing to diabetes risk, there remains uncertainty about how their function is developmentally regulated in cells and tissues relevant to the disease. Human pancreas development is known to involve spatially- and temporally-coordinated changes in gene expression, but the precise molecular mechanisms underlying this process have not been fully described. Our study aimed to characterize the patterns of DNA methylation - a key epigenetic mark which plays a key role in orchestrating transcriptional regulation during human development - in the human pancreas across prenatal development and into adulthood.
Materials and methods: We quantified DNA methylation at over 850,000 sites across the genome using the Illumina EPIC array in 101 fetal pancreas samples (45 male, 56 female) obtained from the Human Developmental Biology Resource (HDBR) ranging from Carnegie Stage 18 to 21 weeks post conception. We also profiled DNA methylation in 23 adult pancreatic samples obtained from the Barts Pancreas Tissue Bank (9 male, 14 female, ages 34-80 years). Parallel DNA methylation data was

also available on a large cohort of fetal and adult brain (cortex) as part of ongoing work in our lab.
Results: We found dramatic changes in DNA methylation associated with human pancreas development, with over 140,000 differentially methylated positions (DMPs) and 8,000 differentially methylated regions (DMRs) identified across the genome. Of note, these DMRs were enriched in functional domains annotated to maturity-onset diabetes of the young (MODY) and annotated to gene pathways involved in diabetes, metabolism and development. Highly significant differences in DNA methylation were observed between males and females at a number of autosomal sites. A comparison with data generated on human fetal cortex highlighted both synergistic and tissue-specific developmental changes in DNA methylation.
Conclusion: We have performed the first systematic analysis of the dynamic changes in DNA methylation occurring across human pancreas development. Our data highlight regulatory networks important for pancreatic function and disease. Our data, which includes matched genetic data, represents a unique resource for understanding development of the human pancreas and the identification of epigenomic variation in diabetes and other diseases.
Supported by: E3 Fund
Disclosure: **A. MacCalman:** None.

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Increased risk of fetal abdominal obesity (FAO) in old and/or obese pregnant women with normal glucose tolerance
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Background and aims: We previously reported that fetal abdominal overgrowth was detected at the time of GDM diagnosis at 24-28 weeks of gestation, especially in old and/or obese women and persisted until delivery despite appropriate GDM management. In this study, we investigated the fetal abdominal growth in old and/or obese pregnant women with normal glucose tolerance (NGT).
Materials and methods: Medical records of 7820 singleton pregnant women including 380 GDM subjects, delivering at CHA Gangnam Medical Center were reviewed retrospectively. GDM and NGT were diagnosed by 100-g oral glucose tolerance test after 50-g glucose challenge test (GCT) based on Carpenter-Coustan criteria. Data on maternal anthropometry, fetal biometry measured at 50-g GCT and near term, and pregnancy outcomes were collected. Fetal abdominal obesity was investigated by assessing the fetal abdominal overgrowth ratios (FAORs) of the ultrasonographically estimated gestational age (GA) of abdominal circumference (AC) per actual GA by the last menstruation period (LMP), biparietal diameter (BPD) or femur length (FL), respectively. FAO was defined as ≥90th percentile of FAORs of total subjects. GDM and NGT subjects were divided into four study groups: group 1 (age<35 and pre-pregnant BMI < 25), 2 (<35 & ≥25), 3 (≥35 &<25), and 4 (≥35 &≥25). All analyses were conducted using STATA version 15.1 (StataCorp LP, College Station, Texas).
Results: 1) FAORs of old group 3 and 4 NGT subjects were significantly higher than those of young and non-obese group 1 NGT subjects. Comparing with each NGT groups, FAORs of group 3 and 4 GDM subjects were significantly higher than each NGT groups (p<0.05). 2) Relative to group 1 NGT subjects, odds ratios for FAO by FAOR of GA-AC/GA-LMP at 50-g GCT was 1.42 in group 3 NGT and increased to 2.90 and 3.81 in group 3 and 4 GDM subjects respectively (p<0.001). At near term, odds ratios for FAO by FAOR of GA-AC/GA-LMP were 2.83, 1.66, and 2.41 in group 2, 3 and 4 NGT subjects (p=0.001) and these ratios increased to 5.04, 3.18, and 4.47 in each group of GDM subjects (p=0.001), but the FAORs of group 1 GDM were not different from those of group 1 NGT subjects. 3) Relative to group 1 NGT subjects, odds ratios for LGA at birth were 3.06, 1.47, and 2.83 in group 2, 3, and 4 NGT subjects and these ratios increased to 1.98, 4.81, 3.77, and 6.01 in group 1- 4 GDM subjects. Odds ratio for macrosomia was 2.53 in group 2 NGT subjects and these ratios were 2.30 and 4.62 in group 3 and 4 GDM subjects. Odds ratio for primary cesarean delivery was 1.33 in group 3 NGT and the ratio increased to 2.26 in group 3 GDM subjects (p<0.001).
Conclusion: Fetal abdominal overgrowth and adverse pregnancy outcomes were observed even in old and/or obese NGT subjects even though those were milder than in GDM subjects. It could be suggested that old and/or obese pregnant women with normal glucose tolerance need intervention to prevent fetal abdominal obesity.
Disclosure: **Y. Kim:** None.

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Both gestational diabetes exposure and maternal methylome interaction impacts offspring epigenetic signature
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Background and aims: Gestational diabetes mellitus (GDM) is associated with a future offspring risk for the development of obesity and insulin resistance, which could be explained by an epigenetic mechanism in response to hyperglycaemia exposure during pregnancy. Although several loci have been implicated in GDM, due to the small sample size and genetic heterogeneity of the majority of these studies, this link remains undetermined. Therefore, we sought to perform the largest GDM epigenetic study using a single Finnish FinnGeDi cohort.
Materials and methods: We designed a case-control study with a total of 536 mother-offspring pairs, of which 298 pairs had GDM exposure; mothers were matched by age and pre-pregnancy BMI. DNA extracted from whole blood from mothers and cord blood from offspring was subjected to methylome analysis using the Illumina Infinium Methylation-EPIC 850K arrays. We performed three epigenome-wide association studies in mothers (adjusted for age, BMI, maternal weight gain and blood cellular composition) and offspring (adjusted for gestational week, birthweight, sex and cord blood cellular composition) to identify differentially methylated sites associated with GDM exposure in 1) mothers and offspring, separately, and 2) shared in both mother-offspring pairs, and 3) offspring-specific effects, by including maternal methylation as a covariate.
Results: We did not identify any significantly differentially methylated sites associated with GDM in the offspring and mothers, nor a shared effect in mother-offspring pairs. We then adjusted offspring methylation marks for the maternal methylome to account for the impact of the maternal current and past environment and identified a single FDR-significant site at the cg22790973 probe located in the TFCP2 gene. Additionally, we included an interaction term in our statistical model to identify sites that are not only affected by GDM status, but also by maternal methylation according to GDM status. We identified a further seven genes, with the strongest association at the same cg22790973 probe (TFCP2), plus cg03456133, cg24440941 (H3C6), cg20002843 (LOC127841), cg19107264, cg11493553 located in the UBE3C gene and cg17065901 in FAM13A, both susceptibility genes for type 2 diabetes and BMI and cg23355087, within the DLGAP2 gene, a gene associated with insulin resistance during pregnancy.

Conclusion: Our study does not support robust and simple epigenetic associations in offspring and mothers in response to GDM. However, in terms of the offspring-specific epigenetic signature, our study demonstrates for the first time the potential complexity of epigenetic transmission in response to GDM. The epigenetic signatures in the offspring are likely to be not only determined by GDM status, but also by factors that have influenced maternal epigenetic status in the past, implying that the maternal metabolic and glycaemic long-term history matters.
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Disclosure: **A. Khamis:** None.

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New clinical score to predict glucose intolerance at postpartum reclassification in women with gestational diabetes

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Background and aims: Women with gestational diabetes mellitus (GDM) have a higher risk of future type 2 diabetes mellitus (T2DM). An oral glucose tolerance test (OGTT) at 4-12 weeks postpartum is recommended in all women but compliance can be poor. We aimed to develop a clinical score system to help predict the presence of glucose intolerance at postpartum OGTT.
Materials and methods: Retrospective study of the Portuguese GDM registry. From a total of 19545 women, we excluded 6841 (35.0%) with no data on postpartum OGTT, and 2599 (13.3%) with missing data on BMI, first-degree familiar history of T2DM, previous GDM or arterial hypertension (AHT), academic degree, or medication used. Glucose intolerance was defined based on the OGTT at 4-12 weeks postpartum. Glycaemic increment (GI) was defined as the difference in mg/dL between the glycaemia of the patient and the diagnostic cutoff for GDM (in either fasting glucose at the 1st trimester or the OGTT at 24-28 weeks). Patients with and without glucose intolerance at reclassification were compared. A multivariate logistic regression analysis was used to study predictors of glucose intolerance. Variables included were those associated with abnormal glucose. A clinical score was derived from the regression model using the smallest regression coefficient as 1 point. A receiver-operating characteristic (ROC) curve was used to determine the best score cutoff for glucose intolerance prediction.
Results: A total of 10105 women were studied and 793 (7.8%) had glucose intolerance at postpartum reclassification. Women with glucose intolerance were older, less often had an academic degree, and more frequently had a personal history of GDM or AHT, and a family history of T2DM. Their BMI and GI were higher, and they more often needed insulin during pregnancy. In the multivariate regression model, age, family history, previous GDM, AHT, insulin therapy and GI were independent predictors of glucose intolerance. Based on this regression a scoring system was developed. Points were given based on age (≤30 years, 0; 31-35, 5; 36-40, 7; 41-45, 9; and >45, 11 points); BMI (<25.0Kg/m2, 0; 24.0-29.9, 1; 30-34.9, 2; 35-39.9, 3; and >40, 4 points); familiar history of T2DM, 10 points; previous GDM, 8 points; AHT, 13 points; Academic degree, minus 4 points; Insulin therapy, 11 points; and GI (0-2 mg/dL, 0; 3-5, 4; 6-10, 8; 11-20, 8; 21-30 13; 31-40, 19; 41-50, 24; 51-60, 29; 61-70 35; and >70, 40 points). The area under the ROC curve was 0.71 (95% CI: 0.69-0.73), p<0.001. Median score was 19 (11-29) ranging from -4 to 88 points. The number of patients with glucose intolerance was 2.6%, 3.6%, 4.3%, 5.2%, 6.6%, 8.1%, 11.1%, 17.4%, and 26.5% for score results of ≤5, 6 to 10, 11 to 15, 16 to 20, 21 to 25, 26 to 30, 31 to 35, 36 to 40 and >40, respectively. A score ≤10 had a sensitivity of 90.4%, specificity 74.5%, negative predictive value 96.9%; and a score ≥40 had a sensitivity of 29.5%, specificity 7.4%, positive predictive value 25.2%.
Conclusion: We propose a new simple score based on widely available clinical data that allow clinicians to identify women with GDM at higher risk of future diabetes mellitus.
Disclosure: **C. Cidade-Rodrigues:** None.

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Postpartum screening of women with gestational diabetes in specialised diabetes practices in the period from 2015 - 2017: data from 12,991 women from the GestDiab registry

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Background and aims: Women who had gestational diabetes (GDM) have an increased risk of developing manifest diabetes mellitus and cardiovascular diseases later in life. Given the increased risks associated with GDM, the postpartum screening for diabetes 6 to 12 weeks after childbirth is important to monitor women's metabolism. Therefore, our aim was to assess the proportion of women with GDM who were screened postpartum for diabetes.
Materials and methods: For our observational study we used data of the nationwide GestDiab registry for the years 2015-2017. We assessed the proportion of postpartum diabetes screening among first-time pregnancies during the study period that were associated with GDM. In addition, we investigated whether postpartum screening was associated with maternal characteristics by using univariate and multiple logistic regressions considering cluster adjustment by random effects for the diabetes specialized practices
Results: Our final sample consisted of 12,991 first-time pregnancies. A total of 38.2% (95% CI 32.8% - 43.7%) of our sample were screened for diabetes postpartum, regardless of the timing. Around 50% of these women attended the postpartum diabetes screening within the recommended time frame of 6 to 12 weeks postpartum. We found that women older than 27 years were more likely to attend postpartum screening, with little difference between the age groups above 27 years. Women whose mother tongue was Turkish or Arabic were less likely to attend postpartum screening. Similarly, women with higher fasting blood glucose and women with HbA1c above 5.5% at diagnosis versus ≤4.9% were less likely to attend (OR 0.80 (0.70 - 0.91)). In addition, women who smoked were less likely to participate (OR 0.39 (CI 0.34 -0.45)). Women with a BMI of 35 or higher were also less likely to participate. Treatment with insulin was associated with more frequent attendance at postpartum screening (OR 1.79 (1.63 - 1-98)), while a previous pregnancy that had been complicated by GDM was associated with lower attendance (OR 0.85 (0.75 - 0.96)).
Conclusion: With a total of 38.2% of women in our sample undergoing postpartum diabetes screening, the screening rate appears to be smaller than expected. Thus, in more than 60% of cases, opportunities were missed to diagnose manifest diabetes and provide appropriate medical care or to identify those at increased risk of developing manifest diabetes in the coming years. Factors associated with participation in postpartum screening could be largely related to women's general health awareness, but there is a lack of knowledge about the reasons why women miss the opportunity for postpartum screening. Further research should explore barriers and facilitating factors from both the patient and healthcare provider perspectives, particularly in Germany, in order to develop a multi-level approach to postpartum GDM care.
Disclosure: **U. Linnenkamp:** None.

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A lifestyle intervention to prevent deterioration of glycaemic status among women with previous gestational diabetes: the LIVING trial

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Background and aims: This study aimed to determine whether a pragmatic resource and culturally appropriate lifestyle intervention programme offeredto women with recent prior gestational diabetes mellitus (GDM)in South Asia would reduce the incidence of deterioration in glycaemic status.

Materials and methods: Women with GDM from 19 hospitals in India, Sri Lanka and Bangladesh were invited for an oral glucose tolerance test (OGTT) 6±3 months post-partum. Those who had not developed type 2 diabetes mellitus (T2DM) and provided written informed consent were randomised to a 12-month low-intensity group (4 sessions) and individual (2 sessions, if needed, plus text/audio messages) lifestyle intervention programme, or usual care. Due to the impact of COVID-19 pandemic, a substantial proportion of group sessions were individualized and planned face-to-face sessions were delivered remotely. The primary outcome was proportion of women with a worsening in glycaemic category based on American Diabetes Association OGTT criteria:1) normal glucose tolerance (NGT) to impaired fasting glucose (IFG), impaired glucose tolerance (IGT),or both; or 2)NGT to T2DM; or 3)IFG, IGT or both to T2DM. Secondary outcomes included development of T2DM, body weight, waist circumference and systolic blood pressure.

Results: 1832 potentially eligible participants underwent an OGTT at a median of 7 months post-partum. After exclusion of 162 (8.8%) with T2DM, 1612 agreed to be randomized, but 11 patients were subsequently excluded from analysis due to ineligibility. At baseline, the mean age of randomized participants was 30.8 years (SD 4.9), mean body mass index (BMI) was 26.6 (SD 4.7), 62.5% had NGT, 15.0% had IFG, 11.7% had IGT and 10.7% had both IFG and IGT. Among the 808 women randomized to the lifestyle intervention, 79.7% received the content of all planned group or individual sessions. The proportions participating in only 3, 2, 1 and no group sessions, respectively, were 3.7%, 1.9%, 0.9% and 13.6%, respectively. As a result of the COVID-19 pandemic, 39% of sessions were delivered remotely by telephone, and 27% of group sessions were individualized. After a median of 14.5months follow-up, 1308 (81.2%) had an end-of-study (EOS) visit or at least one follow-up OGTT. The lifestyle intervention, compared to usual care, was not associated with a reduction inthe incidence of worsening glycaemic category (25.5% vs. 27.1%; hazard ratio, 0.92 [95%CI: 0.76 to 1.12]). The lifestyle intervention, compared to usual care, was also not associated with significant reductions in any of the secondary outcomes. There was no evidence of heterogeneity of intervention effect on the primary outcome by baseline glycaemic status, age, gravida, BMI, insulin use during pregnancy, time since GDM-affected pregnancy, or country. Only 24 serious adverse events were reported during follow-up (13 in intervention and 11 in control group).

Conclusion: After a GDM-affected pregnancy, 9% of women were T2DM at a median of 7 months post-partum and over one-third of the remainder had pre-diabetes. At a median of 15.5 months subsequent follow-up, about one-quarter of women without T2DM were found to have a deterioration in glycemic status. A pragmatic low-intensity lifestyle intervention, substantially modified due to the COVID-19 pandemic, did not prevent this deterioration.

Clinical Trial Registration Number: NCT03305939
Supported by: GACD
Disclosure: **N. Tandon:** None.

SO 03 Diet, lifestyle and behaviour

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Vitamin C intake and the risk of islet autoimmunity and type 1 diabetes: The Environmental Determinants of Diabetes in the Young (TEDDY) Study

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Background and aims: High plasma vitamin C status in childhood might protect against islet autoimmunity. We studied whether childhood vitamin C intake is associated with the risk of islet autoimmunity and progression to type 1 diabetes and whether single nucleotide polymorphisms (SNPs) in vitamin C metabolism related genes modify these associations.

Materials and methods: The Environmental Determinants of Diabetes in the Young (TEDDY) birth cohort study follows children genetically at risk for type 1 diabetes (n=8,676) in the U.S., Finland, Germany, and Sweden. Blood samples were collected every 3-6 months from birth for the assessment of type 1 diabetes related autoantibodies. Vitamin C intake was assessed from diet and dietary supplements from 3 months to 10 years of age by means of 3-day food records. SNPs in vitamin C metabolism genes were genotyped using the ImmunoChip and TEDDY-T1DExome custom microarray.

Results: Based on Cox proportional hazard regression including 8,081 children, mean total energy-adjusted vitamin C intake was not associated with the risk of islet autoimmunity [hazard ratio (HR) 1.000; 95% CI 0.998-1.001], primary insulin autoimmunity (HR 1.001; 95% CI 0.998-1.003), or progression from islet autoimmunity to type 1 diabetes (HR 0.998; 95% CI 0.994-1.001). SNPs in vitamin C metabolism genes were not associated with outcomes but ascorbic and dehydroascorbic acid transport and glutathione S-transferase genotypes modified the association between vitamin C intake and islet autoimmunity (Table 1).

Conclusion: Findings suggest that genotype involved in free-radical detoxification in combination with high vitamin C intake may protect against islet autoimmunity while the combined role of vitamin C intake and genotypes involved in vitamin C transport might increase the risk of islet autoimmunity.

Table 1 - Interaction of vitamin C metabolism related genes and energy-adjusted vitamin C intake[a] on the risk of islet autoimmunity (IA), and the association between vitamin C intake and the risk of islet autoimmunity according to genotypes. P-values are corrected for multiple testing by the false discovery rate-method.

Gene SNP	*p* Value for interaction[b]	Number of minor alleles (% of children by genotype)	HR (95% CI) for IA associated with vitamin C intake	*p* Value[c]
SLC2A2 (GLUT2) rs1604038 (A allele)	**0.037**	GG (50.93)	0.998 (0.995-1.001)	0.162
		GA (41.03)	0.999 (0.996-1.002)	0.512
		AA (8.04)	**1.003 (1.001-1.005)**	**0.008**
SLC2A2 (GLUT2) rs5400 (A allele)	**0.027**	GG (75.38)	0.999 (0.996-1.001)	0.214
		GA (22.78)	0.999 (0.996-1.003)	0.713
		AA (1.83)	**1.007 (1.003-1.011)**	**0.001**
SLC23A2 rs1776963 (A allele)	**0.037**	GG (68.69)	0.998 (0.995-1.000)	0.098
		GA (28.02)	1.001 (0.999-1.003)	0.184
		AA (3.29)	**1.015 (1.005-1.026)**	**0.004**
GSTP1 rs79512139 (A allele)	**0.037**	GG (82.41)	1.001 (0.999-1.002)	0.430
		GA (16.88)	**0.992 (0.986-0.998)**	**0.009**
		AA (0.70)	0.989 (0.957-1.022)	0.515

Abbreviations: HR, hazard ratio; IA, islet autoimmunity; SNP, single nucleotide polymorphism.
[a] Energy-adjusted using residual method.
[b] Interaction of childhood vitamin C intake with the genotype on the risk of islet autoimmunity in model adjusted for vitamin C intake, sex, HLA genotype DR3/4, family history of type 1 diabetes, country, and three largest principal components for ethnicity.
[c] Adjusted for sex, HLA genotype DR3/4, family history of type 1 diabetes, country, and three largest principal components for ethnicity.

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Disclosure: **M. Mattila:** None.

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A high-protein and -unsaturated fatty acid diet increases fasting and postprandial insulin sensitivity independently of weight loss: 6-months results of the NutriAct Trial

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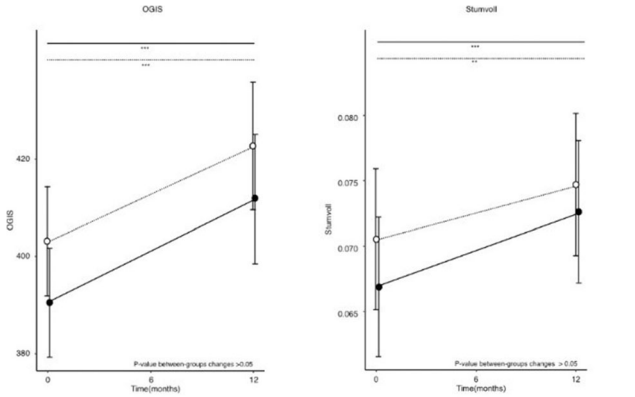
Background and aims: It is still unclear whether a long-term dietary intervention not aiming weight loss with high- protein- and unsaturated fatty-acids (UFA) intake improves insulin sensitivity independently of weight loss.
Materials and methods: 502 participants were randomized into either usual care control group including dietary recommendations of the German Nutrition Society (DGE) or an intervention group, which used supplementation of rapeseed oil and specifically designed foods as well as repetitive advices to implement a food pattern based on high intake of predominantly plant proteins, UFA and fiber (NutriAct pattern). Food intake was repeatedly assessed by 3-day food records at months 0, 3 and 6. Participants with data on dietary intake and who underwent a 3h-oral-glucose-tolerance-test (OGTT) at baseline and month 6 were included in the present analyses. Insulin-sensitivity-indexes (ISI) based on post-prandial insulin and glucose levels, Stumvoll and OGIS, as well as the fasting insulin resistance index HOMA were calculated based on published formulas with OGTT- and antropometric-data collected at each study visit.
Results: 471 participants (235/236 control/intervention, 35% male, median age 66y, 10% with T2D, 41% obese) were included in the present analyses. In the first 6 months of the trial, the intervention but not the control group showed improvements in the three studied indexes (all within-group $p<0.001$). Participants from the intervention group had a significantly higher HOMA decrease ($p=0.02$) as well as greater increase in the Stumvoll and OGIS indexes ($p=0.01$ and $p<0.01$) after adjustment for age, sex and BMI changes. Irrespective of the study arm assignment, participants with greater increases in poly- and mono-unsaturated fatty-acids (PUFA and MUFA) intake showed a higher decrease in the HOMA index, independently of weight changes ($p<0.05$). Individual macronutrients changes were not associated to Stumvoll or OGIS indexes.
Conclusion: The NutriAct intervention led to an improvement of the post-prandial ISI Stumvoll and OGIS as well as in the fasting HOMA-Index in 6 months. Higher increases in PUFA- and MUFA-intake were associated to a steeper HOMA decrease, but not with changes in OGIS and Stumvoll indexes. All these associations were independent of weight changes.



Circles indicate predicted least square means and bars indicate their respective 95% confidence intervals derived from linear mixed models. Models adjusted for age, sex and changes in BMI. * indicates p<0.05; **p<0.01, *** p<0.001.

Clinical Trial Registration Number: DRKS00010049
Supported by: German Ministry for Education and Research
Disclosure: **L. Pletsch-Borba:** None.

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Habitual intake of dietary advanced glycation endproducts is not associated with generalised microvascular function: the Maastricht study

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Background and aims: Endogenously-formed advanced glycation endproducts (AGEs) are important drivers of microvascular dysfunction and the microvascular complications of diabetes. AGEs are also formed in food products, especially during preparation methods involving dry heat. However, whether these dietary AGEs also contribute to microvascular function is currently unknown.
Materials and methods: In 3140 participants of The Maastricht Study (age 60 ± 8 years, 51% men, normal glucose metabolism n=1779, prediabetes n=462, and T2DM n=870) we used multiple linear regression to estimate cross-sectional associations between intake of dietary AGEs and generalized microvascular function while adjusting for demographical, cardiovascular, lifestyle, and dietary factors. Intake of dietary AGEs N^{ε}-(carboxymethyl)lysine (CML), N^{ε}-(1-carboxyethyl)lysine (CEL), and N^{δ}-(5-hydro-5-methyl-4-imidazolon-2-yl)-ornithine (MG-H1) was assessed using the combination of our UPLC-MS/MS dietary AGE database and a food frequency questionnaire.
Results: Overall, intake of CML, CEL, and MG-H1 was not associated with microvascular function of the retina (flicker light-induced arteriolar and venular dilatation, and central arteriolar and venular equivalent), in plasma (z-score of endothelial function biomarkers sVCAM, sICAM, eSelectin, and vWF), in skin (heat-induced skin hyperemic response), and in urine (24-hour albuminuria). These associations were not dependent on glucose metabolism status.

Conclusion: We did not show any strong association between habitual intake of dietary AGEs and generalized microvascular function, regardless of glucose metabolism status.
Clinical Trial Registration Number: NL31329.068.10
Supported by: The Maastricht Study is supported by grants, see Schram et al. Eur J Epidomiol 2014 for an overview
Disclosure: **A. Linkens:** None.

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Oral glucose tolerance test results are altered by the size of the last meal before the test

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Background and aims: The oral glucose tolerance test (OGTT) is widely used in epidemiological studies globally and in sub-Saharan Africa to determine prevalence of diabetes and glucose intolerance. In many households in sub-Saharan Africa, obtaining sufficient food remains a challenge. It is not clear how restrictions in food availability affects OGTT results. Aims: To determine the impact of the size of evening meal the day before OGTT.
Materials and methods: 40 adult participants (mean age 43.7yrs, mean BMI 23.3/kg/m2, 21 male) with no history of diabetes were recruited from rural Uganda for a randomized cross-over study. The day prior to the OGTT participants were admitted and given a normal midday meal (1065 kcal, 139g carbohydrate) and then randomly either a normal calorie/normal carbohydrate (1065 kcal, 139g carbohydrate) or a small (58 kcal, 14g carbohydrate) evening meal. OGTT was performed the next morning with 30-minute glucose sampling. The protocol was repeated after one week with the alternative evening meal.
Results: Glucose tolerance was worse after the small evening meal (mean area under plasma glucose-time curve 925 mmol min/L(small) v 841mmol min/l(normal) P<0.001) with a higher 2 hr OGTT value (7.2 v 6.3 mmol/l P=0.003) and the diagnosis of impaired glucose tolerance (>7.8mmol/L) doubled (10 v 5 P= 0.18).
Conclusion: This randomized cross over study demonstrates that abnormal glucose tolerance results may not reflect sustained hyperglycaemia, but rather response to the glucose load as a result of the previous short-term food restriction. This indicates that glucose tolerance results might not reflect the actual burden of diabetes in some African populations.
Clinical Trial Registration Number: PACTR202007803347704
Supported by: NIHR using ODA funding
Disclosure: **W. Nakanga:** None.

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Tobacco use and interaction with genotypes of human leucocyte antigen in the risk of latent autoimmune diabetes in adults: results from two population-based studies

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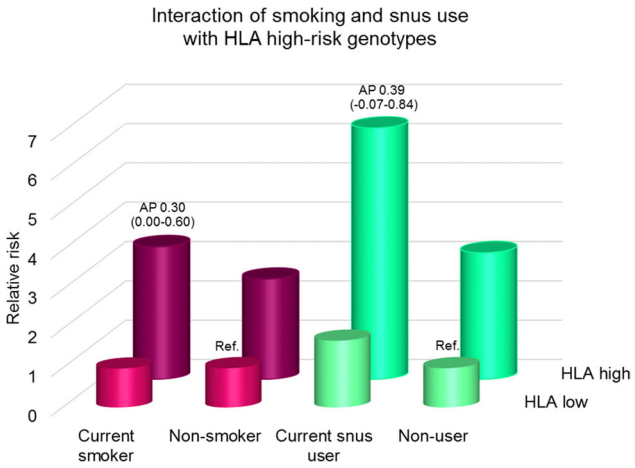
Background and aims: Smoking and use of smokeless tobacco (snus) are associated with increased risk of type 2 diabetes but their influence on the risk of latent autoimmune diabetes in adults (LADA) is unclear. Our aim was to clarify the association between smoking, snus use, and LADA and to investigate potential interactions with human leucocyte antigen (HLA) high-risk genotypes.
Materials and methods: We used data from a Swedish case-control study including incident cases of LADA (n=591) and type 2 diabetes (n=2026) and 3009 matched non-diabetic controls, and a Norwegian prospective study with incident cases of LADA (n=143) and type 2 diabetes (n=1959) over 894,099 person-years of follow-up. Pooled relative risks (RRs) with 95% confidence intervals (CIs) were estimated for smoking, and odds ratios (ORs) for snus use (case-control data only). Potential interactions between tobacco use and HLA high-risk genotypes were assessed by attributable proportion due to interaction (AP). Models were adjusted for age, sex, BMI, education and alcohol consumption.
Results: Both current vs. never smoking (RR 1.31; 95% CI 1.05, 1.65) and long-term, heavy snus use (OR 1.93; 95% CI 1.17, 3.18 for ≥15 box-years vs. never use) were associated with an increased risk of LADA. There were indications of interaction between smoking and HLA high-risk genotypes (AP 0.30; 95% CI 0.00, 0.60) in relation to LADA and a similar tendency for snus use and HLA (AP 0.30; 95% CI -0.07, 0.84) (Figure). Smoking was positively associated with insulin resistance (HOMA2-IR) in LADA patients (β 0.23, p 0.008 for ≥15 pack-years vs. never smoking). Smoking and snus use were also associated with increased risk of type 2 diabetes, but there was no sign of interaction with HLA genotypes.
Conclusion: Our findings suggest that smoking and snus use increase the risk of LADA, and that tobacco use acts synergistically with genetic susceptibility in the promotion of LADA.



Supported by: Swedish Research Council, FORTE, Novo Nordisk Foundation, Swedish Diabetes Foundation

Disclosure: **J. Edstorp:** None.

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Clustering of risk behaviors and associations between risk behaviors and cardio-metabolic risk factors in adult individuals with type 1 diabetes

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Background and aims: Management of type 1 diabetes (T1D) calls for balancing several, seemingly independent, self-management practices. We investigated clustering of risk behaviors in adults with T1D and assessed whether such clustering is associated with cardio-metabolic risk factors.

Materials and methods: Subjects were participants with T1D in the FinnDiane Study. Included, in these cross-sectional analyses, were all with eGFR ≥30 ml/min/1.73 m^2 and health behavior data (diet, alcohol consumption, leisure-time physical activity [LTPA], smoking, and sleep). Diet data were collected by two 3-day food records. Those with macronutrient and fiber intakes according to the Finnish dietary recommendations were considered adherent. High alcohol consumption was defined as alcohol intake >10 g/day in women and >20 g/day in men. LTPA was self-reported. Those at least moderately active (10-40 metabolic equivalent of task hours), reporting >2 weekly sessions were considered adherent. All but those reporting sleeping as well as usual, in the Beck Depression Inventory, were defined as having poor sleep. Current smoking was self-reported. Generalized linear regression was used to study the associations between risk behaviors and cardio-metabolic risk factors.

Results: In 956 individuals (40% men, mean age 46 years) diet non-adherence was reported by 84%, high alcohol consumption by 15%, low levels of LTPA by 54%, smoking by 11%, and poor sleep by 42%. In all, 4.3% reported no risk behaviors, while 26%, 37%, 25%, 6.8%, and 1.0% had 1, 2, 3, 4, and 5 risk behaviors, respectively. Any combination of ≥4 risk behaviors occurred more frequently than expected by chance. After adjustments, those with ≥2 risk behaviors had higher BMI, waist circumference (WC), and diastolic blood pressure (DBP). Those with ≥3 risk behaviors had larger waist-hip ratio (WHR), and higher HbA_{1c}, and triglycerides (TG). Those with ≥4 risk behaviors had higher cholesterol. Dietary non-adherence was associated with higher WC (odds ratio 2.447, 95% confidence interval 0.329-4.564), WHR (0.016, 0.004-0.029), and HDL-cholesterol (0.100, 0.024-0.175), but lower systolic (-3.005, -5.864- -0.145) and DBP (-1.706, -3.347- -0.065). High alcohol consumption was associated with higher HDL-cholesterol (0.090, 0.013-0.167). Low LTPA was associated with higher BMI (1.258, 0.713-1.803), WC (3.735, 2.192-5.277), WHR (0.016, 0.007-0.025), DBP (2.013, 0.814-3.212), HbA_{1c} (2.866, 1.242-4.490), and TG (0.170, 0.075-0.266), but lower HDL-cholesterol (-0.078, -0.133- -0.023). Smoking was associated with lower BMI (-1.155, -2.011- -0.299), but higher HbA_{1c} (4.328, 1.778-6.879) and TG (0.162, 0.013-0.312). Finally, poor sleep was associated with higher BMI (0.566, 0.023-1.109) and larger WC (1.595, 0.059-3.132).

Conclusion: Clustering of ≥4 risk behaviors was observed in T1D. Clustering of ≥2 risk behaviors was adversely associated with several cardio-metabolic risk factors. Our observations support a beneficial role of adopting an overall healthy lifestyle including prudent diet, physical activity, sufficient sleep, moderate alcohol intake, and avoidance of smoking.

Supported by: AF; NNF; SAGF; FRF, HUHRH, WESF, LHS, PSS

Disclosure: **A.J. Ahola:** Grants; The study was supported.

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Occupational and domestic physical activity and the risk of diabetes in adults: results from a long-term follow-up cohort

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Background and aims: Leisure-time physical activity (PA) has been associated with decreased incidence of diabetes. Few studies exist evaluating the influence of occupational and domestic PA on the risk of diabetes with a long-term follow-up. We aim to examine the association between occupational and domestic PA and the incidence of diabetes in a long-term prospective cohort of Chinese adults.

Materials and methods: A total of 10343 adults (age ≥ 18 year) who were prospectively followed up in China Health and Nutrition Survey from 1997 to 2015 were included in our analysis. Occupational and domestical PA were collected with detailed seven-day data and were converted into metabolic equivalents values. Diabetes cases were identified by self-reported doctor/health professional diagnosis of diabetes, and/or fasting blood glucose ≥7.0 mmol/L, and/or glycosylated hemoglobin (HbA1c) ≥ 40 mmol/mol (6.5%). Cox proportional hazard models were used to calculate hazard ratios (HR) and 95% confidence intervals (CI).

Results: With the adjustments for age, sex, body mass index, blood pressure, socio-economics and lifestyle factors (Table 1), the highest quartiles of occupational PA were significantly associated with lower risk of diabetes (HR 0.765 [95% CI, 0.596-0.982]) compared with lowest quartiles. Age-specific differences were observed that the association was stronger among the older (age> 42years) (HR 0.611 [95% CI, 0.445-0.838]) than the younger (age≤42 year) (HR 0.973 [95% CI, 0.634-1.493]), but no gender-specific differences were observed. The association between domestic PA and the risk of diabetes was insignificant (P >0.05).

Conclusion: Higher amounts of Occupational PA were associated with decreased risk of diabetes in Chinese population, especially among middle-aged and older adults. Domestic PA were shown no association with the incidence of diabetes.

Table 1. Hazard ratio (95% CI) of incidence of diabetes according to the quartiles of occupational and domestic physical activity

	Hazard ratio (95% CI)			
	Model 1 [a]	Model 2 [b]	Model 3 [c]	Model 4 [d]
Occupation PA				
Q1(lowest)	Reference	Reference	Reference	Reference
Q2	1.024 (0.817, 1.284)	1.000 (0.796, 1.256)	1.014 (0.807, 1.274)	1.014 (0.806, 1.274)
Q3	0.865 (0.691, 1.083)	0.877 (0.697, 1.103)	0.909 (0.723, 1.144)	0.957 (0.761, 1.204)
Q4(highest)	0.657 (0.581, 0.834)	0.684 (0.533, 0.877)	0.699 (0.544, 0. 899)	0.765 (0.596, 0.982)
Domestic PA				
Q1(lowest)	Reference	Reference	Reference	Reference
Q2	0.967 (0.761, 1.230)	0.931 (0.732, 1.185)	0.939 (0.737, 1.195)	0.968 (0.760, 1.232)
Q3	0.974 (0.753, 1.260)	0.935 (0.723, 1.210)	0.907 (0.700, 1.175)	0.901 (0.695, 1.170)
Q4(highest)	1.001 (0.767, 1.308)	0.945 (0.726, 1.240)	0.929 (0.711, 1.214)	0.887 (0.678, 1.160)

Note: Abbreviations: SD, standard deviation; HR, hazard risk; CI, confidence interval; PA, physical activity.

[a] Model 1 adjusted for age, sex.

[b] Model 2 adjusted for Model 1, marriage status (married or not), educational attainment levels (low, medium, high), household income per capita levels (low, medium, high), and urbanization index.

[c] Model 3 adjusted for Model 2, smoking status (ever/current or never smoker), alcohol consumption (yes or not), total energy intake, systolic blood pressure (SBP), diastolic blood pressure (DBP).

[d] Model 4 adjusted for Model 3 and body mass index (BMI).

Supported by: National Natural Science Foundation of China; China Health and Nutrition Survey

Disclosure: **L. He:** None.

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Association between social jetlag and change in glycaemic control in people with type 2 diabetes. The Hoorn diabetes care system cohort

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Background and aims: Social jetlag has recently gained interest as a novel risk factor in type 2 diabetes. It is characterized by a discordance between the social and biological rhythm and has been associated with the metabolic syndrome, in the general population, and with glycemic control in a few small cross-sectional studies in people with type 2 diabetes. This study aimed to assess the prospective association between social jetlag and changes in glycemic control in a large cohort of people with type 2 diabetes.

Materials and methods: The study sample consisted of 936 people with type 2 diabetes. Social jetlag was derived from sleep diaries and was defined as the difference in midpoint of sleep between week and weekend days. HbA1c (mmol/l) was assessed at baseline and after one-year follow-up. Social jetlag was categorized as ≤1 hour, between 1 and 2 hours, and ≥2 hours of social jetlag. The association between categories of social jetlag and HbA1c was analyzed using linear regression analysis adjusted for baseline HbA1c, sex, age, diabetes duration, neuropathy, eGFR, medication use, depression score and work, and educational status. Effect modification by age and work status was assessed, and alcohol consumption, physical activity, and smoking were tested as mediating factors.

Results: In the fully adjusted model, the high social jetlag group had an increase in HbA1c levels of 0.88 mmol/mol (-0.74;2.49), compared to the low social jetlag group. However, this association is small and not statistically significant. Additionally, no effect modification and no mediating effects were observed.

Conclusion: We did not observe a statistically significant association between social jetlag and change in HbA1c in this study. We will continue with analyses with two-year follow-up data and with more elaborate measures of metabolic control, including changes in medication use.

Supported by: Dutch Diabetes Foundation

Disclosure: **E.J. Bouman:** None.

SO 04 Prediction models and precision medicine

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Phenotypic and genetic determinants of glycaemic deterioration among Asian Indian type 2 diabetes population

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Background and aims: Type 2 diabetes (T2D) is a complex heterogeneous disease condition with high variability in diabetes progression. The Asian Indian phenotype is characterized by an earlier age of onset with lower adiposity than the Western European diabetes phenotype. No studies to date have investigated how the Asian Indian diabetes phenotype differs in rates of glycaemic deterioration. We aimed to identify phenotypic and genetic markers associated with glycaemic deterioration rate (coefficient of failure) among Asian Indians.

Materials and methods: We used electronic health record (EHR) data of 10,339 individuals with T2D treated at a chain of diabetes centers across India, following standard treatment protocols and linked through the same EHR system. To derive the annual glycaemic deterioration rate a linear mixed model was constructed with HbA1c (%) reading from diabetes diagnosis to insulin initiation or end of follow-up. The HbA1c readings were adjusted for change in Body Mass Index (BMI) and oral hypoglycaemic agent (OHA) use at the time of each HbA1c measurement. For detecting phenotypic associations, we used a simple linear regression model with glycaemic deterioration rate and phenotypes recorded at the time of diabetes diagnosis. To identify genetic markers associated with glycaemic deterioration rate we conducted a genome-wide association study (GWAS) with minor allele frequency (MAF) <0.05.

Results: The mean annual glycaemic deterioration rate among Asian Indians was 0.098% (95% CI 0.096-0.099) HbA1c per year. Phenotypic factors independently associated with higher glycemic deterioration were (n=9713) younger age of diabetes diagnosis, lower HDL-c, elevated BMI, dyslipidemia, and higher HbA1c at diabetes diagnosis. Lower beta-cell function (log HOMA B) and higher insulin resistance (log HOMA IR) were also significantly associated with higher glycemic deterioration rate in the age and sex adjusted model (n=3390). In a GWAS (n=1010) we identified two SNPs located at *UBE2E2* (*rs41400550* [β=-0.02 per allele, $p=3.93*10^{-8}$] and *rs80144523* [β=-0.02 per allele, $p=4.22*10^{-8}$]*)* that were associated with glycemic deterioration rate among Asian Indians.

Conclusion: The mean annual glycaemic deterioration rates among Asian Indians were comparable with their White counterparts. This is the first analysis reporting phenotypic and genetic determinants of the

rate of glycaemic deterioration in the Asian Indians T2D population; similar phenotypic parameters were identified and previously reported for the White diabetes populations. We have shown for the first time that beta-cell function and insulin resistance at the time of diabetes diagnosis were associated with glycaemic deterioration rate. Further analysis is required to validate the association of the genetic markers with the rate of glycaemic deterioration.

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Disclosure: **A. Thakarakkattil Narayanan Nair:** None.

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Recursive partitioning analysis of the glomerular filtration rate optima: the 10-year-health examinees study

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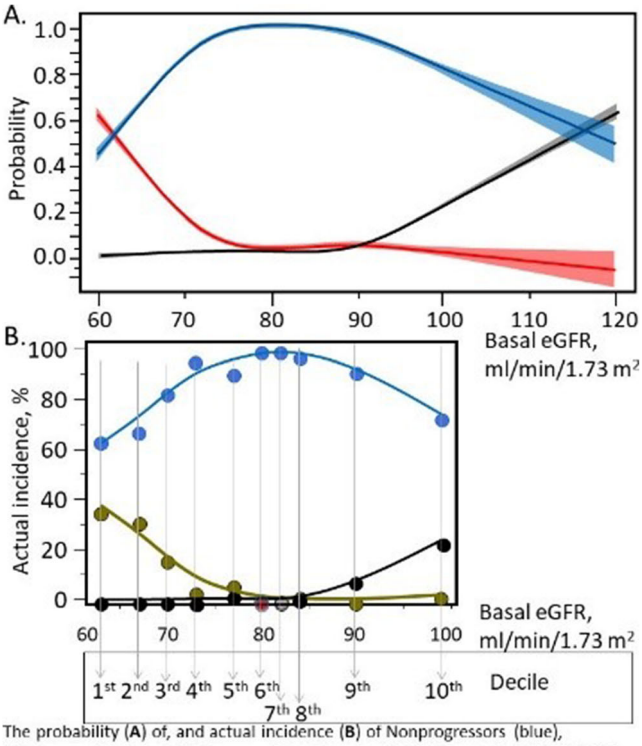
Background and aims: Background and aims: Attenuated glomerular filtration rate (GFR) is a risk for chronic kidney disease (CKD). In addition, excessively elevated GFR, i.e., glomerular hyperfiltration (GHF), has been recognized as a predictor of CKD. Then, there should be the optimal range of GFR for maintenance of normal renal function, which was searched for in this study.

Materials and methods: This is a retrospective, 10-year-longitudinal analysis of 1,563 health examinees without CKD and GHF at baseline, and received a yearly examination for consecutive 10 years from July 2005 to May 2015: males 67%, the median age 50 years, BMI 23.0 kg/m^2, FPG 5.3 mmol/l, HbA1c 5.4%, estimated GFR (eGFR) 78.4 ml/min/1.73 m^2 and known patients with diabetes 6%. eGFR was calculated using the formula developed for Japanese subjects, CKD defined as eGFR<60 ml/min/1.73 m^2 or dipstick proteinuria and GHF defined as age- and sex-specific eGFR≥95% of the Japanese general population. The participants fulfilled the CKD definition consecutively at the year 9 and 10 of the 10-year-follow-up were classified as $CKD_{progressors}$ (n=167), those who exhibited GHF more than twice during the 10-year-observation classified as $GHF_{progressors}$ (n=52), and the rest classified as Nonprogressors (n=1,344). Recursive partitioning analysis with 66% learning and 33% validation set employing eGFR, fasting plasma glucose (FPG), age, systolic BP and insulin sensitivity as explanatory variables targeting the best cutoff for the three renal endpoints was carried out.

Results: The basal eGFR contributed 100% to the outcome and the contribution from the other variables including FPG was literally zero. As to the eGFR, 69.25 ml/min/1.73 m^2 and 94.35 ml/min/1.73 m^2 were the cutoffs maximizing differentiation of the participants' renal outcome with the validation AUC (95%CI) for the receiver operating characteristic curve for $CKD_{Progressors}$ 0.788 (0.723-0.841), Nonprogressors 0.737 (0.676-0.790) and $GHF_{Progressors}$ 0.820 (0.716-0.892), respectively. The probability, obtained by the partitional model in the learning set and the actual incidence of the outcome agreed well (Figure) and application of the cutoff to the validation set yielded 73% sensitivity for 10-year-maintenance of normal kidney function.

Conclusion: In apparently healthy Japanese adults, GFR optima was "between 69.25 and 94.35 ml/min/1.73 m^2" which predicted 10-year-maintenance of normal kidney function with 73% sensitivity and 74% accuracy. Plasma glucose contributed little if any to the long-term renal outcome in this population.



The probability (**A**) of, and actual incidence (**B**) of Nonprogressors (blue), $CKD_{Progressors}$ (red) and $GHF_{Progressors}$ (black), respectively. The probability (95%CI) obtained by the partitioning analysis using the learning set was plotted as a function of the $eGFR_{basal}$ (A) and actual incidence as a function of decile of $eGFR_{basal}$ in the validation set (B). The dots correspond to the mean incidence and the arrows correspond to the mean $eGFR_{basal}$ of each decile.

Disclosure: **Y. Nakasone:** None.

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A serum resistin and multi-cytokine inflammatory pathway is associated with and helps predict all-cause mortality in type 2 diabetes: a step toward precision medicine

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Background and aims: Type 2 Diabetes shows increased mortality, especially of cardiovascular origin in which atherosclerotic plaque instability plays a major role. Discovering novel biomarkers will play the function to identify high-risk patients to expose to more aggressive managements and/or specific treatments. We recently described a serum resistin and multi-cytokine inflammatory pathway (REMAP) (including resistin, IL-1β, IL-6, IL-8 and TNF-α) which is correlated to cardiovascular markers and disease. We here investigated whether REMAP is associated with and improves the prediction of all-cause mortality in Type 2 Diabetes and whether atherosclerotic plaque instability plays a role in this association.

Materials and methods: A serum REMAP score was created (by summing up log-transformed and standardized values of all five inflammatory molecules) and analyzed in three cohorts comprising a total of 1,528 patients with diabetes (409 incident deaths) and in 59 patients who underwent carotid endoarterectomy (CEA; 24 incident deaths). Plaques were classified as unstable/stable according to the American Heart Association. Two well-established prediction models of mortality in diabetes (ENFORCE and RECODe) were used. Discrimination was evaluated by *c* statistic and relative Integrated Discrimination Improvement (rIDI) and reclassification by category-free Net Reclassification Improvement (cNRI).

Results: Serum REMAP was associated to all-cause mortality in each cohort (all P<0.001) and in all 1,528 individuals (fully-adjusted hazard ratio for 1 SD increase [HR]; 95% CI: 1.34; 1.22-1.47 for 1 SD increase). When the pooled sample was stratified according to study-specific tertiles of REMAP a clear trend of mortality incidence rate was observed from 2.1% (96 events/505; 4522.9 person/year - py) to 2.5% (107 events/503; 4220.8 py) and 5.3% (206 events/520; 3851.1 py) in tertile 1, 2 and 3 (P for trend<0.001). In CEA patients, REMAP was associated with all-cause mortality (HR=1.64; 95% CI: 1.01-2.65) and plaque instability (fully-adjusted OR, 95% CI for 1 SD: 3.04, 1.02-9.01). Only a trivial 7% reduction of the association between REMAP and mortality was observed when plaque instability was considered in the model. The REMAP score ameliorated *c* statistic and %rIDI of both ENFORCE and RECODe (P<0.05). Also risk classification was ameliorated in both models, with a clinically meaningful proportion of nonevents being correctly reclassified (P<0.001).

Conclusion: In Type 2 Diabetes, REMAP is independently associated with total death and improves established all-cause mortality prediction models. Our finding makes possible envision a precision medicine approach in which REMAP is used to identify high-risk individuals to target with more aggressive and timely managements and possibly who may be more responsive to treatments with neutralizing monoclonal antibodies against IL-6 and IL-1β which have been recently proved to reduce cardiovascular burden and total mortality.

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Disclosure: **M. Scarale:** Grants; Fondazione Umberto Veronesi.

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Estimation and validation of machine-learning-based retinal image analysis for detection of the risk of undiagnosed diabetes

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Background and aims: Undiagnosed diabetes is a global health issue. Previous studies have shown that about 24.1% to 75.1% of all diabetes cases are estimated to be undiagnosed across various countries. As the situation worsens, and the undiagnosed diabetes cases would lead to more diabetic complications and induce huge healthcare costs to society. It would be highly advantageous if a simple but accurate test for estimating the risk of diabetes is available.

Materials and methods: A preliminary analysis of 1264 subjects was used for training the classification model. They included 661 type 2 diabetes patients obtained from Hong Kong outpatient clinic and a Traditional Chinese Medicine Hospital, and 603 non-diabetes controls were obtained from a lifestyle study and a local community social program. A set of 542 subjects with 284 diabetes and 258 controls were used as testing data. All subjects had their retinal images taken by a non-mydriatic fundus camera. A fully automatic retinal image analysis for diabetes risk (ARIA-DM) was developed to estimate retinal microvascular characteristics and incorporate a machine-learning technique to derive an overall estimation of diabetes risk. In addition to the testing data for diabetes and controls, we have another prediabetes testing data of 101 subjects with fasting glucose of 126 mg/dL (7.0 mmol/L) or higher but a confirmation HbA1c value of 5.7% to 6.4%.

Results: The training analysis using 1264 subjects for model development has very high accuracy. The 10-fold cross-validation using support vector machine (SVM) approach has a sensitivity of almost 100% and specificity of 99.7%. The separate testing data analysis with 542 subjects also achieved very high accuracy with mean diabetes probabilities of 2.7% (95% CI of 2.0%, 3.4%) in the control group and 98.0% (95% CI of 97.5%, 98.5%) in the diabetes group. For the prediabetes testing data, the mean diabetes probability was 10.4% (95% CI of 8.2%, 12.6%). All 101 prediabetes testing subjects have probabilities less than the cutoff value of 50% for diabetes classification. More results on significant retinal characteristics and further external validation using UK testing data will be discussed in the presentation.

Conclusion: Method to estimate the risk of diabetes based on retinal images using ARIA-DM has been developed and validated using both Asian and Caucasian data. ARIA-DM is a fast, convenient and non-invasive alternative for the community pre-screening program for undiagnosed diabetes.

Disclosure: **R.L. Thomas:** None.

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A clinical-genetic score for predicting type 2 diabetes remission after bariatric surgery: the OBEGEN project

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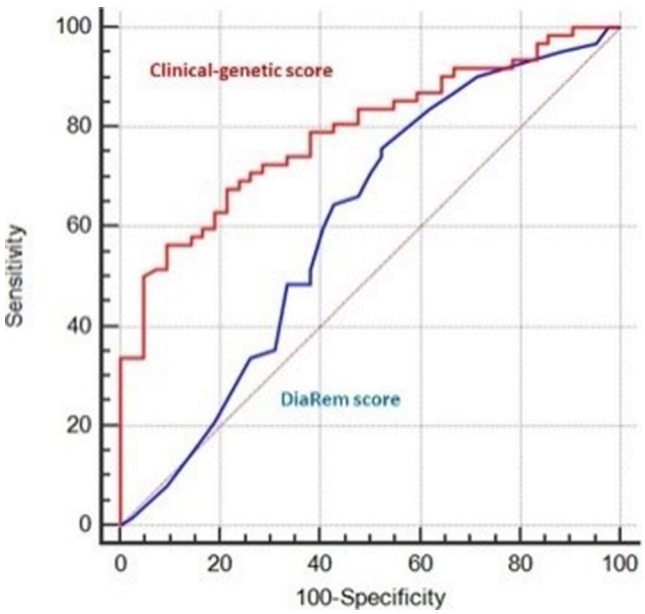
Background and aims: The remission rate of type 2 diabetes (T2D) after bariatric surgery (BS) is around 60-70% after 1 year of follow-up. Our purpose was to assess the role of genetic susceptibility as a potential tool to predict satisfactory response to BS in terms of diabetes remission. Therefore, we propose to develop a genetic scoring system for predicting T2D remission following BS, and to compare our results with the current clinical based prediction score (DiaRem).

Materials and methods: A multicenter, retrospective, and observational study including 161 patients who underwent bariatric surgery (Y-de-Roux gastric bypass or sleeve gastrectomy). DNA was extracted from saliva samples, and 59 SNPs from 39 genes were examined. Receiver Operating Characteristic (ROC) curve analysis were used to calculate sensitivity and specificity. Diabetes remission was evaluated at weight loss nadir and defined according to American Diabetes Association criteria.

Results: T2D remission was observed in 107 patients (66.4%). A good predictive model for diabetes remission [area under ROC of 0.784 (95% CI 0.696-0.856), p<0,0001; sensitivity 70.0% and specificity 75.5%], was

obtained by combining clinical variables (type of surgery, baseline BMI, excess of body weight, dose of insulin) and five SNPs located. This predictive model showed a significant higher area under ROC than the clinical score (p=0.0283) and the genetic score (p=0.0270) alone. An internal validation of the clinical-genetic algorithm was performed using the Bootstrap method: AUC 0.784 (95% CI 0,687-0.856). The AUC to predict diabetes remission of the clinical-genetic algorithm was significantly higher than that obtained with the DiaRem score (0.784 vs. 0.609, p=0.0133).

Conclusion: The OBEGEN project shows how the union of genetic biomarkers plus clinical variables identify patients with obesity who will be "good" or "bad" responders to BS regarding diabetes remission. Our results underline the potential key role of genetic testing in precision medicine to personalize the therapeutic approach to severe obesity and to assure better outcomes of obesity comorbidities after BS.



Clinical Trial Registration Number: NCT02405949

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Disclosure: **A. Lecube:** Grants; Instituto de Salud Carlos III (PI PI18/00964), Fondos FEDER "Una manera de hacer Europa"). CIBERdem is an initiative of the ISCIII.

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Targetome redirection by adenosine-to-inosine editing of miRNAs could unravel the complexity of diabetic complications

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Background and aims: MicroRNAs (miRNAs) are known to play a role in the development of insulin resistance type 2 diabetes (T2D). However, their role in the development and progression of the complications associated with the pathogenesis of T2D remains unexplored, particularly the contribution of miRNA editing, specifically the conversion of adenosine to Inosine (A-I), which is catalyzed by adenosine deaminase acting on RNA enzymes (ADARs). The aim of this study was to study the role miRNAs and their editing in relation to diabetic complications.

Materials and methods: We first identified the differential miRNA expression in patients with T2D (n=23), patients with T2D with complications (n= 93), (nephropathy, neuropathy, liver fibrosis, and lung fibrosis), and healthy controls (n=31). Small RNA-seq data was used to perform pathway enrichments, test for associations with bioinformatically predicted target genes, and to investigate A-to-I miRNA modifications of the identified miRNAs. To compare the gene targets of the wild type and edited versions of top miRNA candidates, binding site prediction of the gene 3'-UTRs was performed by multiple prediction tools. qRT-PCR was used to validate miRNAs and ADARs expression in plasma and tissues of T2D patients, and immunohistochemistry was performed to visualize the expression of of ADAR in the target tissues.

Results: Of 54 miRNAs significantly deregulated between T2D and those with complications, 8 miRNA (miR-200c-3p, miR-378c, miR-122-5p, let-7c-3p, miR-181d, miR-100-5p, miR-3615, miR-885-5p and, miR-9-5p) had a distinct expression signature between the different complications subgroups. targeting equally more than 42 biological pathways, such as cell cycle regulation, TGFß and AGE-RAGE signalling. Such pathways have previously been identified to play an important role in the development and progression of T2D complications. However, the lack of any new pathways, regardless of the type of complication and/or target tissue, could be the result of A-to- I editing. It was subsequently shown that the expression of ADAR was significantly increased in the complication specific tissues. Furthermore, bioinformatic analysis showed that A-to-I editing was at higher frequency for miRNAs identified in the T2D patients and indicated a significantly shift in the targetome. For instance, miR-122-5p which was identified as miRNA specific for liver fibrosis targets 226 genes involved mainly in MAPK, calcium, and Wnt signalings (FDR ≤0.05); however, A-to-I editing (21.66%) redirects the targeting of this miRNA towards 94 target genes which are enriched in the MECP2, post-protein modifications, and p53 signaling pathways (FDR ≤0.05).

Conclusion: Circulating miRNA have the potential to identify and discriminate T2D patients with distinct complications. However, their role in the development of such complications remains unclear, particularly giving the extent to which A-to-I editing can occur. Such editing is predicted to change the targeting of miRNAs and ultimately their biological effect. Editing effects therefore need to be considered when developing miRNA based therapeutic approaches for diabetic complications.

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Disclosure: **A. Abukiwan:** Grants; BMBF and SFB1118-A04.

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Predicting the onset of APECED diabetes

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Background and aims: Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is a rare monogenic autosomal recessive disorder caused by mutations in the autoimmune regulator gene *AIRE*. The translated Aire protein is involved in the regulation of organ-specific antigen expression and negative selection of autoreactive T-cells. APECED patients present with multiple endocrinopathies while diabetes is one of its more rare manifestations. Its prevalence greatly varies between different patient cohorts, and the determinants of time to onset and symptoms of clinical progression to diabetes seem currently unpredictable and abrupt. We aimed to explore clinically detectable glycaemic predictors of diabetes onset in our well-characterised Finnish APECED cohort.

Materials and methods: We reviewed medical records for those with a clinical diabetes diagnosis and investigated change in pre-diagnostic glycaemic parameters (glucose, insulin, C-peptide and glucagon) retrieved from bi-annually conducted 60-minute (') intravenous glucose tolerance tests (ivGTT) and HbA1c measurements over 20 years of follow-up. The homeostasis indices for β-cell function and insulin resistance (HOMA-β, -IR) were calculated based on fasting values during ivGTTs, and fractional glucose turnover rate (K_G) as $0.693/T_{1/2Gluc}$*100; with Pearson correlation coefficient (r) and 2-sided p-value.

Results: Between 1972-1996, a total of 68 ivGTTs were conducted prior to diabetes diagnosis in 13 out of 22 paediatric and adult patients with diabetes in the Finnish APECED cohort of 97 patients. These included six ivGTTs in one asymptomatic, normoglycaemic man followed for up to 20 years with a cursory childhood diagnosis of latent diabetes. The mean (SD) age at diabetes onset was 27.78 (11.73) years; 29.6 (12.9) for women and 26.6 (11.7) for men. Pre-diabetes and diagnostic HbA1c values were rarely measured; the mean HbA1c at the time of diabetes diagnosis (n=5) was 9.8 (2.3) %. Fasting plasma glucose (FPG) values (mmol/l) were mostly normoglycemic prior and up to diabetes diagnosis: 4.4 (0.6) (-20 years to -6 months) and 5.7 (0.9) (-6 months to diagnosis). Mean 60' glucose values were elevated only for those with <6 months to diagnosis: 12.1 (0.7) mmol/l. Post-dose hyperinsulinaemia was present in single ivGTTs up to 20 years prior to diagnosis, while rapid 1st phase insulin secretion (1'+ 3' value; normal >46 mIU/L) was lost or increasingly delayed -5 years prior to diagnosis in some, but not all. However, up to and at diagnosis, adequate fasting insulin (>2 mIU/L) and C-peptide (>0.5 ng/ml) production was still observed in most (85%) patients. IvGTTs included randomly glucagon (18 tests in 6 individuals): a rapid 20-30% drop was observed 1' to 3' post-dosing, with a return of values to fasting level at 5' and with no obvious pattern of change over time. Nevertheless, despite intra- and inter-individual variability, K_G predicted diabetes onset over time (-20 years to diagnosis) among those with confirmed diabetes: r= -0.568 (p< 0.001). HOMA indices were not sensitive enough to detect any change over time in our cohort with most FPG values indicative of normoglycaemia.

Conclusion: APECED, a monogenic disorder, is a model disease for studies in endocrine autoimmunity, including diabetes. Identification of reduced pre-diagnostic glucose removal rate over time, indicative of increased peripheral insulin resistance in the presence of normoglycaemia and normal insulin response, provides first insights into progression of risk of atypical diabetes in patients with APECED.

Disclosure: **P.M. Paldánius:** Honorarium; UPM Biomedicals. Lecture/other fees; UPM Biomedicals, Novartis Pharma.

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Phenotypic and genetic heterogeneity in a Thai glucokinase MODY family reveals the complexity of young-onset diabetes

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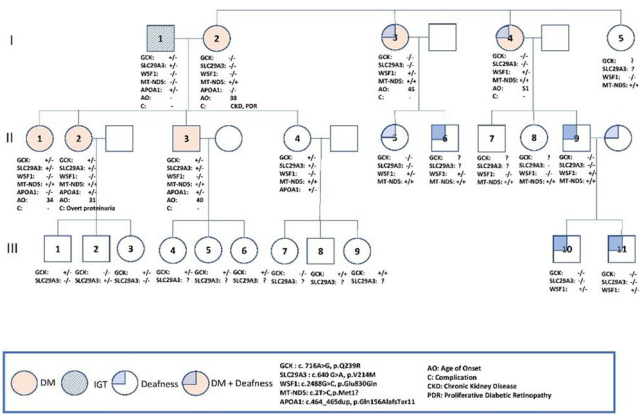
Background and aims: Glucokinase-Maturity-Onset Diabetes of the Young (GCK-MODY) is characterized by asymptomatic, non-progressive and fasting hyperglycemia. However, there are sporadic reports on severe forms of GCK-MODY due to co-existence of autoimmune diabetes or obesity-driven insulin resistance. In this study, we aimed to characterize genetic heterogeneity of a large Thai family with GCK-MODY.

Materials and methods: A total of 25 family members within three generations of young-onset diabetes with sensorineural hearing loss was studied. We conducted a research study to investigate possible genetic causes of diabetes in this family. We used next generation sequencing (NGS) to screen for all coding regions including intron-exon junctions of 35 MODY genes. Whole exome sequencing was also used to detect mutations associated with diabetes and deafness in other family members.

Results: A proband with non-obese diabetes was found to harbor heterozygous missense variant (c.716 A>G) of GCK in exon 7 with amino acid change (Q239R). This same mutation exhibited marked phenotypic heterogeneity ranging from normal glucose tolerance to diabetes with complications in other family members. Moreover, atypical features including deafness and isolated low HDL-C in the family pedigree led to discovery of additional mutations/variants. These included the SLC29A3-1 (equilibrative nucleoside transporter 3), WSF1 (Wolfram syndrome type 1), mitochondrial DNA-encoded NADH-dehydrogenase (MT-ND5) and Apo-A1 mutations.

Conclusion: Our findings highlight the complex causes of familial young-onset diabetes calling for a paradigm shift regarding the mild nature of GCK-MODY.



Supported by: Grant for promoting intramural research in Theptarin Hospital (Grant No.1/2563).

Disclosure: **Y. Thewjitcharon:** None.

SO 05 Therapeutic advances

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Cholecalciferol therapy effect on glucose metabolism in patients with prediabetes

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Background and aims: There have been an increasing number of investigations on the role of vitamin D in glucose metabolism and the possibility of type 2 diabetes prevention with cholecalciferol but the optimal doses have not been determined yet. Aim. To assess the effect of cholecalciferol therapy at various doses on glucose metabolism in patients with prediabetes.

Materials and methods: Sixty-five patients with prediabetes (females, age 53.5±6.4 years) not taking vitamin D and without diseases affecting its metabolism were randomized into two treatment groups: cholecalciferol 500 IU/day (Group 1, n=32) and 4,000 IU/day (Group 2, n=33) for three months. Anthropometric data, comorbidities, and concomitant medications were assessed. Before and after 3-month therapy all patients underwent a standard oral glucose tolerance test with venous blood sampling at points 0', 60', and 120'. Plasma glucose was evaluated by the glucose oxidase method, insulin and glucagon-like peptide 1 (GLP-1) were assessed by the enzyme-linked immunosorbent assay. The indices of insulin resistance (HOMA-IR), insulin sensitivity (ISI-0,120) and functional activity of b-cells (HOMA-B) were calculated. Glycated haemoglobin (HbA1c) was determined by ion exchange chromatography. Serum 25-hydroxycalciferol [25(OH)D] and parathyroid hormone (PTH) were evaluated by the chemiluminescent immunoassay.

Results: Fifty-eight patients completed the study. Most patients (89.2%) had initial vitamin D deficiency/insufficiency. After three months of cholecalciferol therapy, an increase in 25(OH)D and decrease in PTH concentration was found in both groups ($p \leq 0.01$). Normal values of serum 25(OH)D were reached by 3 patients (10.7%) from Group 1 (500 IU/day) and by 22 patients (73.3%) from Group 2 (4,000 IU/day). Reduction in HbA1c (p=0.001) and plasma glucose at points 60' (p=0.04) and 120' (p=0.04), increase in insulin level at point 120' (p=0.03) and gain in HOMA-B index (25.3%) at the end of the study was observed only in patients taking 4,000 IU of cholecalciferol daily. After three-month therapy normal glucose and HbA1c levels had 19 patients (63.3%) from Group 2 and only 2 patients (7.1%) from Group 1 (p=0.001).

Conclusion: Treatment with 4,000 IU of cholecalciferol per day for three months is associated with increase in vitamin D level and glucose metabolism improvement in women with prediabetes.

Disclosure: **A.T. Andreeva:** None.

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Five years of treatment with liraglutide improves glucose tolerance in women with prior gestational diabetes, but effects are lost after wash-out

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Background and aims: Women with prior gestational diabetes mellitus (pGDM) are at high risk of developing type 2 diabetes (T2D). We evaluated whether treatment with the glucagon-like peptide 1 receptor agonist liraglutide for five years reduces the risk of T2D in these women.

Materials and methods: Overweight women with pGDM were randomised to a placebo-controlled, one-year double-blinded trial with a four-year open-label extension period to receive treatment with liraglutide 1.8 mg s.c. once-daily for five years or placebo for one year followed by no treatment (Table). At baseline, one year and five years, a 4-hour 75 g OGTT and an i.v. isoglycaemic glucose infusion (IIGI) were conducted. Liraglutide-treated women completed both an OGTT on drug and after one week of wash-out.

Results: Compared to placebo-treated women, liraglutide-treated women had a better glucose tolerance after five years of treatment (Table). However, this effect was not sustained after one-week wash-out of liraglutide. No significant difference was observed in fasting plasma glucose (FPG), HbA_{1c} or body weight between groups after five years of treatment.

Conclusion: After five years of treatment, liraglutide showed no significant effect on FPG, HbA_{1c} or body weight, but continued to exert a beneficial effect on glucose tolerance in women with pGDM when on drug; but after wash-out, this effect was lost.

	Baseline		1-year follow-up			5-years follow-up		
	Placebo	Liraglutide**	ΔPlacebo	ΔLiraglutide**	ETD	ΔPlacebo	ΔLiraglutide**	ETD
N	65	65	45	42		40	29	
Glucose tolerance (AUC) on drug (mmol/l × min)	1654 (1645;1724)		-38 (-91;14)	-212 (-269;-155)*	-173 (-250;-97, p<0.001)*	146 (51;240)*	-63 (67;200)	-223 (-339;-106, p<0.001)*
Glucose tolerance (AUC) after wash-out of drug (mmol/l × min)	1654 (1645;1724)		-38 (98;23)	20 (-44;85)	58 (-29;146, p=0.192)	146 (51;240)*	165 (56;273)*	19 (-126;163, p=0.794)
FPG (mmol/l)	5.6 (5.5;5.6)		0.0 (-0.1;0.1)	-0.3 (-0.4;-0.1)*	-0.2 (-0.4;-0.0, p=0.006)*	0.2 (0.0;0.4)*	0.0 (-0.3;0.2)	-0.2 (-0.5;0.1, p=0.206)
HbA1c (%)	5.1 (5.1;5.2)		0.0 (-0.1;0.1)	-0.2 (-0.3;-0.1)*	-0.2 (-0.3;-0.02, p=0.019)*	0.2 (-0.0;0.4)*	0.2 (-0.0;0.3)	0.0 (-0.3;0.2, p=0.781)
Body weight (kg)	88.2 (86.3;91.2)		-1.5 (-3.1;0.54)	-4.6 (-6.8;-3.1)*	-3.2 (-5.6;-0.9, p=0.007)*	4.3 (1.6;7.2)*	0.6 (-2.7;4.6)	-3.5 (-7.8;0.9, p=0.114)

*Data at baseline is mean (95% confidence interval (CI)). Data at 1-year follow-up and 5-year follow-up are baseline adjusted mean change from baseline (a with 95% CI) and estimated treatment difference (ETD) for liraglutide (1.8 mg once-daily) vs. placebo; fasting plasma glucose (FPG) and area under the curve (AUC) (for plasma glucose during oral glucose tolerance test; *p-values <0.05 were considered significant; **Liraglutide 1.8 mg once-daily.*

Clinical Trial Registration Number: 2012-001371-37-DK

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Disclosure: **E.S. Andersen:** None.

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Treatment patterns for first- and second-line management of type 2 diabetes in the Middle East and North Africa region: insights from DISCOVER

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Background and aims: To gain insights into the region-specific treatment practices and associated clinical outcomes in patients with type 2 diabetes (T2D), we evaluated real-world data from the Middle East and North Africa (MENA) region cohort (Algeria, Bahrain, Egypt, Jordan, Kingdom of Saudi Arabia, Kuwait, Lebanon, Oman, South Africa, Tunisia, Turkey, United Arab Emirates) of the Global DISCOVER study.

Materials and methods: DISCOVER is a 38-country, prospective, observational study of patients with T2D enrolled at initiation of 2nd-line

therapy and followed for 3 years. In this analysis, we present descriptive data from 12 countries of the MENA region; no statistical testing has been performed.

Results: A total of 3525 patients at baseline and 3333 at 3-year follow-up were evaluated. At baseline, mean±SD cohort age was 54.3±10.8 years, with 52.5% of males and mean T2D duration of 74.6±64.4 months. Mean glycated haemoglobin (HbA1c) at baseline and 3-year follow-up were 8.7 ±1.7% and 7.5±1.3%, respectively. Changes in other metabolic parameters over the 3-year follow-up were (mean ±SD, baseline vs. 3 years): fasting glucose (mg/dL) 182.7±60.3 vs. 137.4±46.7; total cholesterol (mg/dL) 191.3±47.1 vs. 181.4±41.5; low-density lipoproteins (mg/dL) 116.7±40.8 vs. 107.6±34.7; high-density lipoprotein (mg/dL) 43.5±12.5 vs. 44.8±11.5). The most common second-line therapies were metformin in combination with either a sulphonylurea (SU; 844, 24.0%) or a dipeptidyl peptidase-4 inhibitor (DPP-4i; 774, 22.0%), or triple therapy with metformin, an SU and a DPP-4i (506, 14.4%). Drug classes prescribed at first- and second-line are shown in the Table. Major hypoglycaemic events over the prior year were reported in 61 (1.9%) patients at baseline and 15 (0.5%) patients at 3 years.

Conclusion: Similar to the global DISCOVER study programme, most patients in the MENA region cohort were on metformin as first-line glucose-lowering therapy. SUs and DPP-4is were commonly used at first- and second-line, while newer agents with cardiovascular risk reduction were prescribed to only a minority of patients. Despite drug availability challenges in some countries, data from the DISCOVER study in MENA region cohort showed changes in the metabolic control of patients with T2D after 3-years of follow-up.

Table: Antidiabetic medications in the DISCOVER-MENA region cohort, alone or in combination

n (%)	1st Line (prior to enrolment)	At enrolment
	N=3525	N=3525
MET[a]	2955 (83.8%)	2570 (72.9%)
SU	1101 (31.2%)	1550 (44.0%)
DPP-4i	160 (4.5%)	911 (25.8%)
TZD	75 (2.1%)	150 (4.3%)
Meglitinides	63 (1.8%)	126 (3.6%)
SGLT-2i	7 (0.2%)	114 (3.2%)
Alpha-glucosidase	24 (0.7%)	70 (2.0%)
GLP-1 RA	0 (0.0%)	94 (2.7%)
Insulin (basal)	NA	247 (7.0%)

Major therapies are presented. Percentages calculated for patients with data available; missing data are excluded

[a]Does not include metformin fixed-dose combination therapies

MET, metformin; DPP-4i, dipeptidyl peptidase-4 inhibitor; GLP-1 RA, glucagon-like peptide 1 receptor agonist; SGLT-2i, sodium–glucose co-transporter-2 inhibitor; SU, sulphonylurea; TZD, Thiazolidinedione

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Disclosure: **K. Al-Rubeaan:** None.

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The association between hormone therapy and sarcopenia in postmenopausal women: the Korea National Health and Nutrition Examination Survey, 2008-2011

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Background and aims: Menopausal transition contributes to sarcopenia, but the effects of hormone therapy (HT) on sarcopenia in postmenopausal women have not been determined. This study assessed the effect of HT on sarcopenia in postmenopausal women.

Materials and methods: The present study included 4,254 postmenopausal women who participated in the Korea National Health and Nutritional Examination Surveys in 2008-2011. Appendicular skeletal muscle mass divided by weight (ASM/Wt) and the prevalence of sarcopenia were analyzed in groups of women stratified by duration of HT use.

Results: ASM/Wt was higher and the prevalence of sarcopenia was lower in participants with a history of prolonged (≥13 months) HT use than in participants with a shorter duration of HT use or no HT use. After adjusting for multiple confounding factors, prolonged use of HT remained significantly associated with estimated mean ASM/Wt and the prevalence of sarcopenia (odds ratio: 0.60; 95% confidence interval: 0.41-0.88; P = 0.01). In addition, the prevalence of sarcopenia were linearly associated with history of hypertension, duration of hypertension, physical activity, and duration of HT use. Subgroup analysis showed that the association between duration of HT use and the prevalence of sarcopenia was maintained in younger (< 65 years old) and leaner (body mass index < 25 kg/m^2) postmenopausal women.

Conclusion: The present study showed that prolonged use of HT was associated with high muscle mass and a low prevalence of sarcopenia in postmenopausal women.

Table. Odds ratios for the prevalence of sarcopenia in postmenopausal women stratified by duration of hormone therapy.

	No HT use (n=3,656)	HT use for 1–12 months (n=302)	HT use for ≥ 13 months (n=275)	*P* for trend
Unadjusted	1 (ref)	0.93 (0.68 – 1.27)	0.67 (0.47 – 0.95) [b]	0.03
Model 1	1 (ref)	0.95 (0.69 – 1.31)	0.64 (0.45 – 0.92) [b]	0.03
Model 2	1 (ref)	0.93 (0.68 – 1.28)	0.60 (0.41 – 0.88) [b]	0.02
Model 3	1 (ref)	0.90 (0.66 – 1.24)	0.59 (0.40 – 0.87) [a]	0.01
Model 4	1 (ref)	0.89 (0.65 – 1.23)	0.60 (0.41 – 0.88) [b]	0.01

Data were analyzed using complex samples logistic regression and are expressed as odds ratio (95% confidence interval).

Abbreviations: HT, hormone therapy.

Model 1: adjusted for age, age at menarche, age at menopause, and number of pregnancies.

Model 2: adjusted for age, age at menarche, age at menopause, number of pregnancies, past history of OC use, past histories of DM and HTN, smoking history, and physical activity.

Model 3: adjusted for age, age at menarche, age at menopause, number of pregnancies, past history of OC use, past histories of DM and HTN, smoking history, physical activity, and energy intake (total, proteins, carbohydrates, and fats).

Model 4: adjusted for age, age at menarche, age at menopause, duration of menstruation and menopause, number of pregnancies, past history of OC, duration of OC use, past histories of DM and HTN, duration of HTN and DM, smoking history, physical activity, and energy intake (total, proteins, carbohydrates, and fats).

[a]$P < 0.01$ and [b]$P < 0.05$.

Disclosure: **S. Kim:** None.

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A multivalent vaccine does not accelerate the onset of diabetes in NOD mice

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Background and aims: The exact causes of Type 1 diabetes (T1D) remain elusive. Genetic elements play a role however cannot cause the disease alone. Various environmental factors have also been associated with T1D including the common Coxsackievirus B (CVB) family of enteroviruses. Despite evidence that exists documenting their association with the disease, whether CVB infections are causal remains inconclusive. Vaccination strategies with a CVB vaccine that target genetically at-risk populations provide a viable

option for clarifying the involvement of CVBs in T1D. We therefore created a multivalent vaccine containing the six CVB serotypes (CVB1-6), performed proof-of-concept studies and demonstrated that the vaccine is highly immunogenic in mouse models and non-human primates. Moreover, the vaccine protected against virus-induced diabetes in an experimental mouse model. These studies provided important preclinical evidence for the development and clinical use of a similar vaccine, which is currently in use in a recently initiated phase I clinical trial. To further evaluate the safety of this vaccine in a diabetes prone host, here we addressed whether the vaccine impacts immune cell infiltration in the pancreatic islets of Langerhans and/or diabetes onset in the commonly used NOD mouse model for T1D.

Materials and methods: CVB1-6 viruses were formalin inactivated and mixed to create the vaccine. Female NOD mice aged 4.5-7.5 weeks old were randomly divided into 3 groups and left untreated, were mock-vaccinated with vaccine buffer or vaccinated with CVB1-6 vaccine on two to three occasions with two to three-weeks intervals between vaccinations. Weights and blood glucose were monitored weekly. Neutralising antibody titres were measured by standard plaque reduction assay in serum samples collected prior to each vaccination and at the study end. Mice were either followed to 11-13.6 weeks of age when they were removed and pancreas was collected for histological analysis of islet immune cell infiltration (insulitis) or up to 30 weeks of age to monitor diabetes incidence.

Results: As previously seen, the CVB1-6 vaccine was highly immunogenic, inducing neutralising antibodies to all six CVB serotypes and had no adverse effects on weight. Pre-diabetic NOD mice that were buffer treated (n=13) had a comparable mean insulitis score (0.9 ± 0.5) to those that were CVB1-6 vaccinated (n=8; 1.2 ± 0.5) with no significant differences (NS; student's t-test) between the two groups. Furthermore, no significant differences were detected in either the average age at diabetes onset between the untreated (n=10; 22.1 ± 5.0 weeks), buffer-treated (n=15; 20 ± 5.6 weeks) and CVB1-6 vaccinated (n=14; 19.1 ± 5.5 weeks) groups (all comparisons NS; one-way ANOVA with Tukey's multiple comparison) or in the cumulative diabetes incidence curves (NS; Gehan-Breslow-Willcoxon test).

Conclusion: Vaccination did not alter the degree of insulitis in the islets of Langerhans in prediabetic animals, nor the onset of diabetes in the NOD murine model for T1D. A similar vaccine has been made for humans and is currently undergoing phase I clinical trial. The data generated in these preclinical studies further supports the use of such a vaccine to determine whether CVBs have a causative role in T1D and highlights the vaccine safety in the context of diabetes in a diabetes prone model.

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Risk factors for severe hypoglycaemia in adults with insulin-treated type 2 diabetes

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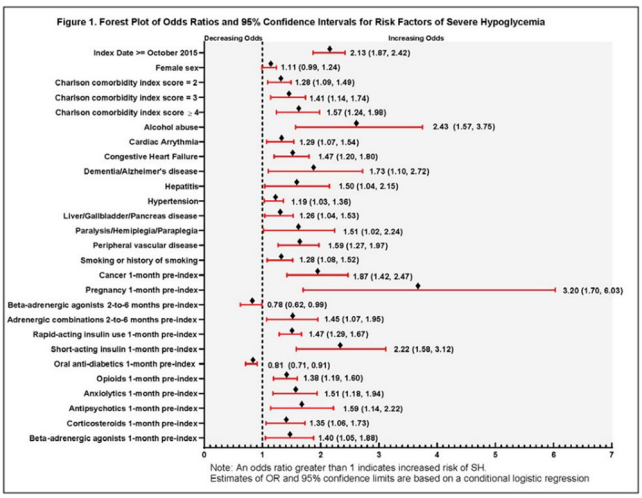
Background and aims: As severe hypoglycaemia has been reported to occur less frequently in people with insulin-treated type 2 diabetes than in those with type 1 diabetes, their risk factors have been less well characterised. A healthcare claims database was therefore used to identify possible severe hypoglycaemia risk factors.

Materials and methods: Adults with type 2 diabetes and ≥1 insulin claim (2012-2018) were identified in IQVIA's PharMetrics® Plus database. Cases with severe hypoglycaemia were matched to controls using incidence density sampling and exact matching on known risk factors (age, sulfonylurea pharmacy claims, previous severe hypoglycaemia, renal disease). A conditional logistic regression model explored associations between potential risk factors and severe hypoglycaemia occurrence. In a sensitivity analysis without exacting matching, the known risk factors were added back to the regression model.

Results: In 3153 cases/3153 matched controls, median age was 57 years old (interquartile range: 51-62). Recognised risk factors were previous severe hypoglycaemia, older age, and renal disease; new or less-recognized factors identified included alcohol abuse, smoking, pregnancy, higher comorbidity scores, dementia, cardiac, hepatic and neurological comorbidities and recent cancer. Various recently prescribed medications were also implicated (as shown in Fig 1 and the sensitivity analysis - results not shown).

Conclusion: While claims database analyses have limitations, some unexpected lifestyle factors, acute and chronic comorbidities and associated medications have emerged as risk factors for severe hypoglycaemia in insulin-treated type 2 diabetes. Although all people with insulin-treated diabetes are at risk of severe hypoglycaemia, additional glucose monitoring and education may be required for specific subgroups of patients with insulin-treated type 2 diabetes.



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Changes in HbA_{1c}, BMI and rates of severe hypoglycaemia in 4,113 adults with type 1 or type 2 diabetes using continuous glucose monitoring systems

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Background and aims: Use of continuous glucose monitoring (CGM) might have beneficial effects on glycemic control and body mass index (BMI) in adults with type 1 or type 2 diabetes.

Materials and methods: Data of the diabetes prospective follow-up registry (DPV) was used to identify individuals with type 1 or 2 diabetes ≥18 years of age starting CGM use as of 2015 and follow-up information available. HbA1c, BMI and event rates of severe hypoglycemia in the year prior to CGM start were compared to a follow-up period of CGM use for >6 months. Repeated measurements linear and negative binomial regression were used and all models were adjusted for sex, age at diabetes onset and respective baseline parameters. Analyses were stratified by diabetes type.

Results: 4,113 adults (2,798 with type 1 and 1,315 with type 2 diabetes) with baseline as well as follow-up information on outcome parameters were identified. Mean age at baseline was 30.9 years in type 1 and 66.8 years in type 2 diabetes. In type 1 diabetes adjusted mean HbA1c decreased significantly from 7.66% (95%-confidence interval: 7.62-7.69%) at baseline to 7.54% (7.51-7.58%) during follow-up. In type 2 diabetes HbA1c decreased from 7.18% (7.14-7.23%) to 6.97% (6.93-7.02%). BMI slightly increased from 25.31 kg/m^2 (25.22-25.40 kg/m^2) to 25.68 (25.59-25.77 kg/m^2) in type 1 diabetes, while remained stable in type 2 diabetes (baseline: 31.94 kg/m^2 (31.71-32.17 kg/m^2), follow-up: 31.86 kg/m^2 (31.63-32.09 kg/m^2)). Event rates of severe hypoglycemia were significantly lower after >6 months with CGM 8.36/100 PY (7.39-9.46/100 PY)) compared to baseline 11.02/100 PY (10.14-11.98/100 PY, p<0.001) in adults with type 1 diabetes and did not change in type 2 diabetes.

Conclusion: Results of the DPV registry provide real world evidence on the use of CGM in adult individuals with type 1 or type 2 diabetes. These results indicate beneficial effects of CGM use on glycemic control in adults with type 1 or type 2 diabetes. Longer follow-up periods might give further insights into other potential beneficial effects.

Supported by: The DPV registry is funded by the German Center for Diabetes Research (DZD) and the Diabetes agenda 2010, which received support from DEXCOM, Sanofi, and Bayer

Disclosure: **S. Lanzinger:** None.

SO 06 Genes and genomic engineering

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A case-cohort of genome-wide association analysis of type 2 diabetes in Qataris: an insight from 1.000 Qataromics cohort of biobank

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Background and aims: Type 2 diabetes (T2D) is a common disease with multiple genome-wide association studies having reported >400 genetic variants associated with the disease. We identified a case-cohort of 468 unrelated individuals sequenced in the framework of Qatar Genome Program (QGP) as incident T2D cases.

Materials and methods: A control-cohort of ~500 subjects were designed for conducting a Genome Wide Association Study to investigate the interplay of genetic factors on T2D risk in Qatar.

Results: Here we describe the results of our association analysis between 6.4 million common autosomal genetic markers and T2D risk in our study cohort. We identified 110 distinct variant-disease associations at an LD-adjusted Bonferroni significance, many of which replicate the known associations with relevant traits for the disease. We report a 130kb region on chromosome 9 that shows multiple independent associations with the disease and harbors many important genes including a microRNA precursor for miR-7 which is a biomarker for T2D and has been shown to regulate secretion and synthesis of insulin-like peptides.

Conclusion: Our results confirm the previously reported associations as well as implicate additional novel loci for the disease in a Middle Eastern context, thus constituting an important resource to facilitate investigations into the genetics of T2D.

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Disclosure: **A.A. Akil:** None.

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Whole genome- and whole exome sequencing analyses provide novel rare genetic variants for coronary artery disease and stroke in type 1 diabetes

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Background and aims: Type 1 diabetes (T1D) increases the risk for cardiovascular disease. In the general population, 163 genetic variants have been associated with coronary artery disease (CAD) and 35 variants with stroke. Genetics of CAD has been studied to some extent also in T1D, but research on strokes and rare variants in T1D are lacking. We aimed to research rare risk variants affecting CAD and stroke in T1D.

Materials and methods: The study is part of the Finnish Diabetic Nephropathy Study; a multicenter, nationwide, and prospective study focusing on the complications of T1D. We performed whole genome sequencing (WGS) and whole exome sequencing (WES) on ~600 and ~500 individuals with T1D, respectively, and aligned sequencing reads to GRCh38 reference genome. We collected cardiovascular phenotypes

from the Finnish Death Registry and Finnish Hospital Discharge Registry until the end of 2017. All strokes were verified from medical records and brain imaging and classified by stroke neurologists. Controls were restricted to those with >34 years of age. After exclusion of samples that lacked phenotypic data or did not fulfil quality control criteria, analyses for CAD comprised 420 individuals with WES (169 with CAD) and 484 with WGS (195 with CAD). Stroke analyses comprised 484 individuals with WES (74 with stroke: 22 hemorrhagic/49 ischemic) and 571 with WGS (110 with stroke: 26/63). We performed single variant association tests outside exons with Firth regression, and meta-analysed WES and WGS exomic region score test results, by adjusting for sex, diabetes onset calendar year and sequencing data principal components. Bonferroni corrected p-values $<4\times10^{-7}$ were considered significant for protein altering variants, and p-values $<5\times10^{-8}$ for the other variants.

Results: We found two suggestive genetic variants for CAD (rs6219 and rs5742714, $p=1.42\times10^{-7}$, $N_{WES+WGS}=904$, MAF=7%) on the three prime untranslated region of insulin like growth factor (*IGF1*), thus likely affecting *IGF1* expression level. For stroke, the top discovery was on a gene desert region (rs4435704, $p=6.42\times10^{-8}$, $N_{WGS}=571$, MAF=4%). The same variant was found suggestively significant for ischemic- ($p=1.69\times10^{-7}$), but not for hemorrhagic stroke ($p=0.66$). For ischemic stroke, we found two significant protein altering variants: rs72646850 (Thr21008Ile, $p=1.60\times10^{-7}$, $N_{WES+WGS}=1055$, MAF=0.3%) and rs200579488 (Val759Met) on *MICALL2* gene ($p=1.24\times10^{-7}$, $N_{WES+WGS}=1055$, MAF=0.4%); rs200579488 was predicted deleterious/ probably damaging by SIFT and PolyPhen. rs72646850 is a missense mutation on *TTN* gene, which codes for muscle connectin and is linked to cardiomyopathy

Conclusion: We performed single variant association tests with sequencing data for CAD and stroke in T1D. We discovered suggestive variants for CAD on *IGF1* gene, and for stroke on a gene desert region. Finally, we found significant protein altering variants on *MICALL2* and *TTN* genes for stroke.

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Disclosure: **A.A. Antikainen:** None.

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Epigenetic mechanisms of hyperglycaemic macrophage programming

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Background and aims: Hyperglycaemia (HG) is a hallmark of diabetes critical for the development of diabetic vascular complications. Macrophages are key innate immune cells responsible for inflammatory reaction in diabetes and development of micro- and macrovascular complications. Inflammatory programming of macrophages can be regulated and maintained by epigenetic mechanisms, in particular histone modifications. The aim of our study was to identify the effect of hyperglycemia on the programming of differentially activated macrophages.

Materials and methods: Using Affymetrix technology we have compared the transcriptional program of human primary monocyte-derived M0, M1 and M2 macrophages differentiated in normal and hyperglycemic conditions. Confirmation of proinflammatory gene expression was performed by RT-PCR and ELISA. Histone modifications were identified by chromatin immunoprecipitation (ChIP).

Results: Hyperglycaemia induced differential expression of 1171 genes in M0, 1573 genes in M1 and 16 genes in M2. The major affected gene families in M0 and M1 were cytokines, receptors and members of S100 family. Hyperglycaemia induced expression of IL1β, key inflammatory cytokine, CCR2, major receptor for macrophage chemotactic factor, CCL2, S100A9 and S100A12 ($p<0.05$). ChIP revealed that three activating histone modifications AcetylH3, H3K4me3 and H3K4me1 are elevated on the promoters of CCR2, IL1β, S100A9 and S100A12 genes. Inhibition of histone methyltransferases SET7/9 and SMYD3 showed that these enzymes differentially regulate S100A9 and S100A12 gene expression with a statistical significance of $p<0.05$.

Conclusion: Our data demonstrate that hyperglycaemia induces epigenetic and transcriptional programs in macrophages that result in the recruitment of proinflammatory macrophages into the sites of low-grade inflammation supporting development of vascular complications.

Supported by: DIAMICOM INTERNATIONAL RESEARCH TRAINING GROUP 1874/2

Disclosure: **E. Badillo:** None.

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Beta cell miR-125b controls glucose homeostasis by targeting lysosomal and mitochondrial genes

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Background and aims: MicroRNAs are tiny RNAs that repress gene expression posttranscriptionally and are often dysregulated in diabetes. High levels of circulating miR-125b associate with hyperglycaemia and, in beta cells, miR-125b is regulated by AMPK, a proposed metformin target. Nevertheless the function of this miRNA in metabolic tissues is contested. We hypothesized that miR-125b contribute to the deleterious effects of hyperglycaemia in beta cell function and aimed to determine whether miR-125b is regulated by glucose and its role in mouse and human beta cells.

Materials and methods: We modulated miR-125b activity in beta cell lines (MIN6 and EndoCBH1) and human islets using mimics, adenovirus and CRISPR-Cas9 and generated mice with beta cell specific overexpression of miR-125b (MIR125B-Tg). Functional characterization included glucose tolerance, glucose stimulated insulin secretion (HTRF/ELISA), mitochondrial and lysosomal morphology (confocal and electron microscopy) and gene expression (RNAseq). To identify miR-125b targets in a high-throughput manner, we performed inmmunoprecipitation of miRNA-induced silencing complex and RNAseq (RIP-seq).

Results: MiR-125b levels in human islets strongly correlate with BMI and is induced by glucose in mouse and human islets while feeding mice a ketogenic (low sugar) diet reduced islet miR-125b, only in the presence of AMPK. Overexpression and knockout of miR-125b in MIN6/EndoC-BH1 cells impaired and improved GSIS, respectively, whereas MIR125B-Tg mice were hyperglycaemic, glucose intolerant and presented strongly reduced insulin secretion and content and loss of β-cell identity. RIP-seq revealed novel miR-125b targets involved in lysosomal and mitochondrial function and gene ontology analysis, as well as EM, confirmed the role of miR-125b in these pathways.

Conclusion: miR-125b is an important regulator of mouse and human beta cell function and may contribute to the negative effects of hyperglycaemia in these cells

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Disclosure: **A. Martinez-Sanchez:** None.

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WFS1 gene engineering reverts ER stress related to autophagy dysfunctions in an iPSC-derived beta cell model of Wolfram syndrome

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Background and aims: Wolfram syndrome (WS) is a rare genetic neurodegenerative disease characterized by juvenile-onset diabetes mellitus and optic atrophy. It is associated to mutations in the *WFS1* gene, encoding for an endoplasmic reticulum (ER)-resident protein involved in unfolded protein response (UPR). WFS1 protein deficiency leads to overall dysfunction and apoptosis in pancreatic β-cells because of increased ER stress and inappropriate UPR. Understanding molecular mechanisms leading to human β-cell degeneration in WS could help to develop effective therapies. Using a patient's iPSC-derived β-cell model of WS, we aimed to explore the molecular signaling of ER stress control, proposing CRISPR/Cas9-mediated correction of *WFS1* as an effective strategy to revert the pathological phenotype.

Materials and methods: iPSCs were generated from a WS diabetic patient carrying two WFS1 heterozygous mutations. Stem cells from healthy donor were used as wild type (WT) control. Differentiation into β-cells was performed by using an *in vitro* protocol recapitulating pancreas ontogenesis. ER stress was assessed in basal conditions and after treatment with Thapsigargin (500 nM) or inflammatory cytokines (1000 U/ml IFNγ, 100 U/ml IL-1β). UPR and autophagy pathways were analyzed by qPCR and western blot. Apoptosis was measured by FACS staining with AnnexinV/PI and by assessing caspases activation in western blot. Wfs1 gene correction was achieved through CRISPR/Cas9-directed recombination using ssODN as donor template.

Results: No significant differences in basal ER stress response were found between WS and WT iPSCs. Likewise, WS iPSCs efficiently differentiated into β-cells (91.7±2.83 $PDX1^+$; 28.27±4.26 $NKX6.1^+$; 24.00±6.08 INS^+), showing no increased ER stress markers compared to WT. However, both WS iPSC and iPSC-derived β-cells resulted in higher susceptibility to ER stress. Precisely, WS cells showed increased apoptosis (47,1±5.9 $AnnexinV^+/PI^+$; $p < 0.01$) and impaired UPR after prolonged exposure to TG (>16h) or cytokines (48h). Moreover, sXBP-1 after 16/24h ($p < 0.05$) decreased in WS cells, following the induction of PERK/ATF4/eIF2α axis ($p < 0.05$) and a marginal increase of ATF3, ATF5 and ATF6. Over time, uncontrolled ER stress response resulted in higher caspase-3 cleavage. Without cell-stress signals, changes in autophagy markers Beclin-1, ATG10, ATG12, LC3-II (downregulated) and p62 (upregulated) were found in both WS iPSC and iPSC-derived β-cells compared to WT. Impaired and prolonged (over 16-24h) activation of such autophagy proteins after stress induction may explain the inability of WS cells to resolve ER stress. Finally, we used CRISPR/Cas9 to correct the WFS1 mutation, restoring functioning WFS1 protein expression. Gene edited cells showed to respond to ER stress, activating autophagy correctly within the first 8-16 hours of TG treatment and preventing caspase-3 cleavage and apoptosis (11.8±3.4 $AnnexinV^+/PI^+$; $p < 0.01$).

Conclusion: We designed an iPSC-based model for WS to investigate mechanisms underlying WSF1 mutation and combine CRISPR/Cas9 with stem cell technology as a promising therapeutic strategy. Differentiation of gene edited patient-derived iPSCs into high functioning β-cells could represent an unlimited, fully compatible source to effectively treat diabetes in WS patients.

Disclosure: **S. Torchio:** None.

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CRISPR editing of the PPARGC1A Gly482ser (rs8192678) polymorphism in human white adipose cells shows differential effects on mitochondrial function and adipogenesis

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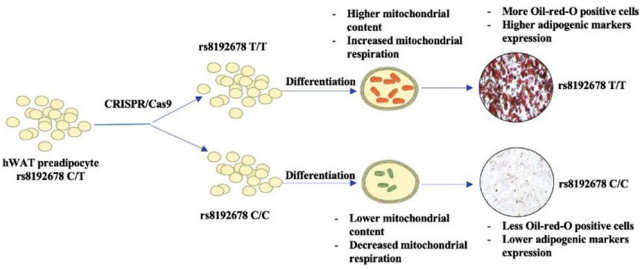
Background and aims: *PPARGC1A* encodes PGC-1α (peroxisome proliferator-activated receptor γ coactivator 1-α), a central regulator of energy metabolism and mitochondrial function. A common polymorphism in *PPARGC1A* (rs8192678, C/T, Gly482Ser) has been associated with obesity and related metabolic disorders, but no published functional studies have investigated direct allele-specific effects in adipocyte biology.

Materials and methods: We used CRISPR-Cas9 to perform allele switching (C-to-T or T-to-C) at rs8192678 in an isogenic human pre-adipocyte white adipose tissue (hWAT) cell line; we then evaluated the allelic effects at rs8192678 on adipocyte adipogenesis and mitochondrial function. Accordingly, single-cell clones were expanded and screened to obtain homozygous T/T (482Ser) and C/C (482Gly) isogenic cell populations. The effect of the allele editing on white adipocyte differentiation and on mitochondrial function was then studied in three cell populations of the respective genotype. In ongoing experiments, CRISPR/Cas9 was also used to append a luciferase tag to C/C and in T/T cells. The luciferase will be used as a reporter for the endogenously expressed PGC-1α protein stability, and will therefore provide insights into mechanisms by which rs8192678 alleles affect PGC-1α activity.

Results: At the end of the differentiation, the C/C adipocytes were visibly less Oil-Red-O positive than T/T adipocytes under optical microscopy; they had 78.5% lower triglyceride content ($p<0.0001$, n=9), and lower expression of adipogenic markers (all markers $p<0.0001$, n=3). Furthermore, C/C adipocytes had lower mitochondrial content ($p<0.001$, n=9), which coincided with decreased oxygen consumption rate (OCR) at basal ($p<0.0001$, n=3) and maximal respiration ($p<0.0001$, n=3). Also, C/C adipocytes had lower ATP-linked OCR ($p<0.0001$, n=3).

Conclusion: Our data show discriminatory causal effects of the two rs8192678 alleles in adipocytes. The C allele confers lower *PPARGC1A* expression, and consequential impaired adipocyte differentiation, at least in part due to disrupted mitochondrial biosynthesis and function. Our study is the first to give experimental insights into the molecular mechanisms behind observational epidemiological studies the Gly482Ser variant and obesity and metabolic disorders.



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Disclosure: **M. Huang:** None.

SO 07 Risk factors and consequences of poor glycaemic control

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Clinical characteristics of COVID-19 patients in a regional population with diabetes: the ACCREDIT study

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Background and aims: Diabetes is associated with greater COVID-19 morbidity and mortality. However, it is unclear if characteristics specific to this cohort affect the prognosis of COVID-19. ACCREDIT study explored clinical and biochemical characteristics associated with 7- & 30-day mortality and intensive care amongst diabetes patients hospitalised with COVID-19.

Materials and methods: This retrospective cohort study involved hospitalised diabetes patients with COVID-19 across Mersey-Cheshire region. We collected data directly from medical notes from 7 hospitals from 1 January to 30 June 2020 with approval from regional research ethics committee. We calculated mortality rates and made Kaplan-Meier curves to assess the mortality rate trend over time. We also explored the impact of COVID-19 on inpatient diabetes team resources. To examine the effect of characteristics on the primary outcome, univariate and multivariate logistic regression analyses were done and optimised by splitting the dataset into training, test, & validation sets to develop a robust predictive model.

Results: We analysed data from 1004 diabetes patients (mean age 74.1 (± 12.6) years, predominantly men 60.7%). 45% of the patients belonged to the most deprived deciles in the UK - greater than regional average (34.75%). Median BMI was 27.6 (IQR 23.9-32.4) kg/m2. Microvascular and macrovascular complications of diabetes were seen in 49.6% and 56.2% respectively. The primary outcome of death by day 7 was observed in 24%, increasing to 33% by day 30. Only 7.5% of patients needed intensive care. Roughly 1 in 10 patients required insulin infusion (9.8%) and diabetes therapy escalations (11.9%). In univariate analyses, patients with type 2 diabetes had a higher risk of 7-day mortality ($p < 0.05$, OR 2.52 [1.06, 5.98]). Patients requiring insulin infusion had a lower risk of death ($p = 0.02$, OR 0.5 [0.28, 0.9]). CKD in younger patients (<70 years) had a greater risk of death (OR 2.74 [1.31-5.76]). No significant association was seen in BMI, diabetes complications, latest HbA1c, and use of RAS blockers. On multivariate analysis, CRP and age remained associated with the primary outcome (OR 3.44 [2.17, 5.44]) and were used in developing a validated predictive model for death by day 7.

Conclusion: CRP and age were predictive of in-hospital death by day 7. Using these two variables in the validated model enables early prognostication of this cohort during their hospitalisation. Higher 7-day mortality rate was observed compared to other studies, possibly due to greater socioeconomic deprivation and older age. Young patients with CKD had a greater risk of early death. A high proportion of these patients required diabetes treatment escalations warranting increased input from the inpatient diabetes teams.

Disclosure: **D. Llanera:** None.

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Impact of COVID-19 lockdown on glycaemic control in patients with type 1 diabetes

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Background and aims: In 2019, a new coronavirus known as severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) has emerged and was classified as a pandemic in a short period of time. Since then, there have been 126,890,643 million people diagnosed with coronavirus disease 2019 (COVID-19) and 2,778,619 million deaths (as of March 30, 2021). In order to reduce the spread of COVID-19, many countries have imposed a lockdown with movement restrictions, home confinement, social distancing, which has affected routine healthcare activities. The aim of this review was to examine the impact of the COVID-19 lockdown on glycemic control in patients with type 1 diabetes (T1D).

Materials and methods: Empirical analysis were performed by investigating databases (Cochrane Library, MEDLINE via PubMed, Web of Science Core Collection, EMBASE, and CINAHL until March 2021). From $n = 567$ citations, $n = 540$ were excluded (duplicates; wrong topic; outcomes and patients unsuitable). We included $n = 27$ studies published in English and German; observational, cohort, clinical, and cross-sectional studies.

Results: In total, $n = 2,526$ patients with T1D were analyzed. Of $n = 27$ studies, $n = 20$ (74%) showed clear improvements in glycemic control, $n = 4$ (15%) showed stability and $n = 3$ (11%) deteriorations. Overall, $n = 14$ studies observed significant improvements ($P < 0.05$) in time in range (TIR), $n = 13$ studies noted significant improvement ($P < 0.05$) in mean/average blood glucose, and $n = 11$ studies showed significant improvement ($P < 0.05$) in HbA1c values, before and during lockdown. Deterioration in $n = 3$ papers could be due to an interruption in health care (e.g. due to non-availability of insulin in India). Improvement is likely related to the use of digital diabetes therapies, as all studies used at least one of the following: mostly continous glucose monitoring (CGM) and flash glucose monitoring (FGM) as well as continuous subcutaneous insulin infusion (CSII), hybrid closed loop system (HCL) and web platform.

Conclusion: Glycemic outcomes in people with T1D improved in most cases during COVID-19 lockdown, which may be associated with positive changes in self-management and digital diabetes management. Further research is required.

Disclosure: **S. Stichling:** None.

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Impact of COVID-19 lockdown on metabolic control and access to healthcare in patients with diabetes from a tertiary care centre: the CONFI-DIAB study

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Background and aims: Restrictive measures to slow down the COVID-19 spread have been established including stay-at-home orders and

hospital routine care cancellation. Neutral or beneficial effects of lockdown on glucose metrics have been suggested in T1DM, but no comprehensive assessment of diabetes management during lockdown was performed.

Materials and methods: CONFI-DIAB was an analytical cross-sectional mono-centric study in a tertiary care center aiming at describing the impact of COVID-19 lockdown on metabolic control and access to healthcare in a population sample of real-world patients with diabetes. We analyzed data from a self-administered questionnaire, which was sent the week before the end of lockdown, along with data from medical records. The primary outcome was the change in HbA1c levels between the 6-months preceding and the 6-weeks following the end of lockdown. Data are expressed as median associated to quartile values. The protocol was approved by the local Institutional Ethics Committee.

Results: This analysis focused on 870 patients with diabetes: 520 (59.8%) were male, age was 65.0 (57.0,72.0) years, BMI was 28.6 (25.1,32.9) kg/m2, 549 (63.1%) had T2DM (T1DM, 30.7%), and diabetes duration was 20.0 (10.0,30.0) years. HbA1c levels pre- and post-lockdown were respectively of 7.7% (7.1,8.4) and 7.4% (6.8,8.2), which translated into a significant reduction of -0.1% (-0.6,0.15) ($p<0.0001$) (mean: -0.21±0.80%; $p<0.0001$), independently of diabetes type. A significantly different reduction in HbA1c was found in participants who either lost weight, had stable weight or weight gain during lockdown, respectively -0.3% (-0.8,0.0), -0.1% (-0.5,0.1) and -0.1% (-0.5,0.3) ($p=0.0029$). Weight changes according to T1DM and T2DM subgroups were respectively as follows: baseline weight of 74.0kg (64.0,83.5) and 87.5kg (78.0,100.0); weight gain in 30.2% (+2.0kg (1.5,3.0)) and 34.0% (+3.0kg (2.0,4.0)) of patients; weight loss in 18.1% (-2.0kg (-1.5,-3.0)) and 15.0% (-3.0kg (-2.0,-4.0)) of patients. Severe hypoglycemia episodes have been reported by 36 (4.3%) of 840 patients, while 21 (2.8%) of 756 reported an episode of ketosis/ketoacidosis. Regarding health care consumption, 423 (49.4%) and 790 (92.3%) patients did not consult their general practitioner (GP) and diabetologist, and 195 (23.0%) patients were supported with home nursing care. Blood tests were done by 379 (44.8%) patients, while 673 (78.3%) did refill their prescriptions, while no patient indicated difficulty accessing a community pharmacy. Only 1.3% and 3.5% of diabetes specialist nurses and biomedical laboratories were unavailable. Teleconsultation services were used by 269 (32.1%) patients. Among them, 43.4% reported an online visit with their GP, 47.9% with their diabetologist, 30.5% with their diabetes specialist nurse. Among the 569 patients who did not use teleconsultation services, 87.6% reported lack of need, 10.0% did not have Internet access.

Conclusion: Despite the implementation of a lockdown and disruption in healthcare, no deterioration, rather an improvement, in metabolic control was observed in a large sample of real-world patients with T1DM and T2DM, particularly in patients who lost weight.

Clinical Trial Registration Number: NCT04485351

Supported by: This work was supported by funds from Asten Santé SA, Dinno Santé, Elivie, Homeperf, ISIS Diabète, Linde Homecare France, Nestlé Home Care, ORKYN', Vitalaire Groupe Air Liquide.

Disclosure: **L. Ludwig:** Grants; Asten Santé SA, Dinno Santé, Elivie, Homeperf, ISIS Diabète, Linde Homecare France, Nestlé Home Care, ORKYN', Vitalaire Groupe Air Liquide.

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Diabetes prevalence is rising among young residents in Malmö, Sweden

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Background and aims: The prevalence of diabetes type 2 is increasing in many parts of the world. The population of Malmö has increased in recent years due to people moving in from other parts of Sweden and the world, in combination with increased birth rates. The current study is a part of the Cities Changing Diabetes Malmö project. Aim: To explore diabetes prevalence in Malmö 2011-2018 as well as achieved treatment targets for selected diabetes related outcomes.

Materials and methods: Prevalence data was retrieved from the Region's Primary Care and Hospital Diagnose Register, and data on treatment targets from the National Diabetes Register. The inclusion criteria were either being a resident of Malmö or using primary care health center located in Malmö.

Results: The prevalence of diabetes type 2 in 2018 has doubled from 2011 in the entire Malmö population. During the same time the prevalence of diabetes type 1 has decreased slightly from 0,68% to 0,61%. In 2011 the diabetes type 2 prevalence was 2,52% (2,76% for males and 2,28% for females) and in 2018 it was 4,33% (4,84% for males and 3,82% for females). For residents between 0 and 29 years the increase was 7 times, for residents between 30 and 39 years 5,5 times, for residents between 40 and 49 years 4 times, for residents between 50 and 59 years 3 times, for residents between 60 and 69 years 2,5 times and for those between 70 and 79 years 2 times. For those between 80-84 only a slight increase was observed, and a decrease in residents between 85 and 109 years of age. The National Diabetes register reported that during 2019, 58% of all patients with diabetes using primary care in Malmö reached Hba1c <52 mmol/mol, 20% had albuminuria, 36% had retinopathy and 21% had not had their feet inspected by a health care professional during the last year. Median Hba1c was 52,6 mmol/mol and 17% of the patients were registered as active smokers.

Conclusion: Diabetes prevalence has increased markedly during the last years, driven by type 2 diabetes mainly in the younger population. Treatment targets regarding p-glucose lowering treatments was not met by 42%. One patient out of three has microvascular complications in the eye, one out of five has impaired kidney function, one out of five had not had their feet inspected and one out of five is active smokers. Active diabetes treatments need to be improved preventing an increasing number of younger patients with microvascular complications. Diabetes preventive activities need to target younger populations in Malmö.

Disclosure: **M. Annersten Gershater:** Other; Partly funded by Novo Nordisk as part of the Cities Changing Diabetes Malmo partnership which includes the City of Malmo, Region Skåne and Malmo University.

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Insulin secretion with increasing age: a comparison between Middle Eastern immigrants and native Swedes

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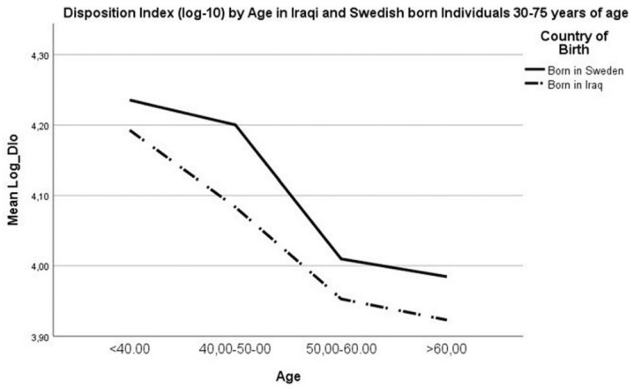
Background and aims: Middle Eastern immigrants in Europe are at high risk of developing early onset type 2 diabetes and show a higher prevalence of insulin deficient type 2 diabetes. In general, little is known how insulin secretion and action changes over time in people without diabetes and in specific if it differs across populations of different ethnicity. Assessing age as a proxy of time, the aim was to study changes in insulin secretion in relation to increasing age comparing a population cohort of Iraqi born immigrants with native Swedes. Further the aim was to investigate if the associations were modified by country of birth.

Materials and methods: Citizens of Malmö, Sweden, aged 30-75 years born in Iraq or Sweden were invited to participate in the population-based study MEDIM, between 2010 to 2012. Insulin secretion (i.e. adjusted for

insulin for insulin action i.e. oral disposition index DIo) was based on Matsuda indices assessed by oral glucose tolerance tests.

Results: The level of DIo was generally lower in the Iraqi born immigrant population (N=1154) vs. native Swedes (N= 649), (median DIo 21 574.3 vs. 25 559.2, p=.004). Further, DIo decreased faster with increasing age in Iraqi born immigrants compared to native Swedes, confirmed by an interaction between age and country of birth $P^{age*country\ of\ birth}$<0.001. Data adjusted for age, gender, BMI, smoking and physical activity.

Conclusion: This study shows that insulin secretion declines with increasing age irrespective of Middle Eastern or European ancestry. However, there seems to be a faster decline in beta cell function with increasing age in Middle Eastern immigrants than native Swedes. Preventive actions addressing impaired insulin secretion may impact future type 2 diabetes risk in Middle Eastern immigrant populations.



*Data filtered by glc-30 > f-glc and glc-30 > 4.44

Disclosure: **N. Fadhel Dhaher:** Grants; ALF medel.

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Fetuin-a and risk of diabetes-related vascular, including microvascular, complications

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Background and aims: Fetuin-A is a hepatokine which has the capacity to bind calcium and phosphate, preventing pathological vascular calcification. Moreover, it is linked to the induction of metabolic dysfunction, insulin resistance and associated with increased risk of diabetes and cardiovascular disease. It is unknown whether fetuin-A associates with higher or lower risk of vascular complications, specifically microvascular complications, in patients with diabetes.

Materials and methods: Participants with incident type 2 diabetes and free of micro- and macrovascular disease from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam cohort (n=587) were followed for microvascular (n=203; median follow-up time 12.8 years) and macrovascular (n=60; median follow-up time 13.4 years) complications. Plasma fetuin-A was measured 4.1 (interquartile range: 2.2-5.8) years prior to the diagnosis of diabetes. Prospective associations between baseline fetuin-A and risk of complications were assessed with Cox regression.

Results: In multivariable models, fetuin-A was linearly inversely associated with incident total and microvascular complications, HR (95% CI) per SD increase: 0.86 (0.74; 0.99) for total, 0.84 (0.71; 0.98) for microvascular and 0.92 (0.68; 1.24) for macrovascular complications. After additional adjustment for cardiometabolic plasma biomarkers, including triglycerides and high-density lipoprotein, the associations remained largely unchanged: 0.88 (0.75; 1.02) for total, 0.85 (0.72; 1.01) for microvascular and 0.95 (0.67; 1.34) for macrovascular complications.

Conclusion: Although higher fetuin-A levels have been linked to higher risk of diabetes and cardiovascular disease, in persons who developed diabetes, fetuin-A was inversely associated with incidence of microvascular complications, independent of potential confounders. No definitive relationship with macrovascular complications was observed.

Supported by: DZD grant 82DZD00302

Disclosure: **A. Birukov:** None.

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Risk of type 2 diabetes in polycystic ovary syndrome is associated with obesity: a meta-analysis of observational studies

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Background and aims: The exact risk of type 2 diabetes in women with polycystic ovary syndrome (PCOS) is unknown. It is also unclear if obesity independently increases the risk in this population. The aim of this study was to systematically review and synthesize the best available evidence regarding the association between PCOS and type 2 diabetes, stratified according to obesity status.

Materials and methods: A comprehensive search was conducted in PubMed, CENTRAL and Scopus databases up to October 31, 2020. Data are expressed as relative risk (RR) with 95% confidence interval (CI). The I^2 index was employed for heterogeneity.

Results: The eligibility criteria were fulfilled by 23 studies (319,780 participants; 60,336 PCOS and 8847 type 2 diabetes cases). Women with PCOS demonstrated a higher risk of type 2 diabetes than those without PCOS (RR 3.45, 95% CI, 2.95-4.05, p<0.001; I^2 81.6%). This risk remained significant both in studies matched or unmatched for participants' age. With regard to body mass index, the RR for developing type 2 diabetes in obese and non-obese PCOS women compared with their non-PCOS counterparts was 3.24 (95% CI 2.25-4.65; p<0.001; I^2 30.9%) and 1.62 (95% CI 0.14-18.50; p=0.70; $I^2$89.9%), respectively. The RR in obese compared with non-obese women with PCOS was 3.85 (95% CI 1.99-7.43; p<0.001; I^2 46.2%). This was also the case for overweight compared with normal-weight women with PCOS.

Conclusion: Women with PCOS present an increased risk of type 2 diabetes compared with non-PCOS women only if they are obese/overweight.

Disclosure: **P. Anagnostis:** None.

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Prevalence of and factors associated with undiagnosed stage 3 chronic kidney disease in patient with type 2 diabetes: a report from REVEAL-CKD

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Background and aims: Chronic kidney disease (CKD) is a serious debilitating condition affecting 10% of the world's population, yet it remains largely under recognised even among patients with pre-existing comorbidities. A large proportion of patients with CKD have type 2 diabetes (T2D), however factors associated with undiagnosed CKD in patients with multimorbidity remain unclear. This analysis aims to assess prevalence and factors associated with undiagnosed stage 3 CKD in patients with T2D.

Materials and methods: REVEAL-CKD is a multinational initiative to assess early stage undiagnosed CKD. From the US, we utilised TriNetX, a federated research network providing statistics on electronic health records. Adult patients, with two consecutive estimated glomerular filtration rate (eGFR) measurements ≥30 and <60 mL/min/1.73^2 recorded at least 90 days apart were identified between 2015-2020. T2D status was ascertained prior to the index date (date of the second eGFR measurement). Those with no CKD diagnosis code at any time before or up to 6 months after the index date were considered to have undiagnosed CKD.

Results: The study cohort included 66,815 patients with eGFR values indicating stage 3 CKD and pre-existing T2D with mean age of 70 years (standard deviation: 10 years). The overall prevalence of undiagnosed CKD was 49.1% (95% confidence interval: 48.7-49.5). Prevalence of undiagnosed CKD increased with age and ranged between 38% and 48% in patients with other pre-existing comorbidities (Table 1). Compared to patients with diagnosed CKD, the undiagnosed group had more females (46% versus 63%) and a had higher proportion of patients ≥75 years: 33% versus 38%. Fewer undiagnosed CKD patients had pre-existing comorbidities than those with diagnosed CKD.

Conclusion: This study suggests that a large proportion of either older or female patients with baseline T2D have undiagnosed CKD. These results suggest that an opportunity exists for more proactive CKD diagnosis and monitoring of patients with pre-existing comorbidities.

Table 1: Type 2 diabetes patient characteristics and baseline comorbidities by CKD diagnosis status.

Baseline characteristics	Overall population N= 66,815	Diagnosed CKD N= 34,009	Undiagnosed CKD N = 32,806	Undiagnosed prevalence[1]
Age, years	n (%)	n (%)	N (%)	%
≥75	23579 (35.29)	11211 (32.96)	12368 (37.70)	52.45
65 - <75	25245 (37.78)	12496 (36.74)	12749 (38.86)	50.50
45 - <65	16869 (25.25)	9491 (27.91)	7378 (22.49)	43.74
< 45	1122 (1.68)	811 (2.38)	311 (0.95)	27.72
Sex: Male	36400 (45.52)	18248 (53.66)	12167 (37.09)	33.43
Female	30415 (54.48)	15761 (46.34)	20639 (62.91)	67.86
Comorbidities: Hypertension	62049 (92.87)	32382 (95.22)	29667 (90.43)	47.81
Heart failure	15563 (23.29)	9668 (28.43)	5895 (17.97)	37.88
MI	9037 (13.53)	5519 (16.23)	3518 (10.72)	38.93
Stroke	8423 (12.61)	4712 (13.86)	3711 (11.31)	44.06

[1](Undiagnosed CKD/Overall population)*100, MI: myocardial Infarction

Supported by: AstraZeneca

Disclosure: **E. Wittbrodt:** Employment/Consultancy; Employed by AstraZeneca. Stock/Shareholding; AstraZeneca Shareholder.

SO 08 Preservation versus destruction of beta cell mass

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A fatty acid modified apelin-13 analogue demonstrates benefits on pancreatic islet cell morphology and beta cell preservation in diabetic mouse models

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Background and aims: The adipocytokine apelin, exists in multiple molecular isoforms including apelin-13, which has potential for improving metabolic status in diabetes. In this study we examined potential pancreatic benefits of treatment with a fatty acid modified apelin-13 analogue in both high fat fed diet-induced obese (DIO) and streptozotocin-induced (STZ) diabetic mouse models.

Materials and methods: Islet cell apoptosis, proliferation and transdifferentiation were examined using Ins1$^{Cre/+}$;Rosa26-eYFP transgenic mice and DIO and STZ-induced diabetic mice. Diabetes was induced in groups (n=6-8) of DIO (45% fat, 20% CHO and 35% protein, energy content 26.15 kJ/g, fed over 3 months) and STZ-induced (following 5 once daily low dose i.p. injections of STZ 50 mg/kg) mice. Both groups of diabetic mice then received once-daily injection (25 nmol/kg) of the acylated apelin-13 peptide analogue, pGlu(Lys^{8}Glu-PAL)apelin-13 amide, for 12 or 10 days, respectively.

Results: pGlu(Lys^8 Glu-PAL)apelin-13 amide treatment partly reversed the expected STZ-induced weight loss and helped normalise circulating insulin concentrations. In contrast, these variables were not altered in DIO diabetic mice, however pancreatic insulin content was enhanced. Apelin analogue treatment also fully, or partially, reversed the detrimental effects of STZ and high-fat feeding on plasma and pancreatic glucagon concentrations, respectively. In DIO mice, the fatty acid modified apelin analogue decreased dietary-induced elevations of islet, β-cell and α-cell areas (P<0.05-P<0.01), whilst reducing α-cell area in STZ-induced diabetic mice. In terms of islet cell lineage, pGlu(Lys^8 Glu-PAL)apelin-13 amide effectively reduced β- to α-cell transdifferentiation in STZ-induced diabetic mice and helped maintain β-cell identity, which was linked to elevated Pdx-1 expression (P<0.001). These islet effects were coupled with decreased β-cell apoptosis and α-cell proliferation in both diabetic mouse models and furthermore, there was an associated increase of β-cell proliferation in STZ-induced diabetic mice.

Conclusion: Of note, sustained APJ receptor activation in diabetic mouse models, using a stable acylated apelin-13 analogue was linked to pancreatic islet benefits, including favourable islet cell morphology and maintenance of β-cell mass.

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Disclosure: **F.P.M. O'Harte:** Other; FOH, PF hold patents in the field of apelin therapeutics.

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High-fat diet-induced upregulation of chemokine Ccl4 in mouse visceral adipose tissue: potential crosstalk with beta cells

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Background and aims: In addition to acting as an energy reservoir, white adipose tissue has an important role in metabolic homeostasis through the synthesis and secretion of adipose tissue-derived peptides, known as adipokines. Little is known about adipokine function at β-cells or whether this is altered in obesity, but several adipokines have actions

that are transduced by binding to islet G protein-coupled receptors (GPCRs), and levels of some adipokines are altered in obesity. This study aimed to establish the expression profile of islet GPCR peptide ligand mRNAs in visceral adipose tissue from lean and diet-induced obese mice to better understand the metabolic crosstalk between adipose tissue and β-cells via GPCR activation.

Materials and methods: RNA was purified from epididymal adipose tissue retrieved from 24-week-old C57BL/6 male mice fed either a control diet (CD: 10% fat) or a high-fat diet (HFD: 60% fat) for 16 weeks, and reverse transcribed to cDNA. Islet GPCR peptide ligand mRNAs were quantified by RT-qPCR, relative to expression of the housekeeping gene, *Actb*, in the same samples. MIN6 β-cell mRNA levels of Ccl4-targeted GPCRs, *Ccr1*, *Ccr5* and *Ccr9*, were analysed by RT-qPCR. The effects of Ccl4 on cytokine- and palmitate-induced apoptosis in MIN6 β-cells were investigated by quantifying Caspase 3/7 activities using a luminescent assay.

Results: Mice fed a HFD for 16 weeks were obese (CD: 29.8±0.15g; HFD: 51.1±0.59g, n=5, p<0.0001) and had fasting hyperglycaemia (CD: 5.3±0.8mM glucose; HFD: 8.3±0.5mM glucose, n=5, p<0.05). 45 and 40 islet GPCR peptide ligand mRNAs were detectable in CD and HFD epididymal adipose tissue, respectively, and expression levels of several mRNAs were significantly modified by the HFD: (control: 1; upregulation (>1): *Ccl5* (3.42-fold), *Ccl3* (8.19-fold), *Ccl4* (11.13-fold) and *Npy* (20.12-fold); downregulation (<1): *Ccl24* (0.12-fold), *Ccl17* (0.04-fold), *Agt* (0.03-fold) and *Bglap* (0.02-fold). Ccl4 was further characterised due to its significant upregulation with HFD (p<0.001, n=5) and unknown effects on β-cell function. MIN6 β-cells expressed mRNA encoding *Ccr9*, but not *Ccr1* and *Ccr5*. Ccl4 demonstrated concentration-dependent protective effects against palmitate-induced MIN6 β-cell apoptosis (Caspase 3/7 activities, % control: no palmitate: 100±6.2; +palmitate: 291.5±14.3; +5ng/mL Ccl4: 232.2 ±17.7; +10ng/mL Ccl4: 222.7±13.6; +50ng/mL Ccl4: 197.5±12.7; +100ng/mL Ccl4: 195.1±9.7; p<0.0001, n=3 experiments). Ccl4 also significantly attenuated cytokine-induced MIN6 β-cell apoptosis (Caspase 3/7 activities, % control: no cytokines: 100±7.9; +cytokines: 595.2±37.0; +5ng/mL Ccl4: 532.8±25.0; +10ng/mL Ccl4: 502.8 ±18.0; +50ng/mL Ccl4: 497.8±19.5; +100ng/mL Ccl4: 469.1±24.5; p<0.01, n=3 experiments).

Conclusion: Adipokine expression is altered in obesity and upregulation of adipose-derived Ccl4 may promote β-cell survival through its observed anti-apoptotic effects.

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Disclosure: **T. Ashik:** None.

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Proteomic profiling of glucose regulated ACC1 phospho-sites in pancreatic beta cells

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Background and aims: Acetyl-CoA-Carboxylase (ACC1) is an enzyme that couples glucose metabolism with de novo lipogenesis (DNL) in lipogenic tissues, by converting acetyl-CoA to malonyl-CoA. In beta cells, ACC1 plays a critical role in beta cell growth and insulin secretion. However, very little is known about how ACC1 activity is regulated in beta cells. The aims of the current project were to 1) assess the role of the AMP-activated protein kinase phospho-site serine 79 (ACC1S79) in beta cell function and whole body glucose homeostasis; and 2) screen for novel phospho-sites that may regulate ACC1 activity in beta cell.

Materials and methods: 1) ACC1S79A knockin mice, with ACC1S79 mutated to alanine, were obtained by crossing ACC1S79A/ACC2S212A double knockin mice with wild type mice to isolate the ACC1S79A point mutation. 2) ACC1 protein was purified from INS1 beta cells cultured with different glucose concentrations. Unbiased quantitative phosphoproteomics was performed to characterise ACC1 phospho-sites: LC-MS/MS was undertaken using a Q-Executive mass spectrometer and data analysed using MaxQuant and Perseus. 3) We generated phospho-specific antibodies against key phospho-sites identified by our screen that exhibited a dynamic response to glucose stimulation, which were validated by western blotting.

Results: 1) Female ACC1S79A mice exhibited no alternations in glucose homeostasis. In male mice we found no differences in serum insulin, glucose tolerance (ipGTT) or insulin action (ipITT), although a small but significant reduction in fasting blood glucose was recorded in ACC1S79A mice compared to littermate controls. There was no significant change in glucose-stimulated insulin secretion (GSIS) from isolated ACC1S79A islets at baseline (2 mmol/l glucose) or in response to glucose-stimulation (7.5 and 20 mmol/l glucose), suggesting that ACC1S79 phosphorylation does not play a major role in GSIS. 2) Using quantitative phosphoproteomics, we identified twenty phospho-sites on the ACC1 protein in beta cells. ACC1S1215 was highly phosphorylated at 2 mmol/l glucose and showed a marked and significant reduction in phosphorylation in response to 15 mmol/l glucose, in contrast to the more modest changes in phosphorylation of ACC1S79. Phosphorylation of ACC1S25 was lower at basal glucose and increased upon glucose stimulation. 3) Validation by western blotting confirmed that ACC1S1215 phosphorylation was highly regulated by glucose, with ACC1S79 phosphorylation more subtle in INS1 beta cells and primary mouse islets.

Conclusion: Our data demonstrate that ACC1S79 phosphorylation does not play a major role as a glucose-regulated phospho-site in beta cells. However, other phospho-sites identified by our phosphoproteomics screen show greater dynamic regulation by glucose and may play a role in regulating beta cell ACC1 activity and insulin secretion.

Supported by: DiabetesUK

Disclosure: **R. Bany Bakar:** None.

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The role of TSC2 acetylation and its subcellular localisation in mitochondrial turnover of pancreatic beta cells

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Background and aims: Eukaryotic cells need to regulate cell growth by the presence of growth factors and a correct sensing of nutrients such as glucose and aminoacids. An alteration in any of these factors generates a dysfunction in cell maintenance and is associated with different diseases such as Type 2 Diabetes Mellitus (T2DM). One of the main controllers of cell size and cell proliferation is the mechanistic target of rapamycin complex 1 (mTORC1). Acetylation is a post-translational modification found in a high variety of proteins, altering its function and stability. Depending on the protein, the introduction of the acetyl group can activate or inactivate its catalytic activity. Our group has previously uncovered that TSC2 acetylation inhibit its activity and hence, stimulate mTORC1 signaling pathway and downregulate autophagy processes,

with important consequences in pancreatic β cells. The main objective is the study of TSC2/mTORC1/p70S6K pathway and the role of TSC2 acetylation in the regulation of pancreatic β cell expansion and viability, focusing on the implication of autophagy and mitochondrial turnover to understand the progression to Type 2 Diabetes Mellitus *in vitro*.

Materials and methods: We used different cell lines generously donated by either David Kwiatkowsky (MEF TSC2 WT and KO) or Leonard Guarente (MEF SIRT1 WT and KO). Furthermore, we have generated stably transfected MIN6 with either scrambled or TSC2 and SIRT1 shRNA in our laboratory. To perform our studies, cells were treated with different drugs: resveratrol (50 uM), a sirtuin activator; nicotinamide (5 mM) as a negative control of resveratrol; acetyl-CoA in different doses; chloroquine (20 uM) to block autophagy flux and CCCP (20 uM) as a mitophagy inducer. Different experiments were performed in fibroblasts (MEF) and pancreatic β cells (MIN6) with a knock out of TSC2 and SIRT1 proteins.

Results: We have determined that TSC2, in its unacetylated form due to resveratrol action, can be recruited to the lysosomal membrane, where it inhibits mTORC1 signaling pathway in MEF and in pancreatic β cells. This mechanism of TSC2 doesn't work when TSC2 is hyperacetylated at the basal state using MEF SIRT1-/- cells or β cells with a knock down of SIRT1. In this regard, when we pretreated the cells with acetyl-CoA, we reverted the effect on mTORC1 signaling by resveratrol in a dose-dependent manner. Furthermore, we have observed that resveratrol can activate mitophagy in both TSC2- and SIRT1-dependent manner.

Conclusion: Collectively, our data indicate that resveratrol, by facilitating TSC2 recruitment to the lysosome, can inhibit mTORC1 and trigger autophagy. In addition, we have observed a significant activation of mitophagy under these conditions, facilitating mitochondrial turnover, depending on both TSC2 and SIRT1 proteins.

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Disclosure: **P. Marqués:** None.

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Regulation of autophagy activity by PERK attenuation contributes to insulin synthesis with an Atg7-dependent manner

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Background and aims: Low-dose PERK inhibitors (PERKi) enhance glucose-stimulated insulin secretion from pancreatic islets through BiP induction and ER calcium regulation. Long-term administration of low-dose PERKi demonstrated increased plasma insulin levels and improved hyperglycemia in rodent models of diabetes. Because autophagy insufficiency of pancreatic beta cells is regarded as one mechanism of diabetes, we evaluated PERKi effects on autophagic activity in insulin-secreting cells.

Materials and methods: Autophagy activity was inhibited by 3-methyladenine (3-MA) treatment to mouse islets or INS1 cells. After PERKi treatment as long as 72 h, cellular insulin content was measured with ELISA and autophagy activity was evaluated with western blot. *Atg7, Bip,* and *Insulin* expression were evaluated by quantitative PCR and western blot. To examine association of ATG7 in the effects, islets were used isolated from beta cell-specific *Atg7* deleted mice.

Results: Treatment of low-dose PERKi recovered insulin content suppressed by 3-MA. It was associated with induction of *Insulin* transcription, *Atg7* transcription, conversion of LC3-B from type-1 to type II, and reduction of p62 protein, however, not with induction of *Bip* expression. When PERKi was treated to *Atg7*-deleted islets, induction of autophagy activity was not observed. In contrast, slight recovery of insulin content was found, which suggested that mechanisms other than autophagy regulation could have contributed.

Conclusion: PERKi increased insulin contents of insulin-secreting cells by *Atg7*-dependent autophagy induction and *Atg7*-independent mechanisms which would be further elucidated.

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Disclosure: **S. Moon:** None.

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Exposure to bisphenol-A induces pancreatic beta cell apoptosis

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Background and aims: Bisphenol-A (BPA) is a widespread endocrine-disrupting chemical that has been associated with type 2 diabetes development. Accumulating evidence suggests that low doses of BPA induce pancreatic beta-cell dysfunction and insulin resistance. However, little is known about BPA effects on pancreatic beta-cell survival. Here we aimed to investigate whether treatment with low doses of BPA could affect beta-cell survival in different models.

Materials and methods: rat and human beta-cell lines, namely INS-1E and EndoC-βH1 cells, respectively, as well as dispersed mouse islets, were exposed to low doses of BPA (1 pM to 1 μM). Viability was assessed by Hoechst 33342/Propidium iodide upon 24 h of treatment with BPA.

Results: BPA doses as low as 1 pM were able to induce beta-cell apoptosis upon 24 h of exposure in rat INS-1E cells, human EndoC-βH1 cells and dispersed mouse islets. Compared to vehicle, the highest BPA dose, i.e. 1 μM, increased apoptosis by 2.2-fold ($n=5$; $p<0.001$) in INS-1E cells, by 1.7-fold ($n=4$; $p<0.05$) EndoC-βH1 cells, and by 1.9-fold ($n=3$; $p<0.05$) in dispersed mouse islets. In INS-1E cells, short treatment with BPA for 1 h followed by its removal from the medium still resulted in beta cell death 24 h post-BPA removal, suggesting that BPA-induced apoptosis is not reversible upon BPA withdrawal. Moreover, treatment with ICI 182,780, a high-affinity oestrogen receptor antagonist, prevented BPA-induced apoptosis both in INS-1E and EndoC-βH1 cells ($n=4$; $p<0.05$). To investigate whether BPA might potentiate cytokine-induced beta-cell death, INS-1E and EndoC-βH1 cells were treated with proinflammatory cytokines (IL-1β + IFN-γ) in the absence or presence of BPA. As expected, IL-1β + IFNγ induced beta-cell apoptosis in INS-1E and EndoC-βH1 cells. Intriguingly, BPA at two different doses (1 pM and 1 nM) protected beta cells against cytokine-induced apoptosis ($n=5$; $p<0.05$). Similar protective results were observed when cells were treated with 17β-oestradiol.

Conclusion: Taken together, these results suggest that, in addition to its effects on beta-cell function, BPA also induces beta-cell death, which may be part of its diabetogenic action described in animal models.

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Disclosure: **R.S. dos Santos:** None.

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Novel islet staging defined by progression of beta cell destruction

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Background and aims: Currently, there is no standardized staging procedure to evaluate pancreatic islet health and insulitis progression. Often, H&E insulitis grading is used, however, we demonstrate that without a more specific staining, H&E fails to distinguish later stages of beta cell destruction. We established a new staging based on islet health characterized by sections stained with fluorescent multiplexed immunohistochemistry (fm-IHC). Additionally, we automated the procedure of detecting and staging islets by using a specialized fm-IHC compression method that enables rule-based detection and staging of islets.

Materials and methods: Formalin fixed paraffin embedded (FFPE) pancreatic tissue slides of NOD mice were stained with a multiplexed, fluorescent panel that simultaneously targets markers for insulin, glucagon, CD45, CD8 and CD4 in addition to nuclear staining with DAPI. Pancreatic tissue sections of female, NOD mice aged 5, 10, 20, 30 (n=4 each) and 35 (n=5) weeks with blood glucose below 200 mg/dl as well as diabetic NOD mice with blood glucose above 350 mg/dl and healthy C57BL6 mice were used. We stained a minimum of 5 FFPE slides per mouse, with sections approx. 80 µm apart. Adjacent sections were also H&E stained to directly compare both staining methods. We applied Cell2Grid, a novel fm-IHC image compression algorithm, followed by cell-based rules for islet detection and staging, enabling quantitative high-throughput analysis.

Results: We were able to define 5 different stages of beta cells destruction using fm-IHC in NOD pancreas sections (fig. 1). We found that H&E insulitis grading was insufficient to evaluate islet health because it cannot differentiate between insulin producing and insulin deficient islets. Our staging classifies islets by presence and absence of stained markers (insulin and immune cell markers). We verified stage 0, the "healthy islet", with C57BL/6 mice and stage 4, the "pseudo-atrophic, glucagon-only islets", with diabetic NOD mice with blood glucose above 350 mg/dl. Stages 1 to 3 occur mostly in NOD mice with blood glucose values under 160 mg/dl. We characterized a non-diabetic NOD cohort regarding islet stages and correlated it to the last blood glucose of the mice. We used Cell2Grid to compress the large data set of fm-IHC images and defined cell phenotypes which made an automated rule-based analysis possible.

Conclusion: Evaluating the overall health and functionality of the pancreas requires an islet staging that reflects the actual beta cell destruction. Our staging reflects the process of autoimmune destruction of beta cells and minimizes inter-observer variability by using automated analysis and rule-based algorithms. Our findings suggest that our staging correlates with increasing blood glucose in NOD mice.

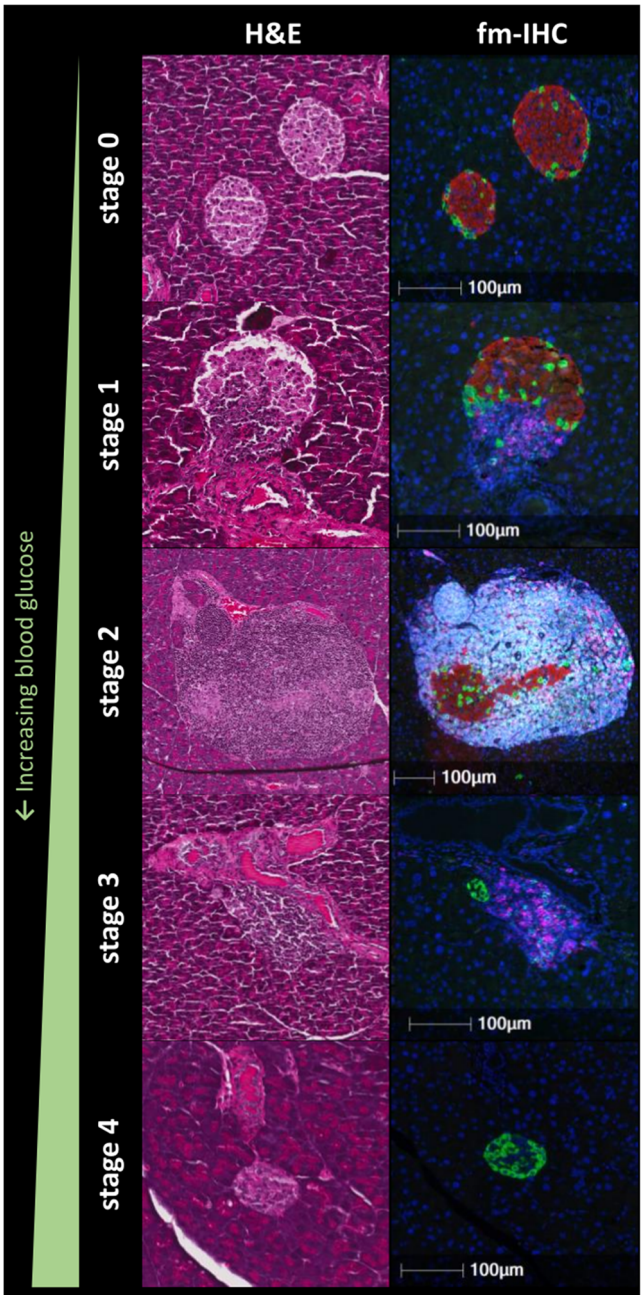

Figure 1: Functionality-based staging of islets established on a previous dataset and exemplified on representative islets. **H&E**: Stages 0 and 4 are indistinguishable, as are stages 1, 2 and 3. **Fm-IHC: insulin, glucagon, CD45, CD4, CD8 and DAPI. Stage 0** – healthy islets; **Stage 1** – starting immune cell presence; **Stage 2** – islet is fully surrounded by immune cells and is losing classic structure and composition; **Stage 3** – pseudo-islet with only alpha cells remaining while immune cells are still present; **Stage 4** – pseudo-islets with only alpha cells.

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Disclosure: **B. Ehall:** None.

SO 09 The ins and outs of insulin secretion

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Loss of maturity in overworked beta cells of normoglycaemic mice

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Background and aims: Progressive loss of beta cell function and mass is central to the pathogenesis of type 2 diabetes (T2D). Pancreatic beta cells adapt to rising metabolic demand by means of increased insulin secretion and cell replication to expand the functional beta cell mass, and glucose homeostasis is maintained by overworking beta cells. Beta cell dedifferentiation has been found in pancreases of T2D patients, which could be attributed to chronic hyperglycemia. However, loss of maturity in overworked beta cells may initiate at earlier stages of the disease and contribute to the progression to overt diabetes. The aim of this study was to investigate whether increased beta cell workload promotes beta cell dedifferentiation in normoglycaemic mice.

Materials and methods: Two mouse models of beta cell overload in euglycemic milieu were studied: chronic stimulation of insulin secretion by continuous glibenclamide treatment with a 2.5mg pellet (G, n=14; Sham-operated (S-G), n=13) and increased insulin demand by 60% pancreatectomy (Px, n=12; sham-operated (S-Px), n=6) in adult $Ins1^{Cre}$; Rosa26-eYFP mice (6-14 months old). Blood glucose and body weight were monitored from the day of pellet implantation, pancreatectomy or sham surgeries until the end of the study (14 days) when pancreases were harvested and underwent islet isolation or OCT embedding for further analysis. Glucose tolerance and *in vivo* insulin secretion were determined on day 12 after surgery (2mg/Kg glucose i.p. injection). Gene expression of beta cell identity and "disallowed" markers was determined by RT-qPCR in isolated islets. Beta cell dedifferentiation was determined by double immunofluorescence insulin-YFP in double transgenic mice with YFP-traced beta cells.

Results: : G-mice showed initial hypoglycemia (day 1, S-G: 152 ±9mg/dl; G: 99±6mg/dl; p<0.0001) followed by transient moderate hyperglycemia (day 6, S-G: 138±7mg/dl; G: 171±8mg/dl; p<0.0001) and euglycemia from day 10 (S-G: 140±5mg/dl; G: 156±6mg/dl) to the end of the study. On day 12, G-mice were glucose intolerant (AUC, S-G: 24401±941, G: 39900±1949, p<0.0001) and lost glucose-induced insulin secretion (plasma insulin ratio t=30 min/t=0, S-G: 1.61±0.13; G: 1.08±0.13, p=0.001). Islets from G-mice showed reduced mRNAs of *ins2*, *nkx2.2* (p< 0.05) and increased levels of disallowed genes *hk1* and *ldha* (p< 0.05). Gene expression of *nkx6.1* and *mafa* was 40% and 94% lower in G-mice, respectively, but differences did not reach statistical significance. Genetic beta cell tracing experiments showed higher percentage of beta cells that lost insulin expression in G-mice (S-G: 0.65±0.08%, G: 1.3±0.2%; p=0.03). Px-mice showed normal blood glucose levels throughout the study (day 14, S-P: 144±8mg/dl; Px: 149±6mg/dl), IPGTT response (AUC, S-P: 24214±1393; Px: 23454±1644) and glucose-induced insulin secretion (plasma insulin ratio t=30 min/t=0, S-P: 1.41±0.17; Px: 1.65 ±0.39). Gene expression of *pdx-1*, *nkx6.1*, *nkx2.2* and *mafa* was significantly reduced in islets of Px-mice, and mRNA levels of *hk1*- and *ldha* were increased (p< 0.05). mRNA levels of ins2 gene were similar between islets from S-P and Px-mice.

Conclusion: Increased beta cell workload in normoglycaemic mice, either induced by direct membrane hyperexcitability or by nutrient metabolism, results in metabolic reprogramming of pancreatic beta cells and loss of beta cell identity.

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Novel miR-29 mitochondrial-associated gene pathways predicted to regulate beta cell insulin secretion

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Background and aims: Defective β-cell insulin secretion is commonly regarded as the definitive trigger for Type 2 diabetes (T2D) and microRNAs (miRNAs) are small non-coding RNAs and regulators of β-cell function. Importantly, a bioinformatics study pinpointed disturbed mitochondrial metabolism as a key signature of hampered islet function and highlighted 27 cis-eGenes implicated in mitochondrial function (*ACP6, AMACR, ASAH2, CCBL2, DNAJC15, FAHD1, NDUFA10, NDUFV3, PCCB, QRSL1, SARDH, TDRKH, WARS2, TSFM, ACSM1, AGXT, AKR1B15, KIAA0141, MRPL39, MUT, DACT2, EFHD1, FOXRED1, MRPL21, NUDT2, PITRM1 & TFB1M)* in human islets, 5 of which associated with HbA1c and/or BMI. Here, to further understand β-cell miRNA-mitochondrial pathways, we investigated whether miRNAs already implicated in β-cell metabolism regulate these genes.

Materials and methods: TargetScan (http://www.targetscan.org/) was used to predict if 16 miRNAs involved in β-cell metabolism (miR-15a-3p, miR-15b-3p, miR-29-3p, miR-29a-5p, miR-29b-1-5p, miR-29b-2-5p, miR-124- 3p.1, miR-124- 3p.2, miR-124- 5p, miR-130a-3p, miR-130b-3p, miR-130b-5p, miR-152-3p, miR-152-5p, miR-184 and/or miR-206) target the listed genes. Thereafter, INS-1 832/13 rodent β-cells were pre-cultured for 1 or 24h in 2. 8mM, 11.1mM or 16.7mM glucose (G) supplemented media. These were evaluated for insulin secretion responses to low and high G (2.8 & 16.7mM) and for insulin content. Insulin was quantified by ELISA. Changes in expression of miRNAs predicted to target specific genes were also assessed by qPCR in these cells, and miRNAs observed to have altered expression were subsequently assessed for islet expression in Goto-Kakizaki (GK) & Wistar rats (7 weeks) and db/db & WT mice (10-12 weeks). Data were analysed by unpaired t-test, or by 1- or 2-way ANOVA with Tukey's or Bonferroni's posthoc test respectively.

Results: TargetScan predicted 7 miR-29, miR-15 and miR-124 family members (miR-29a-5p, miR-29b-1-5p, miR-29b-2-5p, miR-15a-3p, miR-124- 3p.1, miR-124- 3p.2 & miR-124- 5p) as regulators of the first 20 listed genes. Of these families, miR-29 was predicted to regulate the highest number of genes (first 14). In INS-1 832/13s, insulin stimulation index increased with pre-culture for 24 v 1h in 2.8mM G (19.5 v 4.7; p=0.0288, n=4), and decreased with pre-culture in 16.7 v 2.8mM G at 24h only (2.2 v 19.5; p=0.0047, n=4). Similarly, insulin content increased with pre-culture for 24 v 1h in 2.8mM G (122%; p<0.0001, n=4), and decreased at 24h only with pre-culture in 11.1 or 16.7mM v 2.8mM G (54 & 70% respectively; p<0.0001, n=4). qPCR assessments at 24h also revealed significant reductions in miR-29a (16.7 v 2.8mM G; p=0.0295), miR-29b (11.1 & 16.7 v 2.8mM G;

p=0.0367 & 0.0026 respectively) and miR-29c (11.1 & 16.7 v 2.8mM G; p=0.0044 & 0.0017 respectively) expression (n=4). These were mirrored by islet expression changes for miR-29a (p=0.0001), miR-29b (p=0.0405) and miR-29c (p=0.0104) in db/db v WT mice (n≥6) and for miR-29c (p=0.0002) in GK v Wistar rats (n≥11).

Conclusion: Our findings strongly imply that miR-29 is involved in regulating insulin secretion via miR-29a/b/c targeting of key genes in β-cell mitochondrial metabolism in *in vitro* & *in vivo* models of glucotoxicity/T2D. Immediate future plans are to modulate miR-29a/b/c expression *in vitro* and functionally confirm novel miRNA-gene pathways.

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Disclosure: **E. Cowan:** None.

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An integrated microfluidic sensing system platform for dissecting insulin secretion and extracellular Ca^{2+}dynamics of pancreatic islets

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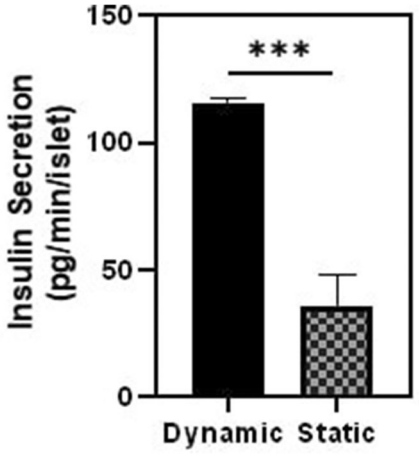
Background and aims: The development of intracellular Ca^{2+} sensors has uncovered the fundamental roles of Ca^{2+} signalling in a variety of biological events. There is a number of evidence that extracellular Ca^{2+} may be an important mediator for inter-cellular communication, and a potential determinant of pulsatile insulin secretion from pancreatic islets. Hitherto, there has been a lack of sensing tools suitable for monitoring extracellular Ca^{2+}. We have developed a microfluidic sensing system for simultaneous monitoring of dynamic extracellular Ca^{2+} and insulin secretion from pancreatic islets.

Materials and methods: The fluorometric properties of the fluorescent indicator, Rhod-5N, were evaluated for detection of extracellular Ca^{2+}. A microfluidic chip was then designed to enable the detection of Ca^{2+} using a fluorescence sensing system, and insulin using enzyme-linked immunosorbent assay (ELISA), in the perfused extracellular fluid. Finally, pancreatic islets isolated from C57BL/6 mice were loaded into the microfluidic chip, and measurements of glucose-stimulated insulin secretion (GSIS; 25mM glucose) were compared between this and a conventional static platform.

Results: Rhod-5N showed a linear and selective fluorescence enhancement in response to Ca^{2+} at concentrations of 0-10mM. In the perfused extracellular fluid, the microfluidic sensing platform had the capacity to record oscillations of Ca^{2+} and insulin with temporal resolutions of 10s and 3min, respectively. GSIS was ~3-fold higher on the microfluidic system than the conventional platform (115.2 +/- 9.18 vs. 35.5 +/- 5.63pg/min/islet, P < 0.0001).

Conclusion: We have developed a novel microfluidic sensing platform that allows simultaneous monitoring of dynamic extracellular Ca^{2+} and insulin in a biologically relevant environment. Pilot data suggest that isolated mouse islets may respond more strongly to glucose stimulation in the biomimetic perfusion environment. Further investigation into the roles of extracellular Ca^{2+} in modulating insulin secretion, based on this platform, is likely to yield an improved understanding of islet biology.



Insulin secretion from pancreatic islets in the dynamic vs. static system in response to 25 mM glucose (n=6, p<0.0001)

Supported by: NHMRC of Australia; the Hospital Research Foundation of Australia; ARC Centre of Excellence for Nanoscale BioPhotonics

Disclosure: **W. Huang:** None.

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The potential role of metal-dependent protein phosphatase, PPM1E in pancreatic B cell function

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Background and aims: Reversible phosphorylation is an important mechanism for regulating the biological activity of many intracellular proteins. Although the molecular mechanisms regulating phosphorylation of proteins involved in the insulin secretory process by the β-cells have been extensively investigated, far less is known about the role of protein dephosphorylation. This is a knowledge gap - for instance, we have found high mRNA expression of metal-dependent protein phosphatase 1E (PPM1E) in human pancreatic islets while it decreased in type 2 diabetic (T2D) patients. So far, the roles of protein phosphatases in general, and PPM1E in particular, have not been examined in β-cell function. The aim of this study is to identify the molecular signaling mechanism by which PPM1E affects β-cell function and whether it is implicated in T2D.

Materials and methods: To explore the role of PPM1E in β-cell function, its expression was silenced or increased in the insulin-producing INS1 832/13 rat cell line, using siRNA or an adenovirus overexpressing *PPM1E*. Pyruvate-, KCl-, forskolin-, and glucose-stimulated insulin secretion were assessed. Plasma membrane potential and cytosolic Ca^{2+} were measured by the dyes PMPI and Fluo4, respectively. Mitochondrial membrane potential was measured using the dye TMRM, and ATP production using the probe Perceval HR. Phosphorylation levels of calmodulin-dependent protein kinase type II (CaMKII) and AMP-activated protein kinase (AMPK), as downstream targets of PPM1E, were measured by western blot in *PPM1E*-silenced INS1 832/13 cells.

Results: Treatment with high glucose and high palmitate for 24h decreased insulin secretion (p<0.001), as well as expression of PPM1E (p<0.01), in INS1 832/13 cells. Silencing of *PPM1E* raised high glucose- and high palmitate-inhibited insulin secretion, suggesting that decreased PPM1E expression in T2D is a compensatory response of β-cells to maintain insulin secretion. Overexpression of *PPM1E* decreased insulin

secretion ($p<0.01$) while *PPM1E* knockdown increased ($p<0.001$) pyruvate-, KCl-, forskolin-, and glucose-stimulated insulin secretion. Increased insulin secretion following *PPM1E* knockdown was associated with elevated cytosolic Ca^{2+} ($p<0.001$) and ATP ($p<0.01$) levels, and also a further lowering of the mitochondrial membrane potential ($p<0.001$). *PPM1E* knockdown, however, did not affect either the plasma membrane potential or total insulin content of INS1 832/13 cells. Phosphorylation levels of both CaMKII and AMPK were higher in *PPM1E* knockdown INS1 832/13 cells, demonstrating an inhibitory effect of PPM1E on activation of these kinases.

Conclusion: PPM1E exerts an inhibitory effect on insulin secretion. Consequently, its reduced expression in T2D patients is likely a compensatory response of β-cells to increase insulin secretion. Modulation of PPM1E activity in the β-cells may thus represent a novel therapeutic strategy for the treatment of T2D.

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Mouse strain- and Ffar1-independent changes of islet transcriptome induced by overnight culture transcriptome induced by overnight culture

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Background and aims: Transcriptome analysis is a useful method for detection of new candidate genes of biological processes and disease development. Previously, it has been described that *Ffar1* deletion as well as the inactivating *Ffar1* mutation R258W abrogated palmitate-mediated stimulation of insulin secretion. Despite a reduced stimulation of insulin secretion, high fat diet-impaired glucose tolerance was ameliorated in both mouse models of *Ffar1* deficiency. This study aims to examine expression changes which are linked to *Ffar1* in order to extend the understanding of the contribution of Ffar1 to islet function. The analysis of beta cell transcriptome is challenging, as islets and beta cells are isolated from the tissue with digestive enzymes, and they are often used after culture to reduce stress-related changes caused by the isolation process.

Materials and methods: We performed RNAseq-based comparative transcriptome analysis ($0.5 >$ fold change (FC) > 2) of freshly isolated and overnight cultured (in RPMI 1640 supplemented with 10% FCS) islets of C57BL/6 $Ffar1^{(+/+)}$, C57BL/6 $Ffar1^{(-/-)}$, C3H $Ffar1^{(+/+)}$ and C3H $Ffar1^{R258W}$ mice.

Results: The analysis revealed stable and comparable mRNA levels of *Ins1*, *Sst*, *Gck*, *Kcnj11*, *Pcsk1*, *Pcsk2* in all islet preparations. Islets of C57BL/6 $Ffar1^{(+/+)}$ contained lower mRNA levels of *Ppy* compared to C3H $Ffar1^{(+/+)}$ mice. The mRNA levels of *Nr4a1* (NUR77)) and *Atp4a* were 3-fold lower in islets from C57BL/6 $Ffar1^{(-/-)}$ mice compared to $Ffar1^{(+/+)}$ mice regardless of freshly isolated or cultured. This reduction was not observed between C3H $Ffar1^{(+/+)}$ and C3H $Ffar1^{R258W}$ mouse islets. However, the R258W mutation was accompanied by an increase of *Gcg* and a reduction of *Ppy* mRNA levels of about 30-50%. In contrast to *Ffar1* deletion, overnight culture induced pronounced changes, i.e. upregulation of 199 genes and downregulation of 180 genes ($p<0.05$, unpaired t-test). The mRNA levels of*Igf1r*,*Slc3a1*(GLUT1),*Tgfb1*,*Aldoa*,*Ldha*,*Cdkn1a*(p21),*Ddit3*(Chop),*Junb* and *Nupr1* were higher, while mRNA levels of *Slc2a2* (GLUT2), *Ucn3*, *Insrr*, *Gjd2* (Cx-36) and *Tgfbr3* were lower in cultured islets compared to the respective fresh islet preparations. In addition, culture reduced mRNA levels of *Ins2* and *Gcg* in C3H islets.

Conclusion: These results suggest that overnight culture of isolated rodent islets specifically affects beta cell differentiation, while the lack of Ffar1 only leads to minor changes in the islet transcriptome.

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Therapeutically relevant concentrations of atypical antipsychotic drugs, aripiprazole and clozapine, promote beta cell mass expansion

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Background and aims: Atypical antipsychotic drugs (AAPs) are used to control various psychiatric conditions, such as schizophrenia, or to augment the treatment of major depressive disorder. The prevalence of type 2 diabetes (T2D) is increased among people suffering from mental illnesses, and there are concerns about whether antipsychotic therapy increases the risk of developing T2D. However, AAPs vary in side effects related to glucose dysregulation and the direct effects of individual AAPs at beta-cells are not well understood. Therefore, the aim of this project was to investigate the effects of two AAPs, aripiprazole and clozapine, on mouse and human beta-cell function.

Materials and methods: Beta-cell proliferation was measured by quantifying BrdU incorporation into MIN6 beta-cell DNA after 48h exposure to 1μM aripiprazole or 2μM clozapine using a BrdU ELISA, and viability and ATP generation were measured by Trypan blue exclusion tests and the CellTiter-Glo assay, respectively. Apoptosis of MIN6 cells, mouse and human islets was quantified following a 48h incubation without or with AAPs using the Caspase-Glo assay. Effects of 1h exposure to aripiprazole and clozapine on insulin secretion from mouse and human islets were investigated by static incubation experiments and radioimmunoassay.

Results: Aripiprazole and clozapine significantly increased MIN6 beta-cell proliferation (OD 450nm, control: 0.59±0.02; +1μM aripiprazole: 0.65±0.01; +2μM clozapine: 0.67±0.02, n=8, $P<0.05$) and ATP generation (by 15.2±1.4% and 14.5±2.0%, respectively), without any effect on Trypan blue dye uptake (% viability after 48h, control: 95.5±2.6; +1μM aripiprazole: 99.0±1.0; +2μM clozapine: 97.7±2.3, n=3, $P>0.2$). These AAPs had a protective effect against apoptosis induced by the saturated free fatty acid, palmitate (MIN6 cells: luminescence units, 0.5mM palmitate control: 653,970±28,403; +1μM aripiprazole: 541,061±13,281; +2μM clozapine: 555,632±16,741, n=8, $P<0.01$; mouse islets: 0.5mM palmitate control: 62,424± 7,921; +1μM aripiprazole: 40,799±4,841; +2μM clozapine: 41,627±3,857, n=8, $P<0.05$) and they also protected against apoptosis induced by the proinflammatory cytokines IL-1β, TNF-α and IFN-γ (MIN6 cells: luminescence units, cytokine control: 1,516,536±27,263; +1μM aripiprazole: 1,337,537±33,275; +2μM clozapine: 1,398,455±22,066, n=8, $P<0.05$; mouse islets: cytokine control: 154,899±13,123; +1μM aripiprazole: 90,644±10,705; +2μM clozapine: 115,269±8,220, n=8, $P<0.05$; human islets: cytokine control: 535,023 ±46,645; +1μM aripiprazole: 334,178±22,224; +2μM clozapine: 357,009±24,797, n=8, $P<0.01$). Acute exposure to aripiprazole and clozapine had no effect on insulin secretion from mouse islets (ng/islet/h, 20mM glucose: 1.79±0.26; +1μM aripiprazole: 1.96±0.36; +2μM clozapine: 2.03±0.43, n=8, $P>0.2$), but they did potentiate glucose-induced insulin secretion from human islets (ng/islet/h, 20mM glucose: 1.44 ±0.15; +1μM aripiprazole: 2.32±0.29; +2μM clozapine: 2.64±0.08, n=8, $P<0.01$).

Conclusion: Aripiprazole and clozapine have direct effects at beta-cells to induce proliferation and protect against palmitate- and cytokine-induced apoptosis, with no impairment of viability, which is of benefit to restore functional beta-cell mass. Our data support these AAPs being recommended for treating psychiatric conditions in patients predisposed to, or with, T2D.

Supported by: MRC

Disclosure: **K.W. Toczyska:** None.

SO 10 Beta cells to the grave in type 1 diabetes

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Proinflammatory cytokines have a toxic effect on human stem cell-derived beta cells

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Background and aims: A potential therapy for type 1 diabetes mellitus is the replacement of beta cells with differentiated human pluripotent stem cells (hPSC). However, it is unclear whether these cells will survive in an environment with persistent autoimmunity after implantation. An encapsulation of stem cell implants could provide protection against the cellular components of the immune system. Soluble substances could, however, overcome this encapsulation barrier. The effect of proinflammatory cytokines on hPSC-derived beta cells has not yet been adequately clarified.

Materials and methods: A human hPSC line, with mCherry knock-in in the insulin locus, was differentiated into hPSC-derived beta cells. The cells were then treated with a mix of IFN-g, TNF-a and IL-1B for 24 hours and purified. The viability was then measured using the Caspase 3/7, Caspase 8 and Caspase 9 tests and transcriptional changes were measured using a whole transcriptome microarray and RT-qPCR. An increase of reactive oxygen species (ROS) was determined by detecting oxidation of 2',7'-dichlorofluorescin-diacetate (DCF-DA). Protein expression of the cytokine receptors was determined by Western blot.

Results: Within 12 hours caspase8 and caspase 9 activities were increased by 4-fold compared to the control. Within 24 hours, the incubation with cytokines led to an almost 3-fold increase in caspase 3/7 activity compared to the control. DCF measurement revealed an increase of ROS in cytokine treated cells compared to untreated cells. The expression of the cytokine receptors IL1R1, TNFRSF1A and IFNGR1 could be detected. At the gene expression level, activation of the STAT1 and NFkB signaling pathways, induction of inflammation markers and increased expression of cytokines such as IL1-B, TNF-a and IL32 and chemokines such as CCL2, CXCL9 and CXCL10 were measured. In addition, genes of the MHC family were induced.

Conclusion: This study shows that IFN-g, TNF-a and IL-1B have a toxic effect on hPSC-derived beta cells. Apoptosis is induced by the intrinsic and extrinsic pathway via caspase 8 and 9 activation. An increase of ROS could also be detected and indicates oxidative stress of cytokine-treated cells. The OMICS analysis indicates that the induced expression of cytokines and chemokines stimulates communication with immune cells and at the same time promotes inflammation. It is possible that hPSC-derived beta cells are involved in their destruction in an environment with persistent autoimmunity.

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Disclosure: **R. Dettmer:** None.

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Identification of new therapeutic targets for the treatment of type 1 diabetes based on the survival strategies of pancreatic alpha cells

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Background and aims: Type 1 diabetes is an autoimmune disease characterized by progressive beta cell loss. During type 1 diabetes progression, both pancreatic alpha and beta cells are exposed to the same stressors, such as proinflammatory cytokines (e.g. IL-1β, IFNγ, and TNFα), but only alpha cells survive in this environment. Of note, the mechanisms underlying this alpha cell resistance are yet to be clarified. In the present study, we sought to identify proteins highly expressed in alpha cells that could protect alpha cells against proinflammatory cytokines.

Materials and methods: An unbiased bioinformatics analysis was performed using mouse or human RNA sequencing data from purified alpha and beta cells obtained from four different studies. Candidates genes were selected based on the following selection criteria: 1) mean expression (RPKM) ≥ two; 2) expression in alpha cells ≥ 2-fold the expression in beta cells; 3) the gene should be expressed in all samples; 4) the gene should be confirmed in the four selected RNA sequencing data. mRNA expression was measured in FACS-purified rat alpha and beta cells as well as in alphaTC1-9 and MIN6 cell lines by quantitative RT-PCR. Small interfering RNAs (inhibition of >50%) were used to inhibit gene expression. Cell viability was evaluated by Hoechst/Propidium iodide staining.

Results: Twenty-five candidate genes met the established selection criteria; from these 25 genes, four genes were selected based on their known functions, namely *Itpr1* (Inositol 1,4,5-trisphosphate receptor type 1), *Pdk4* (Pyruvate dehydrogenase kinase 4), *Vim* (Vimentin), and *Ttr* (Transthyretin). In FACS-purified rat cells, all four genes presented higher mRNA expression in alpha cells than in beta cells (8- to 266-fold change; n=4-7; $p<0.05$). In cell lines, *Itpr1*, *Pdk4*, and *Vim* expression was higher in alphaTC1-9 than in MIN6 cells (n=6-12; $p<0.05$), whereas *Ttr* expression was lower in alphaTC1-9 than in MIN6 cells (n=7-12; $p<0.05$). Interestingly, silencing of *Itpr1*, *Pdk4*, *Vim*, or *Ttr* exacerbated apoptosis under the basal condition in alphaTC1-9 cells (1.5- to 3-fold increase; n=3-8, $p<0.05$). Upon exposure to the cytokines IL-1β + IFNγ, a similar increase in apoptosis was still observed.

Conclusion: These findings suggest that our bioinformatics analysis is a valid approach for the identification of genes that may play important roles in alpha cell survival during the development of type 1 diabetes.

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Disclosure: **L. Marroqui:** None.

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Desensitisation of STAT mediated-signalling during chronic exposure of EndoC-βH1 cells to type I and type II interferons

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Background and aims: The onset of type 1 diabetes is thought to be driven by pro-inflammatory mechanisms operating at the level of pancreatic islets and, in particular, by the presence of interferons (IFNs; both type I and type II) present within the islet milieu. IFN signalling is mediated by the early phosphorylation of the transcription factors STAT1 & 2, but increases in total (unphosphorylated) STAT1 & 2 also occur as a later event in islet cells, both in vitro and in type 1 diabetes in vivo. The significance of the sustained up-regulation of STATs in islet cells is unclear but it may play a role in regulating their responses to chronic inflammation. We have investigated this hypothesis.

Materials and methods: Cultured EndoC-βH1 cells were exposed to IFNα and IFNγ and the extent of STAT1 & STAT2 phosphorylation (at Y701 & Y690 respectively) and the overall expression was examined by western blotting.

Results: Acute stimulation (30min) of EndoC-βH1 cells with IFNα promoted phosphorylation of both STAT1 & 2 but this response was transient and declined within 2-3h. Exposure to IFNγ also promoted early STAT1 phosphorylation but did not change the phosphorylation status of STAT2. Exposure to either IFNα or IFNγ resulted in a secondary increase in the total levels of both STAT1 & 2. The rise in STAT levels was evident within 24h and was sustained for at least 4 days following initial IFN exposure. It persisted even when the initial IFN stimulus was removed after 24h and the cells then washed and incubated in the absence of IFN. Surprisingly, the increases in STAT1 & 2 levels did not lead to heightened responsiveness to IFNs but, rather they were associated with desensitisation. Accordingly, the addition of a second bolus of IFNα, 24h after an initial stimulation, failed to induce renewed STAT1 & 2 phosphorylation despite the marked elevation in total STAT levels seen under these conditions. Intriguingly, this desensitisation occurred in a homotypic manner since the introduction of IFNγ 24h after initial exposure to IFNα was still effective in promoting STAT1 phosphorylation. The reverse situation was also true, such that IFNγ caused a selective loss of responsiveness to itself but not to IFNα. Investigation of the dose-response relationships for this desensitisation revealed that exposure to 1U/ml IFNα resulted in only limited STAT1 & 2 phosphorylation and a modest increase in total STAT levels but that the cells remained responsive to the ligand when it was re-introduced 24h later. However, as the initial concentration was raised further (to 10 or 100 U/ml) a progressively larger increase in total STAT levels occurred and the cells became less responsive upon re-addition of the ligand 24h later. Transfection of cells with a plasmid encoding STAT1 as a means to increase STAT1 levels independently of IFN stimulation did not lead to desensitisation upon subsequent addition of IFNα.

Conclusion: IFN signalling in human β-cells is associated with acute changes in STAT1 & 2 phosphorylation and this is followed by a secondary increase in the total amount of STAT1 & 2. This rise in STAT levels does not lead to a further enhancement of IFN responses but, counter-intuitively, is associated with a marked desensitisation of the response. This desensitisation is selectively homotypic and may serve as a feedback mechanism operating to minimise potentially detrimental effects of sustained IFN signalling during islet inflammation.

Supported by: Diabetes UK

Disclosure: **S. Dhayal:** None.

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Sphingosine-1 phosphate lyase overexpression prevents cytokine-mediated beta cell cellular structure damage and lipidome changes

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Background and aims: The enzyme S1P lyase (SPL), which catalyzes the irreversible degradation of a bioactive sphingolipid sphingosine-1 phosphate (S1P), has been shown to be implicated in the development of many inflammatory disorders. Our former studies indicate that an impaired expression of SPL may be involved in the aggravation of cytokine toxicity in pancreatic beta-cells and that cytokines significantly influence beta-cell lipidome. Proinflammatory cytokines play a crucial role in the autoimmune-mediated specific beta-cell death during type 1 diabetes (T1DM) development. The triggers of the autoimmunity and the involvement of the beta-cell in this process has not yet been fully clarified. Therefore the aim of this study was to analyse the role of SPL in cytokine-mediated ultrastructural and lipid composition changes in beta-cells.

Materials and methods: Insulin-secreting INS1E cells were incubated with 600 U/ml IL-1β or a cytokine mixture (60 U/ml IL-1β, 175 U/ml TNFα, 10 U/ml IFNγ) for 24 h. Cell viability (MTT assay) was assessed in comparison to cells additionally overexpressing sphingosine kinase (SK2, an enzyme generating S1P in mitochondria, ER and nucleus) or S1P phosphatase (SPP, an enzyme de-phosphorylating S1P to shingosine). Cellular response to cytokines was studied by ER and mitochondrial stress marker expression (qRT-PCR), ultrastructure analyses by electron microscopy and lipidomics (a methanol-based lipid extraction followed by LC-MS/MS).

Results: Overexpression of SPL resulted in a significant protection against cytokine-mediated cell viability loss (cytokine mix: INS1E-control 43%, INS1E-SPL 70%, p<0.01). The protective effect of SPL overexpression was counteracted by a parallel overexpression of SK2. A double overexpression of SPL and SPP1 protected against cytokine toxicity to a smaller extend than SPL overexpression alone. The ultrastructure analysis of cells incubated with cytokines revealed significant differences between control cells and cells overexpressing SPL. INS1E-control cells exposed to cytokines were characterized by swollen, dysfunctional mitochondria and impaired ER structure. INS1E-SPL cells treated with cytokines retained more secretory granules, had a large amount of intact mitochondria and a well preserved ER network. These observations went along with diminished cytokine-mediated ER stress response in INS1E-SPL cells as compared to INS1E-control cells. Overexpression of SPL significantly changed the lipid composition of INS1E cells, directing it into the antinflammatory profile. The typical cytokine-mediated rearrangements in the proapoptoticc ceramide species content observed in control INS1E cells were conteracted by SPL overexpression.

Conclusion: Proinflammatory cytokine foster an imbalance in S1P generation and degradation, which significantly contributes to beta-cell damage. SPL overexpression protects against cytokine toxicity via prevention of ER and mitochondrial stress responses as well as protection of cell ultrastructure. SPL overexpression redirects lipid composition of beta-cells into more anti-inflammatory profile.

Disclosure: **E. Gurgul Convey:** None.

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PTPN2 regulates the interferon signalling in pancreatic beta cells and its expression correlates with therapy outcome in autoimmune diabetes

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Background and aims: Type 1 diabetes (T1D) results from autoimmune destruction of β-cells in the pancreas. Protein tyrosine phosphatases (PTPs) are candidate genes for T1D and play a key role in autoimmune disease development and β-cell function. The therapeutic potential of PTP expression and function in T1D, however, remains unclear.

Materials and methods: Diabetic NOD female mice were treated with the combination of anti-CD3 mAB (2.5 μg/day for 5 consecutive days, d0-4 iv) and IL-1RA (10 mg/day for 5 consecutive days, d0-4 ip). PTPs and global protein profiles were determined by mass-spectrometry in the pancreas and confirmed by immunofluorescence. PTPN2 was silenced by transfection of siRNAs in the human EndoC-βH1 cell line and cultured with or without the pro-inflammatory cytokines; human interferon (IFN)-γ (1000U/ml) or human IFN-α (2000U/mL). The global gene expression profile modulated by PTPN2 was determined by RNA-seq at 24h after cytokine exposure. The third exon of the *PTPN2* gene was deleted with CRISPR/Cpf1 in H1 human embryonic stem cells (hESCs) and differentiated into insulin-producing β-like cells.

Results: The anti-CD3 and IL-1RA combination reversed hyperglycemia in diabetic NOD mice. The global protein and individual PTP profiles were assessed in the pancreas of NOD mice treated with anti-CD3 and IL-1RA or anti-CD3 alone used as controls. PTPN2, a T1D candidate gene, was increased and PTPN13 was decreased in the pancreas of anti-CD3 and IL-1RA treated mice ($p < 0.05$, n=3-5). Expression of PTPN2 correlated with insulin-positive β-cells in pancreatic islets from cured mice (r^2= 0.9068, $p < 0.001$). Differential gene expression was determined by RNA-seq analysis in human EndoC-βH1 cells transfected with PTPN2 or control siRNAs (>70% knockdown, p<0.001) and treated with IFN-γ or IFN-α. 1.6-fold and 4.2-fold genes were modulated by PTPN2 deficiency after IFN-γ or IFN-α exposure, respectively (FDR<0.05). Gene ontology analysis demonstrated that T1D-related pathways are regulated by PTPN2 in IFN-treated β-cells (p<0.05). We confirmed our results in hESC-differentiated β-like cells. *PTPN2* deletion by CRISPR/Cpf1 prolonged the activation of STAT1 and downstream signalling after IFN-γ or IFN-α exposure in differentiated H1 β-like cells (n=3, p<0.01). Importantly, we found association by rank-rank hypergeometric overlap analysis between cytokine-treated PTPN2 knockdown EndoC-βH1 cells and the RNA-seq dataset of human β-cells of T1D diabetic patients.

Conclusion: PTPN2 affects gene networks and pathways related to the autoimmune response in β-cells. Our findings demonstrate that reduced PTPN2 activity can play a key role in β-cell dysfunction and support the active role of β-cells in their own demise in T1D.

Supported by: F.R.S-FNRS PhD Aspirant scholarship, European Research Council (ERC) Consolidator grant, JDRF Career Development Award

Disclosure: **V. Vandenbempt:** None.

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The role of NIK in beta cell-mediated type 1 diabetes

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Background and aims: In type 1diabetes (T1D) β-cell destruction results from an aberrant inflammatory crosstalk between β-cells and immune cells partly mediated via activation of the transcription factor NF-κB. NF-κB signaling occurs through two major pathways, the canonical, which was shown to contribute to β-cell death in T1D, and the alternative, which has not been extensively studied in T1D. The alternative pathway is characterized by the stabilization of NF-κB-inducing kinase (NIK) triggering p100 processing into p52, which dimerizes with RelB to regulate gene transcription. Specific ligands activating the alternative pathway are present in the serum of T1D patients and contribute to autoimmunity in non-obese diabetic mice. *In vitro,* pro-apoptotic cytokine treatment, promotes NIK stabilization and activation of downstream NF-κB signaling in β-cells. A recent study revealed that β cell-specific overexpression of NIK results in spontaneous diabetes in mice due to β cell death and insulitis, however physiological NIK expression is extremely low and

thus overexpression of NIK is not an ideal study model. The aim of our study was to evaluate the role of NIK in β-cell demise during T1D in models where physiologic levels of this protein were expressed.

Materials and methods: A β-cell specific NIK KO mouse ($NIK\beta^{KO}$) was generated to verify the *in vivo* role of NIK in β-cells in physiological condition and in T1D induced by multiple low-dose of streptozotocin (MLDSTZ). Weekly glycemia and bodyweight were monitored. IpGTT, β-cell/islet mass and pancreas insulin content were performed. Moreover, immune profiles of T cell and myeloid cell populations were evaluated in blood, spleen and pancreatic draining lymph nodes (pLN). *In vitro*, β-cell death, GSIS and gene expression were analyzed in mouse islets, EndoC-βH1 cells and/or human β-cells exposed to proinflammatory cytokines (IL-1β+IFN-γ) and/or to ligands of the alternative NF-κB pathway (LIGHT and LTβR agonist).

Results: In physiological conditions lack of NIK did not affect β-cell development or function. Moreover, after MLDSTZ treatment, metabolic parameters including glycemia, ipGTT, and recruitment of Foxp3+Treg, CD8+IFN-γ+ and CD4+IFN-γ+ T cells in blood, pLN and spleen were indistinguishable between $NIK\beta^{KO}$ and WT mice. β-cell mass and islet density were also not different between $NIK\beta^{KO}$ and WT mice. These results suggest that lack of NIK does not affect insulitis or β-cell demise in our model. Furthermore, cytokines and specific ligands of the alternative NF-κB pathway did not affect β-cell death and insulin secretory function (GSIS) in mouse islets or human β-cells. NIK mediated NF-κB induction in human β-cells did not regulate downstream proinflammatory gene transcription such as Fas, Cxcl1 and Ccl2.

Conclusion: Overall, our data suggests that ablation of NIK has no major effects in β-cells both *in vitro* and *in vivo*. Therefore, we postulate that NIK and the alternative NF-κB pathway do not play a significant role in β-cell insulitis and diabetes development.

Supported by: Excellence of Science Grant (FNRS, Belgium, convention 30826052)

Disclosure: **P. Xiao:** None.

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The parasite-derived peptide, FhHDM-1, promotes beta cell function and survival via PI3K/Akt signalling to prevent type 1 diabetes

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Background and aims: Our team has previously identified a novel parasite-derived molecule, termed *Fasciola hepatica* helminth defence molecule 1 (FhHDM-1) that prevents onset of type 1 diabetes (T1D) in non-obese diabetic (NOD) mice. While disease prevention was associated with modulation of macrophage function, recent proteomic analyses of pancreatic tissue indicate that FhHDM-1 also exerts significant effects on the pancreatic β-cells, by activation of PI3K/Akt signalling. As this pathway is associated with β-cell metabolism, survival and proliferation, we investigated the putative effects of FhHDM-1 on β-cells via *in vitro* and *in vivo* studies.

Materials and methods: To evaluate β-cell mass, pancreata from 10 wk. old NOD mice treated with six intraperitoneal (i.p.) injections of FhHDM-1 or vehicle control delivered on alternate days were isolated. Pancreata were then sectioned and immunofluorescently stained for quantification of insulin to act as a measure of functional β-cell mass. To study biodistribution, NOD mice were administered one i.p. injection of FhHDM-1 conjugated to a fluorescent dye, or an equivalent volume of vehicle control. The fluorescent signal was then captured using an IVIS Spectrum imaging system. For *in vitro* studies, the NOD mouse derived NIT-1 β-cell line was used. Western blot was used to investigate activation of PI3K/Akt and various cellular assays were employed to assess β-cell proliferation, survival and apoptosis in FhHDM-1 or vehicle control treated NIT-1 cells.

Results: Consistent with the proteomic analyses, FhHDM-1 preserved β-cell mass in NOD mice ($p=0.0034$). Further biodistribution studies of FhHDM-1 following i.p. administration showed that the peptide localised to the pancreas, suggesting it has a direct effect on β-cell survival. These findings were confirmed *in vitro*, as treatment of NIT-1 β-cells with FhHDM-1 increased phosphorylation of Akt. Consequently, β-cell survival was enhanced ($p=0.0004$), independent of proliferation. Moreover, apoptosis induced by pro-inflammatory cytokines, akin to conditions during T1D pathogenesis, was inhibited ($p=0.0013$).

Conclusion: The positive effect of FhHDM-1 on β-cells has significant therapeutic applications for (i) prevention of T1D in predisposed individuals, (ii) preservation of residual β-cell mass in recent onset T1D, (iii) amelioration of β-cell exhaustion in type 2 diabetes, and (iv) enhancement of β-cell viability during pancreatic islet transplantation.

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Disclosure: **I. Camaya:** None.

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Beta cell loss in treatment-naive patients with type 2 diabetes based on disease duration and HbA_{1c} levels: results from 15 clinical trials

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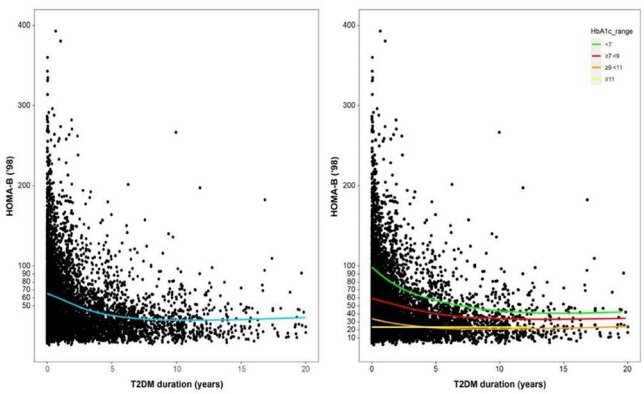
Background and aims: To study the rate of β-cell loss in a pool of 7409 treatment-naïve patients with type 2 diabetes mellitus (T2DM) and explore the impact of metabolic control.

Materials and methods: Data from the baseline visit of treatment-naïve patients were extracted from 15 studies with duration ranging from 3-60 months (mean [SD] duration: 6.4 [15.3] months) with different inclusion criteria for glycated haemoglobin (HbA1c) levels. The homeostatic model (revised version '98) was used to calculate the β-cell activity (HOMA-B) and insulin sensitivity (HOMA-S) over a period of 20 years. Lowess smoothing algorithm was used for line fitting. This analysis was stratified by baseline HbA1c. The "segmented" R package was used to assess the optimal breakpoints and slope coefficients.

Results: Mean age of the pooled population was 54.9 years. The average HbA1c was 7.9% and the mean duration of diabetes (as referred by the patients) was 2.2 years (median 0.75 years). The cross-sectional analysis of HOMA-B and referred duration of the disease revealed a curve with a hyperbolic shape with the intercept at 66% and a slope of −7.2% per year until a breakpoint at 3.8 years and thereafter the slope was −0.8% per year (Figure). Therefore, on this heterogeneous pool of treatment-naïve patients, the β-cell activity at T2DM onset was 66% with β-cell loss of 27% in the first 3.8 years. The overall β-cell loss was 12.3% during the remaining 16 years. In patients with HbA1c <7.0% (n=1886), HOMA-B showed an intercept of 98% and a sustained β-cell activity up to 59% until 3.3 years, thereafter the loss was 1.7% per year.

Conclusion: On an average, the rate of β-cell loss is twice as fast in the first 3.8 years than in the later years. The activity of the β-cell is blunted (<50%) when the diagnosis is delayed and levels of HbA1c are >7%. To leverage the benefit of the endogenous insulin secretion and activity, it is important to detect and treat T2DM intensively when HbA1c is still <7%. These estimates are derived from a cross-sectional analysis and limitations are acknowledged.

Figure: HOMA-B in patients with various T2DM durations



HOMA-B, homeostatic model assessment of β-cell function; T2DM, type 2 diabetes mellitus.

Disclosure: **M. Blüher:** None.

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Beta cell activity modulates treatment response in treatment-naive patients: exploratory analysis from the VERIFY study

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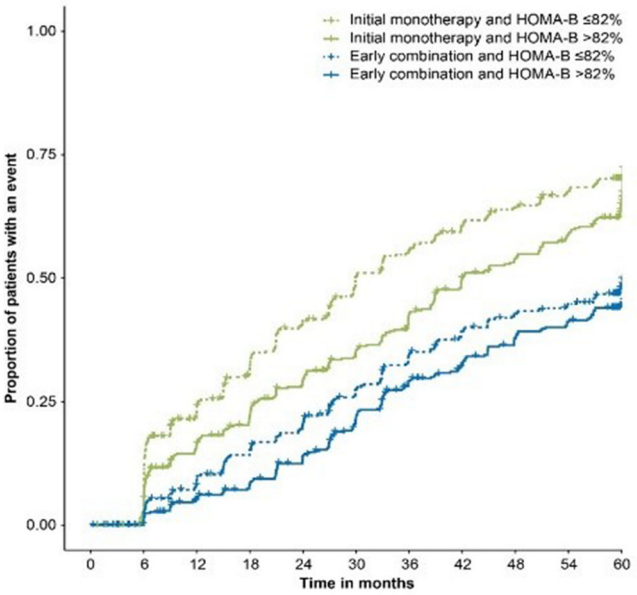
Background and aims: Attempts to preserve insulin secretion in type 2 diabetes (T2DM) are more easily undertaken early in the disease process when β-cell failure may be reversible. We explored the interaction of insulin secretory capacity as assessed by homeostasis index (HOMA-B) with treatment in the context of a long-term clinical study in newly diagnosed individuals by comparing early combination (EC) versus stepwise treatment strategies.

Materials and methods: VERIFY was a multinational, double-blind, placebo-controlled, 5-year parallel-group study, which included treatment-naïve patients with T2DM, randomised (1:1) to receive EC (vildagliptin+metformin) or sequentially intensified initial metformin monotherapy (MET). Adults (aged 18-70 years) with ≤2 years of disease duration, screening HbA1c 6.5-7.5% and body mass index 22-40 kg/m^2 were included. We defined initial treatment failure as HbA1c ≥7.0%, twice, consecutively, 13 weeks apart. The homeostatic model (revised version '98) was used to calculate β-cell activity (HOMA-B). A Cox regression model was used to estimate risk of treatment failure according to baseline HOMA-B and HbA1c values.

Results: Of 1972 patients with initial treatment failure, 1641 had fasting glucose and insulin values available at baseline for HOMA-B calculation. The median baseline HOMA-B was 82%. When MET+HOMA-B ≤82% was set as reference (1.00), the hazard ratios (HR) (95% confidence interval [CI]) per treatment strategy and HOMA-B were: MET+HOMA-B >82%: HR 0.84 (0.67-1.02; p=0.07); EC+HOMA-B ≤82%: HR 0.49 (0.30-0.68; p<0.0001); and EC+HOMA-B >82%: HR 0.40 (0.20-0.61; p<0.0001) (Figure). Baseline HbA1c was strongly associated with the risk of treatment failure (HR 3.28 [3.13-3.42; p<0.0001]). The median time to failure was 911 days with MET and HOMA-B ≤82% and 1884 days with EC and HOMA-B >82%. The sensitivity analysis with a HOMA-B cut-off value of 50% did not substantially change the overall trends or the risk of initial failure.

Conclusion: In the VERIFY trial, the early treatment strategy was the dominant option while the time to treatment failure was prolonged in both groups for patients with higher initial levels of HOMA-B. Thus, HOMA-B should be considered as a candidate biomarker for identification of subjects at greater risk of treatment failure.

Figure: Risk of initial treatment failure with early combination versus initial monotherapy in patients with β-cell activity ≤82% or >82%



HOMA-B, homeostatic model assessment of β-cell activity.

Clinical Trial Registration Number: NCT01528254

Disclosure: **D.R. Matthews:** None.

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Human beta cell dedifferentiation might be induced by noradrenergic stimulus

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Background and aims: β cell dedifferentiation is recognized as one of the mechanisms responsible for the disappearance of β cells from pancreatic islets of diabetic subjects, but the underlying process is still unclear. Noradrenergic innervation is known to inhibit insulin secretion, and in pancreas of diabetic subjects a 3-fold increase of noradrenergic fibers was observed compared to non-diabetic subjects, directly related to the % of dedifferentiated cells within the islets. The increase in fibers has been associated with worsening β cell function, suggesting a role in the pathogenesis of the disease and perhaps in the process of dedifferentiation. The aim of our study was to use *in vitro* human β cell models to explore a direct role of innervation in the process of noradrenergic dedifferentiation.

Materials and methods: The human β cell line EndoC-βH1 was exposed to noradrenaline (NA, at concentrations of 0.4 μM, 4 μM and 40 μM) for 24 and 72 h. The expression of β cell dedifferentiation markers was assessed before and after stimulation of theα2A adrenergic receptor by morphology (immunofluorescence), qPCR and ELISA. FGF2 (100 ng/mL), a knowninducer of β dedifferentiation *in vitro*, was used as a positive control.

Results: NA treatment did not have any impact on β cell viability, while, as expected, it slightly reduced insulin secretionafter 24 h. The expression of the β cell dedifferentiation marker (ALDH1a3) was increased by approximately 30%, while the mature β cell marker NKX6.1was reduced up to 60% after 72 h (by immunofluorescence). Moreover, mRNA expression of FOXO1, the main transcription factor involved in the dedifferentiation process, was reducedup to 35%. We observed a dose and time dependent pattern in all experiments.

Conclusion: Our data suggest a direct role of noradrenergic fibers in the β cell dedifferentiation process, potentially identifying a new target for the prevention and therapy of type 2 diabetes.

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Disclosure: **F. Cinti:** None.

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In vivo and in vitro effect of vertical sleeve gastrectomy upon glucose-insulin homeostasis and beta cell function and survival

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Background and aims: Recent studies demonstrate that vertical sleeve gastrectomy (VSG) directly leads to gene changes in the pancreatic islet and improves insulin secretion in obese mice models. However, the mediators of these effects are still unknown. Here we used VSG obese mice, aiming to explore the involvement of humoral factors released in the bloodstream after the surgical procedure in beta-cell function and survival.

Materials and methods: 30 days old C57BL/6 mice were fed on high-fat diet during 10 weeks. After the development of an obese phenotype, the mice were randomly assigned into two groups: Sham-surgical (Sham) and VSG-surgical (VSG) (n=8). Four weeks after the surgery, we evaluated body composition by analyzing final body weight and perigonadal fat content. We also evaluated glucose-insulin homeostasis through glucose and insulin tolerance tests, fasting glycemia and insulinemia, and insulin secretion. Furthermore, we used a rat pancreatic beta-cell line (INS-1E) incubated with normal culture medium (Control) or medium containing 10% serum from Sham or VSG, for 24h. After the culture treatment period, we analyzed beta-cell function through insulin secretion, expression of genes related to beta-cell function and endoplasmic reticulum (ER) stress, as well as apoptosis measurement (n=4-6). To analyze the data, we used Student´s t-test or One-Way ANOVA with an unpaired Tukey's post-hoc test. Data are mean ± SEM, and the difference between the groups were considered statistically significant if $P \leq 0.05$.

Results: After the surgery, VSG mice displayed reduced body weight (Sham:43.7±0.8;VSG:37.7±0.8,g) and perigonadal fat pad (Sham:4.8 ±0.2;VSG:2.1±0.3,% body weight), which probably contributed to lower fasting glycemia (Sham:129.1±4.1;VSG:101.8±4.9,mg/dL) and insulinemia (Sham:2.2±0.3;VSG:0.9±0.1,ng/mL), improve insulin sensitivity (Sham:1568±81.9;VSG:1015±42.7,AUC) and glucose tolerance (Sham:38154±1603;VSG:27536±1907,AUC), in addition to lower insulin secretion (Sham:1.5±0.10;VSG:0.6±0.03,ng/islet.h) in these mice compared to Sham mice. We hypothesized that the alterations observed in VSG mice were mediated by humoral factors released in the bloodstream after the surgical procedure. Indeed, INS-1E cells incubated with serum from Sham mice secrete more insulin (Control:118.3 ±12.1;Sham:263.6±27.0,ng/μg protein), present increased expression of INS1, INS2, PDX1, BIP, XBP1, ATF4, CHOP and ATF6 (Sham:2.1 ±0.1,2.4±0.4,1.8±0.2,1.6±0.1,1.6±0.2,1.6±0.2,1.6±0.1,1.5±0.1, respectively, fold change of Control), along with increased apoptosis rate, compared with Control cells (Control:1.1±0.04;Sham:5.2±0.40,%Dead cells). Interestingly, in cells exposed to VSG serum, insulin secretion was similar to Control cells (Control:118.3±12.1;VSG:138.5±18.5,ng/μg protein). Moreover, VSG serum-treated cells displayed similar outcomes than Control cells when we analyzed both expression of genes related to beta-cell function and ER stress markers (VSG:1.1±0.1,1.3

±0.1,1.1±0.1,1.0±0.1,1.0±0.1,0.9±0.1,1.0±0.1,1.0±0.1, respectively, fold change of Control), as well as apoptosis rate (Control:1.1±0.04;VSG:1.8 ±0.08,%Dead cells).

Conclusion: Taken together, these data suggest that VSG is able to modulate beta-cell function and survival through a mechanism mediated by humoral factors released in the bloodstream after the surgical procedure.

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Disclosure: **G.M. Soares:** None.

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Extracorporeal islet-based continuous glucose monitoring sensor in rodents

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Background and aims: Continuous glucose monitoring has considerably improved insulin delivery. However, currently used electrochemical sensors react to glucose only. The ideal sensor, ie islets, captures various nutrients and hormonal regulators, reacts in a biphasic manner and is differentially modulated by non-beta islet cells. We have previously demonstrated in-vitro that islet electrical activity, measured extracellularly as slow potentials (SP) by micro-electrode arrays (MEA), faithfully reflects biphasic activation and hormone secretion. As a subsequent step towards a bio-inspired artificial pancreas we have now set out to test this approach as open loop in rodent in-vivo.

Materials and methods: Mouse (C57BL/6J) islets were obtained by standard methods and cultured on microelectrode arrays (MCS, Germany; ca. 10 islets/device) prior to use in a home-made microfluidic PDMS device with either a channel (diameter 800 µm, online experiments) or a small well (15 µl, off-line experiments). Recordings were analysed by MC_Rack (MCS, Germany). Male Wistar rats (8-12 weeks old) were implanted with a linear interscapular subcutaneous catheter (30 mm membrane, 20 kDa cut off; Microdialysis AB) under anaesthesia (1.5% isoflurane, 1mg/kg meloxicam). For dialysis, Ringer dextran-60 was used (pump 107, Microdialysis AB). Intraperitoneal glucose tolerance tests (iPGTT; 2 g/kg) and blood glucose determinations (at 0, 15, 30, 60, 90, and 120min) were performed according to standard procedures. Human blood samples (Sigma) were equilibrated for given glucose values. Statistics were run using PRISM.

Results: We first tested human sera adapted to defined glucose (G) values (G6, G9, G12, G15) and could distinguish each step in terms of SP frequencies (Dunn's, $p < 0.001$; n=5). Testing a wide range of dialysis rates, 1µl/min was found optimal in terms of recovery (85%) and delay within the device. Next we used rat sera and dialysate obtained during iPGTT. SP frequencies were clearly different for initial serum (t=0) with basal 9.4 mM G and subsequent samples (22.9, 11.9 and 8.6 mM G at t=15-60 min; Tukey, p at least <0.05; n=33 electrodes). The corresponding dialysates also were significantly different among them in SP frequencies and amplitudes (mean values +SEM: G18, 0.59+0.11 Hz, 121.7+ 15.3 µV; G16 0.38+0.06 Hz, 22.1+15.4 µV; G 13 0.19+0.03 Hz, 9.2+ 5.5 µV; n=5; Tukey, $p<0.001$). Note that serum and dialysate glucose levels differed due to known diffusion delay of glucose into the interstitial fluid. Experiments are underway to directly link microdialysis to the microfluidic MEA and record continuously during iPGTT and subcutaneously applied insulin. In the longer term, experiments in streptozotocine-treated rats are planned. Finally, open loop recordings in diabetic patients are planned and corresponding authorisations have been granted (ID-RCB 2019-A01837-50; CPP 19.07.18.69425).

Conclusion: Our current data suggest the feasibility of extracorporeal, islet-based continuous glucose monitoring via microdialysis coupled to microfluidic MEAs in rodent.

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Disclosure: **E. Puginier:** None.

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Islet microcapsules with core-shell structure from Microfluidic electrospray for diabetes treatment

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Background and aims: Islet transplantation is a promising method for the treatment of diabetes mellitus, but this method has some limitations, including islet damage and immune rejection during islet transplantation. This study aims to explore a novel method of islet encapsulation to reduce the damage to islets in the process of islet transplantation, improve the survival rate of islets and provide immune isolation.

Materials and methods: Islets were obtained from 6 to 8 weeks of Sprague Dawley rat by density gradient centrifugation, the core-shell structure microcapsules were generated from coaxial electrostatic spray. The outer phase was alginate sodium and internal were sodium carboxymethyl cellulose and cells, collecting liquid was calcium chloride solution. The sodium alginate solution crosslinking with calcium ion to form solid hydrogel shell, cells was wrapped inside the capsule. The collected microcapsules were cultured in an incubator and some microspheres were used to staining for calcein and propidium iodide at the 1st, 3rd, 5th and 7th day. The function of encapsulated and unencapsulated islets (GSIS) at day 7 was measured by ELISA. Using a single large dose (150 mg/kg) streptozotocin (STZ) injected into the abdominal cavity of C57BL/6 mice to establish the type 1 diabetes model. Divided mice into two groups at random, each group has six, one group received islet cells transplantation, another group transplanted encapsulated islet cells, then observing blood glucose of mice.

Results: The core-shell islet cells microcapsules were successfully prepared by microfluidic electrospray method. At the first, third, fifth, and seventh days of culture, the survival rates were more than 90%, suggested that the islet cells encapsulated in core-shell structure microcapsules had a high survival rate in vitro culture. Both unencapsulated islets and encapsulated islets had insulin secretion functions, and there was no statistical difference between the two groups. After transplantation, the two groups of mice lowed blood glucose in the first 3 days, and then the blood glucose of the unencapsulated group increased, while the blood glucose of the encapsulated group decreased steadily. There were significant differences between the two groups and indicated that the core-shell islet microcapsules had an excellent effect in lowering blood glucose of diabetic mice.

Conclusion: Microcapsules with hydrogel shell and liquid core prepared by coaxial electrostatic spray method can improve cells survival rate and provide immune isolation, showing a protective effect on islets, and significantly reduced blood glucose in STZ-induced type 1 diabetic mice after transplantation. The hydrogel also has great biocompatibility and was harmless to humans. The above results showed that the microcapsules with islet cells have the prospect of clinical application in the treatment of diabetes.

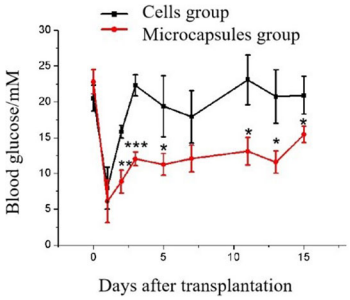

Figure 1. Blood glucose changes in two groups, * P<0.05,** P<0.01,*** P<0.001

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Disclosure: **X. Liu:** None.

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Weight gain following pancreas transplantation in type 1 diabetes is associated with a worse glycaemic profile: a retrospective cohort study

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Background and aims: Information regarding weight changes after pancreas transplantation (PT) in type 1 diabetes (T1D) is scarce. We assessed the weight trajectories post-PT and their relationships with pancreas graft outcomes.

Materials and methods: In this retrospective cohort study, T1D individuals who underwent PT were recruited (T1D-PT; n=194) and divided into three groups according to transplantation date: 1999-2004 (n=57), 2005-2009 (n=79), 2010-2015 (n=58). For weight comparisons, a random sample of T1D without end-stage kidney disease was also recruited during 2015 (n=61; T1D-control). Univariate and multivariate relationships between weight trajectories and graft function were studied.

Results: The median follow-up for the T1D-PT group was 11.1 (6.7-15.3) years. Despite significant weight loss at 6 months (65.7±12.4 vs. 64.1±11.4 Kg; p<0.001), a stepped increase was seen thereafter (weight at 60 months: 68.0±14.0 Kg; p<0.001). Participants from the last period (2010-2015) showed higher weight gain (p<0.001), outweighing that observed in the T1D-control group (at 60 months: 4.69±8.49 vs.- 0.97 ±4.59 Kg; p=0.003). Weight gain between 6-36 months was independently associated with fasting glucose and HbA1c at 36 months and also with HbA1c at 60 and decline in C-peptide between 36-60 months (p<0.05). However, in Cox-regression models adjusted for age, sex, and other recipient and PT intrinsic-related variables, the third tertile of weight gain between 6-36 months showed a nonsignificant increase in the graft failure/dysfunction (HR 2.33 [0.75-7.27]).

Conclusion: In the T1D population, weight gain is common post-PT, especially in the last study period. This finding was associated with biochemical markers of graft dysfunction, which needs confirmation in further studies.

Disclosure: **A. Amor:** None.

SO 12 Sugar Moms

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Diagnosing gestational diabetes: COVID-19 criteria vs oral glucose tolerance test

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Background and aims: During the COVID-19 pandemic, the Royal College of Obstetricians and Gynaecologists (RCOG) published modifications to diagnostic criteria for gestational diabetes (GDM). To balance the risk to patients, the oral glucose tolerance test (OGTT) was superseded by fasting plasma glucose and HbA1c. Variation in glycaemia within the gestational diabetes range is a poor predictor of materno-fetal outcomes. This study compared the National Institute for Health and Care Excellence (NICE) 2015 diagnostic criteria to the modified thresholds, aiming to determine differences in GDM incidence and materno-fetal outcomes.

Materials and methods: We retrospectively reviewed 17294 women at risk of developing GDM who attended our inner city hospital sites (2016-2019). Women were categorised in one of three groups: Group 1: Normal Glucose tolerance, Group 2: GDM diagnosed according to NICE 2015 criteria and Group 3: GDM diagnosed according to COVID-19 RCOG criteria. Materno-fetal outcomes were compared across the three groups. A sub-set of women who had had their HbA1c measured was examined to determine GDM incidence according to revised RCOG criteria (n=1439).

Results: Of the 17294 women, 87.9% (n=15200) had normal glucose status (Group 1). 10.8% (n=1882) fulfilled NICE GDM diagnostic criteria (Group 2) and 4% fulfilled RCOG diagnostic criteria by FPG alone (Group 3, n=710). Of those diagnosed with GDM by NICE criteria, 89.6% (n=1687) were diagnosed by 120minute glucose. Mean (SD) age and mean (SD) early pregnancy body mass index varied significantly across the three groups: 32.4 (±5.6), 34.0 (±5.3) and 33.3 (±5.8) years respectively p<0.001, 25.6 (±5.2)kg/m2, 27.1 (±5.6)kg/m2 and 30.1 (±6.5) kg/m2 p<0.001. Differences in the proportion of women of non-white ethnicity were non-significant: 62.8%, 65.7% and 67.3% (p=0.05). Mean (SD) fetal birthweight varied significantly across the three groups: 3323 (± 503), 3156 (±488) and 3332 (±558) p<0.001. Differences in the proportions of incidence of shoulder dystocia were non-significant: 1.57%, 1.05% and 0.86% respectively, p=0.5. The proportion of neonates requiring input on the special care baby unit varied significantly: 3.78%, 6.96% and 6.03% p=0.001. Mean (SD) Hba1c measured 35.1 (±5.3) mmol/mol. 20.5% (n=295) exceeded the diagnostic threshold for GDM by COVID19-RCOG criteria. 15.2% women with GDM diagnosed by NICE criteria exceeded an HbA1c of 39mmol/mol: 56.2% of women diagnosed by COVID-19 RCOG FPG criteria exceeded the same threshold. Overall, in applying the modified RCOG criteria to this multi-ethnic cohort, 84.8% cases of GDM would have been missed. 5 cases of shoulder dystocia occurred in this cohort compared with 3 in the treated-GDM group (p=0.04). Differences in SCIBU admissions were non significant (n=26 in untreated group versus n=5 in untreated, p=0.6).

Conclusion: This analysis of a multi-ethnic inner-city cohort demonstrates that a higher proportion of women were diagnosed with gestational diabetes by NICE 2015 criteria compared to the number that would have been diagnosed by modified COVID19-RCOG criteria alone. Although neonatal outcomes were similar, it raises concerns of mothers having lost out on appropriate, specialised antenatal care as a result of potential missed diagnoses during the pandemic.

Disclosure: **D. Hirani:** None.

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Diabetes management delivery and pregnancy outcomes in women with gestational diabetes during the first wave of the 2020 COVID-19 pandemic

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Background and aims: The COVID-19 pandemic has forced a rapid adaptation of healthcare services to secure care for many patients' groups. This includes women with Gestational diabetes mellitus (GDM). We evaluated the impacts of the first COVID-19 wave on parameters such as the GDM treatment, glycemic control and pregnancy outcomes.

Materials and methods: In this retrospective study from a reference diabetes centre, we compared patient data from two different time periods: the first wave of COVID-19 pandemic (March 2020 - June 2020) and the preceding five months (October 2019 - February 2020). Data was collected from the medical records and telephone surveys. No patient with concomitant COVID-19 was included.

Results: We included 155 consecutive women - (Group 1 N=73 and Group 2 N= 82 from the COVID-19 pandemic period and non-COVID-19 period, respectively). During the COVID-19 pandemic, almost half of all GDM women (N1=36, 49.3%) used telemedicine as a method of contacting their diabetic specialist while this tool was not utilized in the earlier period. Moreover, these patients reported difficulties in performing blood glucose self-control more often (N1=20, 27.4% vs N2=7, 8.5% $p \leq 0.01$) and spent less time on diabetes education and training than the control group on average (N1=39, 53.4% vs N2=9, 9.8% below 2 hours of training; $p \leq 0.01$). Most analysed glycemic parameters and pregnancy outcomes were similar. Differences were found with respect to the incidence of prolonged labour (N1=12, 16.4% vs N2=3, 3.7% $p \leq 0.01$) and episodes of pre-eclampsia (N1=0 vs N2=7, 8.5% p=0.01).

Conclusion: In this single centre observation, the first wave of the COVID-19 pandemic did not seem to have a negative impact on pregnancy outcomes in GDM women, in spite of difficulties in diabetes management delivery.

Disclosure: **M. Wilk:** None.

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Comparison of clinical and metabolic characteristics, and pregnancy outcomes in women with gestational diabetes depending on the time of diagnosis

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Background and aims: Gestational Diabetes Mellitus (GDM) is associated with higher incidence of adverse pregnancy outcomes. Nowadays GDM is being diagnosed more frequently in the first half of pregnancy. However, until now the causes of this phenomenon have not jet been established. The aim of the study was to compare the clinical and metabolic characteristics, as well as pregnancy outcomes in women with GDM depending on the time of diagnosis.

Materials and methods: A retrospective cohort study was conducted on 393 pregnant women with GDM, treated between 2014 and 2020. In 85 patients GDM was diagnosed before the end of 20th week of pregnancy (group A), and in 308 GDM was diagnosed after 20th gestational week (group B). The clinical and metabolic characteristics of the subjects, as well as pregnancy outcomes were analyzed.

Results: The women in both groups did non differ in terms of the age, mean pre-pregnancy BMI, frequency of each BMI category (≤ 25 kg/m2, 25-30 kg/m2, > 30 kg/m2), venous blood glucose concentrations in 75gOGTT or frequency of insulin therapy (all $p > 0.05$). No differences were observed as concerns the duration of pregnancy, percentage of preterm deliveries, mode of delivery, neonatal birth weight or the incidence of excessive birth weight. In the newborns in group A, compared to group B, signifantly higher incidence of the Small for Gestational Age (SGA) was observed (16.47% vs 8.44%; $p = 0.0302$). Comparative analysis of the infants with SGA with those without SGA (with mean birth weight 2650g (2250 - 2800) vs. 3400g (3150 - 3650), respectively; $p < 0.00001$) showed that in the group of offsprings with SGA, the mothers were diagnosed with of GDM significantly more frequently before the end of the 20th week of gestation (p=0.0302). There were no differences in the remaining parameters. A negative correlation was observed between the occurrence of SGA and the time of GDM diagnosis ($p = 0.0258$), and a positive correlation between the presence of SGA and the diagnosis of GDM before the end of the 20th gestational week (p=0.0302). In the logistic regression models, corrected for maternal age, pre-pregnancy BMI, the time of GDM diagnosis, 75gOGTT glucose concentrations, treatment regimen, and gestational age at delivery, only diagnosis of GDM before the 20th week of gestation was an independent predictor of neonatal SGA (OR greater than 2 for all analyzed models, $p < 0.05$).

Conclusion: Women with GDM, regardless of the time of GDM diagnosis, did not differ in terms of clinical characteristics, GDM diagnostic tests results, or the mode of treatment. In women with GDM diagnosed before the end of 20th gestational week the prevalence of SGA in the newborns is higher compared to the subjects with GDM diagnosed after 20th week of pregnancy. There were no other differences. Further research is needed to explore the cause of the observed phenomenon.

Disclosure: **M. Zurawska-Klis:** None.

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The use of flash glucose monitoring is as effective and safe as self monitoring blood glucose in a cohort of pregnant women with type 1 diabetes using multiple doses of insulin

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Background and aims: Flash glucose monitoring has demonstrated efficacy in improving glycemic control and reducing the time in hypoglycemia in subjects with type 1 diabetes mellitus (T1D). Despite the limited evidence in pregnancy, the Freestyle Libre (FSL) system is approved in pregnant women with T1D. The aim of this study was to evaluate the effect of FSL glucose monitoring on metabolic control and perinatal outcomes in pregnant women with T1D.

Materials and methods: Retrospective cohort study of singleton pregnant women with T1D in treatment with multiple doses of insulin (MDI) controlled in 2 tertiary hospitals. Self-monitoring blood glucose (SMBG) use in the 2013-2018 period was compared with FSL glucose monitoring in the period 2018-2020. 14-day glucometric data downloads per trimester were obtained from FSL group using the Libreview website platform.

Results: 118 pregnant women were included, 60 using FSL. Clinical characteristics were similar in both groups except for active smoking status, which was lower in the FSL group (15% vs. 30%, p = 0.047). There were no differences between groups in the degree of metabolic control (HbA_{1c}) or in the rate of severe hypoglycemia (table). In the FSL group, the time in range (63-140 mg/dl) was 60.15 (53-67)% in the 1st trimester, 63.13 (57-72)% in the 2nd and 68.98 (63.5-80)% in the 3rd trimester; the time above range (>140 mg/dl) was 29.69 (16-37)% in the 1st trimester, 30.79 (22-41)% in the 2nd and 22.91 (12-27)% in the 3rd trimester; the time below range (<63 mg/dl) was 13.36 (6-17)% in the 1st trimester, 9.25 (4-12)% in the 2nd and 9.92 (4-13)% in the 3rd trimester. Regarding obstetric complications, a lower incidence of pre-eclampsia was observed in the FSL group (3% *vs* 16%, *p*=0.023), which was not confirmed when adjusting for confounders (pre-pregnancy body mass index and smoking status). With regard to neonatal complications, there were no significant differences in the incidence of macrosomia, neonatal hypoglycemia, respiratory distress syndrome, malformations and neonatal mortality between both groups.

Conclusion: In our cohort of pregnant women with T1D the use of FSL glucose monitoring was as effective and safe as the use of SMBG in combination with MDI in terms of metabolic control and perinatal outcomes.

	FSL (n=60)	No FSL (n=58)	*P* value
Age (years)	33.46±4.72	33.41±3.68	0.948
Current smoker (%)	9 (15)	17 (30)	**0.047**
Diabetes duration (years)	14.85 (7.11-21.42)	13.39 (9.04-22.61)	0.919
Pre-pregnancy weight (kg)	61.2 (56-71)	62 (56.5-68.5)	0.588
Pre-pregnancy HbA_{1c} (%)	6.7 (6.1-7.4)	6.7 (6-7.4)	0.809
1st trimester HbA_{1c} (%)	6.36 (5.9-6.85)	6.3 (5.9-7)	0.655
2nd trimester HbA_{1c} (%)	5.82 (5.4-6.3)	5.92 (5.5-6.3)	0.248
3rd trimester HbA_{1c} (%)	6.05 (5.5-6.4)	6 (5.7-6.4)	0.795
Severe hypoglycemia (%)	7 (12)	5 (9)	0.701

Data are expressed as mean +/- standard deviation, median (Q1-Q3) or n (%).

P = bivariate analysis

Disclosure: **N. Seguí:** None.

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Risk for ketonaemia in type 1 diabetes pregnancies with sensor-augmented pump therapy with predictive low glucose suspend compared to low glucose suspend: a crossover RCT

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Background and aims: It is unknown whether in pregnancy sensor-augmented pump (SAP) therapy with predictive low glucose suspend (PLGS) is associated with an increased risk for ketonaemia compared to low glucose suspend (LGS). We aimed therefore to determine the frequency of ketonaemia, time of insulin delivery suspension and glycaemic control between PLGS and LGS in pregnant women with type 1 diabetes (T1DM).

Materials and methods: An open-label crossover RCT in 10 women with T1DM on the 640 Medtronic insulin pump with inclusion between 12-30 weeks of pregnancy. Participants were 1/1 randomly assigned to either 2 weeks with PLGS mode or 2 weeks with LGS mode. After the first 2 weeks, participants were switched to the other mode. Ketonaemia in serum was measured 3 times daily (fasting, midday between 1-3 pm and evening between 9-11 pm) during 4 weeks with the Freestyle Abbott meter. The diabetes treatment satisfaction questionnaire (DTSQs), the Hypoglycaemia Fear Survey II (HFS-II) and the Problem Areas in Diabetes-short form (PAID-5) questionnaire were completed baseline and after switch to either mode.

Results: Gestational week at inclusion was 12.5 weeks (12.0-15.0), age of participants of 31 years (24.0-33.0), BMI of 26.6 Kg/m² (24.5-31.8), 9 women were Caucasian and one had a Northern-African background, 8 women were nulliparous, 9 women had a history of diabetic retinopathy, one had microalbuminuria, baseline HbA1c was 5.9% (5.8-6.1) and baseline time in range (TIR 63-140mg/dl) of 64.6% (55.6-68.7). Comparing the LGS mode to the PLGS mode, insulin suspension time per day was 2.0 hours (1.3-2.3) vs. 3.5 hours (3.3-5.0), p=0.002 and frequency of ketonaemia >0.6mmol/l of 0% vs. 0.5% (2), p=1.000, none had ketonaemia >1mmol/l (Table). Comparing the LGS mode to the PLGS mode, TIR was similar with less time in hypoglycaemia with PLGS (Table). In addition, DTSQs, HFS-II and PAID-5 questionnaires showed similar treatment satisfaction and similar fear for hypoglycaemia with LGS and PLGS mode (Table).

Conclusion: SAP therapy with PLGS mode is a safe alternative to LGS in pregnancy since the increased suspension time of insulin was associated with similar TIR with less time in hypoglycaemia, and without increased risk for significant ketonaemia. Treatment satisfaction and fear for hypoglycaemia were similar when using PLGS and LGS mode.

	LGS	PLGS	p-value
Insulin suspension time per day (hours)	2.0 (1.3-2.3)	3.5 (3.3-5.0)	**0.002**
Insulin suspension time over a 2-week period (hours)	28.3 (117.9-32.0)	48.8 (45.8-70.0)	**0.002**
Fasted ketonaemia (mmol/l)	0.08 (0.05; 0.10)	0.07 (0.06; 0.11)	0.432
Midday ketonaemia (mmol/l)	0.07 (0.04; 0.09)	0.09 (0.08; 0.10)	**0.002**
Evening ketonaemia (mmol/l)	0.08 (0.06; 0.11)	0.08 (0.06; 0.11)	1.000
Frequency of ketonaemia >0.6mmol/l (%)	0	0.5 (2)	1.000
Frequency of ketonaemia >1mmol/l (%)	0	0	
TIR (%)	64.7 (58.0-68.7)	61.1 (56.5-67.5)	0.492
Time >140mg/dl (%)	30.1 (23.6-35.2)	33.3 (28.6-36.6)	0.193
Time >180mg/dl (%)	10.3 (6.7-13.7)	14.4 (10.5-16.6)	0.275
Time <63mg/dl (%)	7.5 (4.6-8.3)	4.2 (2.4-6.9)	**0.014**
Time <50mg/dl (%)	2.1 (1.4-2.7	1.1 (0.8-3.1)	0.232
Low blood glucose index	2.8 (1.8-3.5)	1.9 (1.4-2.6)	**0.019**
Coefficient of variation (%)	37.2 (35.3-39.7)	35.1 (32.9-39.0)	0.310
Mean amplitude of glycemic excursions (mg/dl)	120.9 (107.4-1.35.6)	123.5 (114.6-137.6)	1.000
DTSQs	29.5 (26.0-34.0)	32.0 (27.0-33.0)	0.656
HFS-II	20.0 (17.0-22.0)	22.0 (19.0-23.0)	0.547
PAID-5	2.5 (1.0-5.0)	3.0 (1.0-5.0)	1.000

Clinical Trial Registration Number: NCT04292509
Supported by: FWO senior fellowship
Disclosure: **K. Benhalima:** None.

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Analysis of insulin requirement during pregnancy in patients with type 1 diabetes treated with a personal insulin pump

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Background and aims: During pregnancy in the women with type 1 diabetes mellitus (T1DM), there is a dynamic change in the daily insulin requirement (DDI). From the 2nd trimester of pregnancy, a constant increase in DDI is observed, which results from the secretion of pregnancy hormones that increase insulin resistance to ensure the supply of nutrients necessary for the proper development of the fetus. The aim of the study was to assess the change in insulin requirements in women with T1DM treated with a personal insulin pump during pregnancy.

Materials and methods: A single-center, retrospective cohort study was conducted in 93 pregnant women with T1DM treated with a personal insulin pump, 54% of whom were using continuous blood glucose monitoring. Data from the medical history, as well as the course of type 1 diabetes and comorbidities was collected. Insulin requirements, weight gain, and glycated hemoglobin (HbA1c) were analyzed.

Results: The mean age of women was 31.2 ± 4.33 years, mean diabetes duration 15.9 ± 7.43 years, pre-pregnancy Body Mass Index (BMI) 23.8 (23.0-24.6) kg/m2, pre-pregnancy HbA1c 7.12 ± 1.28%, and was 23% of subjects met the pre-pregnancy metabolic control criteria (HbA1c < 6.5%) was 23%. The mean DDI before pregnancy was 39.9 ± 14.9 units (IU) (0.6 ± 0.19 IU per kilogram of the current body weight (IU/kg)), and at delivery was 76.9 ± 34.8 IU (0.95 ± 0.36 IU/kg). The total increase in DDI during pregnancy was 28.8 ± 15.9 IU (0.27 ± 0.19 IU/kg), which constituted 80.6 ± 59.2 % (54.2 ± 50.2% in relation to IU/kg). The basal insulin dose before pregnancy was 18.6 ± 8.0 IU, which was 47.0 ± 13.5% of the DDI. The total increase in the dose of basal insulin during pregnancy was 10.7 ± 8.2 IU, accounting for 71.4 ± 51.9%. The dose of bolus insulin before pregnancy was 21.4 ± 10.8 IU, constituting 52.0 ± 15.2% of the DDI. The total gestational increase in the dose of bolus insulin was 16.8 ± 13.1 IU, which accounted for 96.6 ± 36.7%. The total gestational weight gain was 12.5 ± 5.27 kg. The overall HbA1c reduction during pregnancy was 0.35 ± 1.02%. The HbA1c was 6.44 ± 1.02% in the 1st trimester, 5.93 ± 0.63% in the 2nd trimester and 6.14 ± 0.70% in the 3rd trimester. Target HbA1c values (lower than 6.5% in the 1st trimester, and lower than 6.0% in the 2nd and 3rd trimesters) were achieved by 52%, 57%, and 38% of subjects, respectively. A positive correlation was observed between gestational percentage increase in the dose of short-acting insulin and diabetes duration (r = 0.336, p = 0.0138), and total weight gain during pregnancy (r = 0.471, p = 0.0002), while a negative correlation with the total gestational HbA1c reduction (r = -0.363; p = 0.0104). One case of severe hypoglycaemia was reported.

Conclusion: In women with type 1 diabetes treated with a personal insulin pump, the total increase in the daily insulin requirement is slightly over 50%, with the dose of basal insulin increasing by slightly over 70%, and twice for short-acting insulin. A higher total gestational percentage increase in bolus insulin dose is associated with a lower decrease in HbA1c during pregnancy.

Disclosure: **M. Kosinski:** None.

SO 13 From pregnant women and mice

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Maternal overweight and obesity is associated with impaired glucose homeostasis and fetal overgrowth in the absence of gestational diabetes

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Background and aims: Gestational diabetes mellitus (GDM) is one of the most common causes of excessive fetal growth and metabolic complications in the newborn's later life. However, in addition to the well-known relationship between increased glucose levels and fetal development, recent studies have indicated that increased maternal body mass index (BMI) has considerable impact on the development of large for gestational age (LGA) offspring even in women with normal glucose tolerance. The underlying mechanisms beyond these observations are not completely understood, however, researchers have postulated that maternal insulin resistance at early gestation might be involved in the pathophysiological processes. This study aimed to assess glucose metabolism at early gestation in overweight and obese mothers who remained normal glucose tolerant (NGT) during gestation. Insulin sensitivity and secretion as well as birth weight were compared with normal weight controls and mothers who developed gestational diabetes mellitus.

Materials and methods: 1,132 pregnant women were prospectively included and categorized according to their pregestational BMI and glucose tolerance: 893 were NGT until delivery (570 with normal weight, 220 with overweight, 103 with obesity), whereas 239 women developed GDM. All study participants had a broad metabolic evaluation before 16 weeks of gestation and were followed up until delivery.

Results: Increased BMI was associated with increased levels of HbA1c, fasting glucose, insulin or C-peptide even in mothers who did not develop GDM. Maternal insulin resistance and compensatory hyperinsulinemia at early stage of gestation were markedly associated with overweight or obesity and to a lesser amount with GDM status. Birth weight was associated with maternal obesity (OR 1.90, 95%CI 1.17 to 3.04, p=0.008), as well as with insulin sensitivity and insulin response in women without GDM.

Conclusion: Mothers affected by overweight or obesity but not by GDM had a higher degree of insulin resistance and hyperinsulinemia in early pregnancy which was further associated with fetal overgrowth. An impaired metabolic milieu related to maternal obesity may be associated with fetal development already at early gestation.

Disclosure: **G. Kotzaeridi:** None.

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Impact of prepregnancy overweight and obesity on treatment modality and pregnancy outcome in women with gestational diabetes

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Background and aims: This study aims to evaluate the implications of prepregnancy overweight and obesity on treatment modalities of

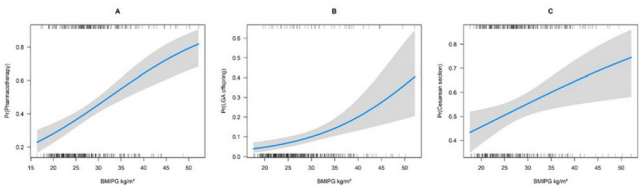
Gestational Diabetes Mellitus (GDM). We assessed the association of increased Body Mass Index (BMI) and dosing of basal and rapid acting insulin formulations as well as pregnancy outcome.

Materials and methods: 509 women with GDM (normal weight: 201, overweight: 157 and obese: 151), attending the pregnancy outpatient clinic at the Department of Obstetrics and Gynecology of a medical university, were included in this retrospective study. We used a prospectively compiled database to assess patient characteristics, treatment approaches (particularly maximum doses of basal and rapid acting insulin or metformin) and pregnancy outcome.

Results: Increased BMI was associated with the need of glucose lowering medication (odds ratio (OR): 1.08 for the increase of 1 kg/m² BMI, 95%CI 1.05-1.11, p<0.001). Obese mothers with GDM received the highest amount of insulin. Metformin, especially in combination with insulin, was more often used in obese patients who also required higher daily doses. Maternal BMI was associated with increased risk of cesarean section (OR 1.04, 95%CI 1.01-1.07, p<0.001) and delivering large for gestational age offspring (OR 1.09, 95%CI 1.04-1.13, p<0.001). Of note, birthweight percentiles were highest in obese patients who required glucose lowering therapy.

Conclusion: Treatment modalities and outcomes in GDM pregnancies are markedly depending on the extent of maternal BMI. Obese patients showed higher requirement of glucose lowering medication and were at higher risk of adverse pregnancy outcomes. It is crucial to further explore the underlying pathophysiologic mechanisms to optimize individual treatment approaches.

Figure 1: Association of pregestational BMI (BMIPG) with the probability of receiving pharmacotherapy (A), for delivering large for gestational age (LGA) offspring (B) and having delivery by cesarean section (C)



Supported by: Research Grant from Sanofi

Disclosure: **T. Linder:** Grants; The study was funded by a research grant from Sanofi.

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Reliance on lipid and protein energy sources is associated with materno-fetal complications in type 1 diabetes pregnancy: a CONCEPTT trial substudy

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Background and aims: To assess metabolomic signatures in maternal and cord blood associated with suboptimal outcomes in the continuous glucose monitoring in women with type 1 diabetes in pregnancy trial (CONCEPTT).

Materials and methods: We analysed serum samples from 162 mothers (12, 24 and 34 weeks' gestation) and 93 cord blood samples for 1049 metabolites and 1041 lipids using ultra-performance liquid chromatography-tandem mass spectroscopy. We used adjusted and unadjusted logistic regression of metabolomic variables using adjudicated outcomes: extremely-large-for-gestational-age (ELGA; >97.5th centile), pre-eclampsia and neonatal hypoglycaemia with modified Bonferroni false discovery rate p≤0.001.

Results: All complications studied were associated with reliance on non-carbohydrate sources of fuel. Through beta oxidation, lipids were the main fuel source in cases of ELGA (24 and 34 weeks), neonatal hypoglycaemia (12 weeks only) and pre-eclampsia (12 and 24 weeks). Marked protein catabolism was evident in cases of neonatal hypoglycaemia (34 weeks) and pre-eclampsia (24 and 34 weeks). Cord blood in ELGA infants showed evidence of simultaneous beta oxidation and de novo lipogenesis, a biologically futile cycle of creating and destroying lipids, which consumes excess energy and substrate. Cord blood from infants with neonatal hypoglycaemia showed evidence of pronounced protein catabolism providing glucogenic amino acids for gluconeogenesis.

Conclusion: Reliance on lipid or protein sources for fuel was associated with ELGA, neonatal hypoglycaemia and pre-eclampsia. Carbohydrate metabolism was insufficient to meet cellular energy demands, possibly due to insufficient insulin, insufficient dietary carbohydrate or both. Improving outcomes in type 1 diabetes pregnancy may require greater focus on normalising carbohydrate metabolism through optimal carbohydrate intake and matched insulin dosing.

Clinical Trial Registration Number: NCT01788527

Supported by: The trial is funded by Juvenile Diabetes Research Foundation (JDRF) grants #17-2011-533, and grants under the JDRF Canadian Clinical Trial Network, a public-private partnership including JDRF and FedDev Ontario and supported by JDRF #80-2010-585. Medtronic supplied the CGM sensors and CGM systems at reduced cost. This project was funded by a Diabetes UK Project Grant. CLM is supported by the Diabetes UK Harry Keen Intermediate Clinical Fellowship (DUK-HKF 17/0005712) and the European Foundation for the Study of Diabetes – Novo Nordisk Foundation Future Leaders' Award (NNF19SA058974). HRM conducts independent research supported by the National Institute for Health Research (Career Development Fellowship, CDF-2013-06-035), and is supported by Tommy's charity.

Disclosure: **Z.A. Stewart:** None.

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Enhanced hepatic insulin resistance may counteract metabolic adaption processes during gestation

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Background and aims: Like type 2 diabetes, gestational diabetes (GDM) can be classified into different subtypes depending on the underlying pathomechanisms. These include adaptation defects in both beta cell mass and insulin secretion, severity of insulin resistance, and the presence of preconceptional (pc.) prediabetes. New Zealand obese (NZO) mice exhibit impaired glucose tolerance (IGT) before and during pregnancy, pc. hyperinsulinemia associated with impaired glucose-stimulated insulin secretion (GSIS) and develop hyperlipidemia with elevated plasma free fatty acids during gestation. Interestingly, after the second trimester (day d14.5), the model shows an improvement of both GSIS and whole-body insulin resistance (determined as HOMA-IR and Matsuda index). The aim of this study was to investigate whether changes in hepatic metabolism in this mouse model contribute to the observed metabolic compensation processes during pregnancy.

Materials and methods: Female NZO mice and healthy NMRI controls were examined pc. and on d14.5. Animals were sacrificed at 9-10 weeks of age, livers were harvested and primary hepatocytes (PH) were cultured.

AKT activity was then determined by ELISA (total-AKT and Ser473 phosphorylation after insulin stimulus, 100 nM) in liver samples and PH. Hepatic glucose production of PH was determined by fluorometric assay after overnight fasting with and without insulin stimulus over a period of 8 hours. Glucose uptake in PH was measured by fluorometric 2-deoxyglucose (2-DG) uptake assay, after initial glucose fasting. In addition, hepatic glycogen content was determined by PAS staining and colorimetric assay.

Results: Whilst no difference was observed pc., NZO mice showed decreased AKT activation (pAKT/total-AKT ratio) compared to NMRI controls at d14.5 (0.24 vs. 0.15, a.u., $p < 0.05$). This alteration could be confirmed in PH after insulin stimulus both pc. (0.41 vs. 0.30, a.u., $p < 0.05$) and at d14.5 (0.38 vs. 0.23 a.u., $p < 0.05$). In NMRI PH, insulin-mediated glucose production was significantly subdued compared to the unstimulated condition at both time points (AUC pc.: $p < 0.01$; d14.5: $p < 0.01$). In NZO PH, glucose production was increased at both time points compared to NMRI controls (AUC pc.: 138.27 vs. 239.94, $p < 0.05$; d14: 158.57 vs. 271.22, $p < 0.01$), demonstrating a diminished response to an insulin stimulus. Contrary to the NZO mice, NMRI PH responded to an insulin stimulus with increased 2-DG uptake both pc. and at d14.5 compared to the unstimulated condition. In addition, after insulin stimulation NZO mice revealed reduced 2-DG uptake compared to NMRI controls at d14.5 (13.26 vs. 8.43 pmol/μg protein, $p < 0.01$). These effects were accompanied by a decreased hepatic glycogen content in NZO mice at d14.5 compared to NMRI controls (55.49 vs. 40.81 mg/g tissue, $p < 0.05$), while it did not differ between the two strains pc. Hepatic PAS staining confirmed reduced glycogen content at d14.5 in NZO mice.

Conclusion: While total body insulin resistance improves in the NZO model at d14.5, decreased AKT activation occurs in the liver. This alteration is accompanied by increased hepatic glucose production along with decreased glucose uptake and low glycogen content. This could explain the observed persistent GIT despite improved GSIS during gestation in the NZO mouse.

Disclosure: **M. Liebmann:** None.

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Serum Elabela is associated with inflammatory abnormalities in gestational diabetes

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Background and aims: Gestational diabetes mellitus (GDM) is a common disease in pregnant women, which mechanisms remain unclear. It has been confirmed that Apelin-APJ system contributes to the energy homeostasis in middle and terminal pregnancy. Elabela (ELA) is another endogenous ligand of apelin receptor (APJ), which is expressed highly in the placenta. Few studies have focused on the role of ELA in GDM and the association between ELA and inflammatory abnormalities.

Materials and methods: The study consisted of 33 patients with GDM (GDM group) and 33 healthy pregnant women as control group (control group). Blood samples were collected for the measurement of fasting plasma glucose (FPG), fasting insulin, total cholesterol (t-CHOL), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride (TG), serum ELA and inflammatory cytokines including TNF-α, IL-6, and IL-1β before performing a 75-g OGTT.And then we collected 1-h and 2-h PG blood samples during a 75-g OGTT.

Results: There were significant differences in serum Elabela ($P<0.05$), fasting plasma glucose (FPG), one-hour glucose, two-hour glucose in the OGTT ($P<0.001$), HOMA-IR ($P<0.001$) and cytokine IL-1β($P<0.05$) between GDM group and control group. ELA had significant correlations with FPG (r=-0.254, P=0.039), fasting insulin (r=-0.258, P=0.036), HOMA-IR (r=-0.275, P=0.026), and IL-1β (r=-0.789, P=0.000). And stepwise multiple linear regression analysis presented IL-1β and FPG most significantly affected the ELA levels.

Conclusion: This study showed that decreased ELA levels were significantly associated with increased glucose levels and insulin resistance in GDM, which might be mediated by inflammatory factors, such as IL-1β. Serum ELA levels might be a clinical biomarker for GDM.

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Disclosure: **Y. Chen:** Grants; National Natural Science Foundation of China (grant numbers 81700723).

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Improvement of insulin and glucagon secretion profiles by estradiol and serotonin in prediabetic mice during gestation

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Background and aims: During gestation, in addition to an increase in various hormone plasma levels, such as estradiol (E2), there is also an increase of serotonin (5-hydroxytryptamine, 5-HT) content in the pancreatic islets. Alteration of these processes may be associated with gestational diabetes. In pancreatic islets, E2 has been shown to increase insulin and inhibit glucagon secretion. The same effects have also been found for 5-HT in most studies. However, their specific role in islet function, in particular of pregnant prediabetic mice, needs to be stressed. For this purpose, female New Zealand obese (NZO) mice, which show an impaired glucose tolerance but no signs of manifest diabetes, are an appropriate model. Interestingly, in vivo but not ex vivo stimulation by glucose shows an improvement of insulin secretion during gestation in NZO mice. In addition, NZO mice exhibit preconceptional (pc.) hyperglucagonemia that improves during gestation. Extrapancreatic modulators may play a role here in balancing glucagon and insulin secretion. The aim of this study was to investigate the role of the pregnancy-associated hormones 5-HT and E2 as compensatory modulators of pancreatic islet hormone secretion.

Materials and methods: Female NZO mice were examined pc. and on day 14.5 of gestation. NMRI mice were used as a healthy control. Hormone concentrations in plasma and pancreas were determined by ELISA. To determine hormone secretion, primary islets of pc. mice were isolated by collagenase digestion technique. The freshly isolated islets were preincubated for one hour with 5 mM glucose and then incubated for one hour in the presence of 5 or 20 mM glucose (control) and 20 mM glucose in combination with 5-HT or E2. Thereafter the insulin and glucagon concentrations were measured by ELISA.

Results: On day 14.5 of gestation, NZO mice showed an increased pancreatic 5-HT concentration compared to control mice (2.65 vs. 1.46 pmol/mg pancreas, $p < 0.01$). Furthermore, NZO mice exhibited elevated plasma 5-HT and E2 levels (5-HT: 20.99 vs. 8.67 nmol/l, $p < 0.05$; E2: 70.07 vs. 45.81 pg/ml, $p < 0.05$). Insulin secretion of isolated islets was significantly enhanced by 10, 100 and 1,000 nM E2 in NMRI mice and by 100 as well as 1,000 nM E2 in NZO mice. E2 showed no significant effect on glucagon secretion in NMRI islets, but there was an increase of glucagon secretion after incubation with 100 nM E2 in NZO islets (5.13 vs. 2.33 pg/islet/h, $p < 0.05$). A concentration of 1,000 nM 5-HT led to an inhibition of insulin secretion in both strains. This effect was significant in NZO mice (0.31 vs. 0.70 ng/islet/h, $p < 0.01$). A comparable inhibitory effect after incubation with 5-HT was observed on glucagon secretion. 1,000 nM 5-HT resulted in an inhibition of glucagon secretion in the NZO strain (0.29 vs. 5.26 pg/islet/h, $p < 0.01$).

Conclusion: E2, but not 5-HT, is most likely part of the compensatory adaptive mechanisms that are not detectable in isolated islets and enhance insulin secretion in NZO mice during gestation in vivo. Interestingly, 5-HT showed an inhibitory effect on insulin secretion and E2 led to an increase of glucagon secretion in NZO mice. However, increased 5-HT concentrations in the pancreas and concomitant inhibition of glucagon secretion could contribute to improved plasma glucagon levels and improved glucose homeostasis in NZO mice during gestation.

Disclosure: **M. Asuaje Pfeifer:** None.

SO 14 Exercise effects beyond blood glucose

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Glycaemic excursions during a standardised bout of hypoglycaemia-inducing physical activity and subsequent hypoglycaemia treatment in adult type 1 diabetes patients

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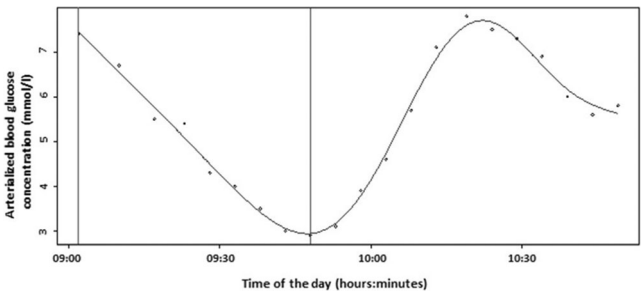
Background and aims: To characterize glucose dynamics during a standardized bout of hypoglycemia-inducing exercise and subsequent hypoglycemia treatment with oral ingestion of glucose in type 1 diabetes patients.

Materials and methods: 10 male patients with type 1 diabetes (mean ±SD: age 34.4±3.9 years, diabetes duration 7.7±1,7 years, BMI 23.4±0.8 kg/m^2, and HbA1c 7.4±0.62% (57±4.8 mmol/mol)) performed a standardized bout of cycling exercise using an electrically braked ergometer at a target heart rate (THR) of 50% of individual heart rate reserve, determined using the Karvonen equation. The exercise was terminated when hypoglycemia was reached, followed by immediate hypoglycemia treatment with oral ingestion of 20g glucose. Arterialized blood glucose (ABG) levels were monitored at 5-min intervals during exercise and for 60-min during recovery. A generalized additive model with smoothing spline was fitted to the ABG data against time to determine glucose dynamics during exercise and in recovery.

Results: The mean ABG value at the end of exercise was 3.63±1.00 mmol/l. During exercise, ABG decreased at a mean rate of 0.11±0.03 mmol/l.min^{-1} (minimum: 0.07, maximum: 0.17 mmol/l.min^{-1}). During recovery, ABG increased at a mean rate of 0.13±0.05 mmol/l.min^{-1} (minimum: 0.06, maximum: 0.19 mmol/l.min^{-1}). An example of glucose excursions during the study (Patients 1) is shown in Figure.

Conclusion: We showed that at a defined level of hypoglycemic-inducing steady-state exercise, glucose concentrations decrease on average by 1mmol/l every ten minutes, and is comparable to the rate of glucose restoration following oral ingestion of glucose. As also substantial variability was found more patients must be examined to analyse glucose changes with respect to the potential "speed group" characteristics.



Supported by: Ministry of Health, Czech Republic, No. 00064203

Disclosure: **J. Broz:** None.

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Effect of 14-week high-intensity interval training on blood lactate concentrations in type 2 diabetes patients

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Background and aims: Patients with type 2 diabetes (T2D) are often faced with chronically increased basal blood lactate concentrations ($[La^-]_b$). Exercise training may contribute to normalizing lactate metabolism of T2D patients by improving oxidative capacity, lactate transport, and pyruvate dehydrogenase activity. Yet, the evidence of the effect of exercise training on basal $[La^-]_b$ is scarce. Besides, the $[La^-]_b$ was shown to be correlated with glucose and lipid metabolism as well as overweightness/obesity, while studies on these relationships responding to exercise training in T2D patients are lacking. Therefore, this study aims to: (i) evaluate the effect of a14-week high-intensity interval training (HIIT) on basal $[La^-]_b$ in T2D patients; (ii) investigate potential associations of changes in $[La^-]_b$ with change in body composition, glucose metabolism indices, and blood lipid profiles.

Materials and methods: Twenty-eight T2D patients (age: 58 ± 5.34 years, BMI: 25.6 ± 3.92 kg/m^2) underwent a 14-week supervised HIIT on a rowing machine, that was performed thrice weekly and consisted of 6 × 1 minute high-intensity bouts at 90% VO_{2max}, interspersed with 2- or 3-minute low-intensity intervals at 40% VO_{2max} (i.e., equaling a total of 20 or 27 minutes per session). Venous blood samples were collected after overnight fasting at baseline and post-intervention. According to basal $[La^-]_b$ at baseline, the patients were divided into a normal lactate group (≤ 2mmol/L, NL, n = 22) and a high lactate group (> 2mmol/L, HL, n = 6).

Results: The ANOVA revealed a significant time × group interaction on $[La^-]_b$ ($p < .001$). Following 14 weeks of training, $[La^-]_b$ were significantly decreased in HL (Baseline: 2.82 ± .44 vs Post-intervention: 2.12 ± .32 mmol/L; $p < .01$), while increased in the NL (Baseline: 1.38 ± .30 vs Post-intervention: 1.76 ± .55 mmol/L; $p < .01$). In HL, 5 out of 6 patients (83%) decreased their $[La^-]_b$, and out of these, three patients (50%) returned to normal ranges (i.e., ≤ 2mmol/L). In NL, the $[La^-]_b$ of 6 out of 22 (23%) patients increased above the normal range. The change in $[La^-]_b$ was positively correlated with the change in total fat mass ($r = .40$, $p = .07$), BMI ($r = .55$, $p < .01$), HOMA-IR ($r = .47$, $p < .05$), and triglycerides ($r = .63$, $p < .01$) respectively.

Conclusion: HIIT can normalize the basal $[La^-]_b$ in T2D patients with hyperlactatemia (i.e., HL group), whereas the effect was heterogeneous in patients with normal basal $[La^-]_b$. This heterogeneous effect may be partly explained by changes in body composition, insulin resistance, and triglycerides.

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Disclosure: **T. Zhao:** None.

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Regular exercise training improves femoral bone marrow metabolism in monozygotic twin pairs discordant for body weight

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Background and aims: High body weight is a protective factor against osteoporosis and risk of fracture. However, obesity suppresses bone metabolism and the association remains unclear. Bone marrow in axial skeleton produces blood cells while the marrow cavity of long bones serves as a specialized fat depot. We studied the effects of regular exercise training on bone marrow (BM) metabolism in twins from monozygotic (MZ) twin pairs discordant for body weight. This study design offers a unique way of studying the impact of environmental factors on metabolism as the effects of genetics and shared exposures can be excluded in the analyses for causal factors.

Materials and methods: Twelve MZ twin pairs, who were discordant for body mass index (BMI) (8 female, 4 male pairs; 40.4 ± 4.5 years; BMI = 32.9 ± 7.6 kg·m^{-2}, mean difference between twins 7.58 kg·m^{-2}), participated in our study. So far, 8 pairs have completed the intervention. They performed a six-month-long controlled training intervention, with four training sessions per week that consisted of endurance, strength and high-intensity training. Lumbar vertebral (vertebrae L2-L4) and femoral BM insulin-stimulated glucose uptake (GU) were measured during euglycemic hyperinsulinemia using ^{18}F-FDG positron emission tomography and BM radiodensity using computed tomography (CT). Lumbar spine (L2-L4) bone mineral density (BMD) was measured from CT images using QCTPro.

Results: At baseline, twins with higher BMI had higher lumbar GU ($p=0.001$) compared to their co-twins with lower BMI but there was no difference in femoral BM GU, lumbar vertebral BM radiodensity, femoral BM radiodensity, or BMD between the groups. Training improved whole-body insulin sensitivity and aerobic capacity ($p<0.05$ for both) similarly in both groups. However, there was no effect on BMI or whole-body fat percentage. Training increased femoral BM GU ($p=0.013$) in both groups similarly but there was no effect on lumbar BM GU or BMD. Training tended to increase lumbar vertebral BM radiodensity in twins with lower BMI ($p=0.067$) while there was no change in their co-twins with higher BMI.

Conclusion: Regular exercise training improves femoral BM GU regardless of body weight while the increase in radiodensity suggests a decrease in adiposity in lumbar spine. Our results suggest that physical activity habits have an important impact on bone health independent of weight and genetics.

Clinical Trial Registration Number: NCT03730610

Supported by: The Academy of Finland (JCH decision 317332, KHP decisions 272376, 314383, 335443, 266286, JK decision 336823), the Finnish Cultural Foundation (JCH, MAH), the Diabetes Research Foundation of Finland (JCH, MAH, KHP), Novo Nordisk Foundation (KHP, NNF20OC0060547, NNF17OC0027232, NNF10OC1013354), Helsinki University Hospital (KHP), Government Research Funds (KHP), Finnish Medical Foundation (KHP), Gyllenberg Foundation (KHP), Sigrid Juselius Foundation (KHP, JK), University of Helsinki (KHP, JK) and State Research Funding/Hospital District of Southwest Finland (JCH).

Disclosure: **R. Ojala:** None.

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Different fueling strategies during racing and training in professional cyclists with type 1 diabetes

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Background and aims: The glycaemic and insulin therapy demands of road cycling during pre-season training and competitive stage racing have recently been profiled in elite athletes with type 1 diabetes (T1D), yet the in-ride nutritional practices employed under training or racing conditions in the same riders have not been explored. This study details and compares the in-ride nutritional practices of professional cyclists with T1D under pre-season training and competitive racing conditions.

Materials and methods: Seven male professional road cyclists with T1D (age: 28±4 years, HbA_{1c}: 6.4±0.4% [45±4 $mmol.mol^{-1}$], BMI: ≤23 $kg.m^{-2}$) undertook 9 days of pre-season training and then went on to compete in a 5-day *Union Cycliste Internationale* road cycling race (Tour of Slovenia). In-ride nutritional, interstitial glucose (Dexcom G6), and cycle performance (Wahoo) variables were collected. The average duration of the rides was similar (Training 4.5±1.4 vs Racing 4.3±0.3 h, p=0.110), while distance (Training 134±10 vs Racing 164±2 km, p<0.001), velocity (Training 28.8±0.7 vs Racing 39.6±1.1 $km.h^{-1}$, p<0.001), and heart rate (Training 132±6 vs Racing 141±4 bpm, p=0.011) were greater in the race event. Paired samples t-tests and stepwise linear regression were used in statistical analysis with p≤0.05 accepted for significance.

Results: Riders consumed a similar number of snacks per hour (Training 1.8±0.2 vs Racing 2.2±0.7, p=0.088) and had a greater reliance on fluid rather than solid food items during racing, compared to training (p<0.001). Macronutrient intake of riders is reported in Table 1. Protein consumption was lower during training (11.4±2.1 g) than race rides (14.5 ±6.0 g, p=0.046). In-ride energy intake was equivalent between training and racing events (Training 932.6±123.9 vs Racing 944.5±324.9 kcals, p=0.909), with an emphasis on carbohydrates as the major source of fuel in both events during exercise (>80% of total energy intake) at a rate of 41.9±6.8 (training) and 45.4±15.5 $g.h^{-1}$ (racing, p=0.548). Relativising macronutrient data to body weight, duration of ride, or distance of ride did not change statistical outcomes. Collapsed data revealed that in-ride carbohydrate intake was a predictor of power output (β=0.395, p=0.002). Riders demonstrated a comparable amount of time within the euglycaemic range ([≥3.9-≤10.0 $mmol.L^{-1}$] Training 77.1 ±32.8 vs Racing 73.4±3.9%; p=0.818).

Conclusion: These data provide a better understanding of the nutritional strategies employed by professional cyclists with T1D who manage their glycaemia well during cycle training and race events. Fluid forms of nutrition are preferred in a race event compared to training - a potentially contributing factor towards the lower protein intake during race rides.

Macronutrient	Training	Racing	P-value
Energy (kcals)	932.6±123.9	944.5±324.9	0.909
Energy ($kcals.h^{-1}$)	202.5±32.0	223.4±77.7	0.419
Carbohydrates (g)	192.5±26.6	192.3±64.2	0.991
% energy	82.7±5.3	81.8±4.4	0.662
$g.h^{-1}$	41.9±6.8	45.4±15.5	0.548
Protein (g)	11.4±2.1	7.8±4.1	*0.046**
% energy	4.9±0.9	3.1±1.0	*0.014**
$g.h^{-1}$	2.6±0.6	1.9±0.9	0.051
Fat (g)	14.5±6.0	15.4±7.5	0.373
% energy	12.4±5.4	15.0±4.8	0.196
$g.h^{-1}$	3.1±1.3	3.6±1.7	0.059

Table 1. In-ride energy and macronutrient intake of professional road cyclists with type 1 diabetes under pre-season training and competitive road cycle racing conditions (n=7). Data are expressed as mean±SD in absolute amounts, as a percentage contribution to the total in-ride energy yield, and after being relativised exercise duration.

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Supported by: Novo Nordisk
Disclosure: **J.P. Pitt:** None.

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The effects of regular excercise training on colon glucose uptake in monozygotic twins discordant for BMI

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Background and aims: Obesity is associated with impaired intestinal insulin-stimulated glucose uptake (GU). Previous studies have shown that short-term training increases colon glucose uptake, but the influence of genetics and the effect of regular exercise training remains unclear. Monozygotic twins offer unique insight to the effects of environmental factors, while controlling for genetic factors. We address the changes in colon glucose uptake with a long fitness program done in monozygotic twins.

Materials and methods: We recruited twelve monozygotic twin pairs discordant for body mass index (BMI) (at least 2 kg/m^2), with at least the other twin in each pair being overweight. Of these recruited pairs 8 have completed the intervention. The leaner twins mean BMI was 29.1 (SD 6.3), heavier twins mean BMI was 36.7 (SD 7.0) ($p < 0.001$) and average age was 40.4 years (SD 4.5). The pairs followed a controlled six-month training program with four training sessions per week (mix of endurance, strength, and high-intensity training). Glucose uptake was measured during euglycemic-hyperinsulinemic clamp using [^{18}F]FDG-PET-imaging before and after the intervention.

Results: At baseline, leaner twins had higher whole-body insulin sensitivity expressed as M-value during the euglycemic-hyperinsulinemic clamp (p = 0.003) and colon GU (p = 0.003) than their heavier co-twins. The post-intervention BMI did not change. Aerobic capacity improved (p = 0.005) in both groups similarly (time x group p = 0.77). M-value increased (p = 0.041) after training intervention. Preliminary data shows that training tends to increase colon GU (p = 0.074) in both groups. The mean GU of all participants increased 24% from 14.28 μmol/(100g*min) to 17.71 μmol/(100g*min). Leaner twins had a greater increase in colon GU than their heavier co-twins (time x group p = 0.030).

Conclusion: Obesity affects colon GU and regular exercise may improve it, independent of genetics. The results indicate that various experiences and exposures, that occur despite genetic similarity in MZ pairs have an important impact on colon metabolism.

Clinical Trial Registration Number: NCT03730610

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Disclosure: **M.S. Lietzén:** None.

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The level of muscle and intestinal damage biomarkers after an exercise with a predominance of eccentric contractions in male patients with type 1 diabetes

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Background and aims: Eccentric exercises plays a significant role in overall fitness, and in rehabilitation programs in restoring lost strength, muscle endurance, and functional kinematics of the limbs. However, compared to concentric or isometric contractions, it leads to a greater muscle damage due to micro-injuries. Additionally, the redistribution of the blood flow during exercise leads to a reduced blood supply to the intestines and oxidative stress. The level of muscle damage can be determined by mioglobin and LDH (lactate dehydrogenase) level. Zonulin is most commonly used to assess the degree of intestinal damage (leakage of the intestinal barrier). We aimed to compare the level of muscle and intestinal damage biomarkers after the exercise with a predominance of the eccentric contractions (PEC) between men with type 1 diabetes (T1DM) and healthy controls.

Materials and methods: We assessed the selected biomarkers after a 30-minute run on the inclined treadmill (10% incline) with a load of 5% of patient BMI with a maximal intensity of 60% of the maximal oxygen uptake. Before starting the test with the predominance of eccentric contractions and 1h, 24h after its completion, the subjects were blood drawn from a vein in accordance with the standards. We enrolled 19 male T1DM patients with a mean disease duration of 12.2 ± 6.0 years characterized by very good glycemic control (mean HbA1c 6.9 ± 0.7%, 52 mmol/mol) and 20 healthy men.

Results: There were no difference in age (25.3 ± 5.4 vs 24.3 ± 2.5, p = 0.91), and BMI (23.9 ± 2.3 versus 22.8 ± 2.0, p = 0.15) between groups. At baseline patients with T1DM had an increased lactate dehydrogenase (LDH) (Table 1). At 60 minutes and 24 hours after the test, T1DM patients had a higher level of myoglobin, LDH and zonulin compared to healthy men (Table 1).

Conclusion: In summary, T1DM male patients seems to be more prone to muscle and intestinal damage after the exercise with PEC.

Table 1. Muscle and intenstinal damage biomarkers before and after the physical exercise with a predominance of the eccentric contractions in the study groups.

Variable	T1DM	Healthy control	p-value
Myoglobin before the test [ng/ml]	25.4 ± 11.1	26.3 ± 10.9	0.790
LDH before the test [U/L]	198.9 ± 54	152.4 ± 41.1	0.004
Zonulin before the test [ng/ml]	20.9 ± 7.3	18.3 ± 7.3	0.361
Myoglobin 60 min after the test [ng/ml]	194.9 ± 62.6	78.2 ± 24.8	<0.001
LDH 60 min after the test [U/L]	298.5 ± 46.4	206 ± 43.4	<0.001
Zonulin 60 min after the test [ng/ml]	36.1 ± 9.5	27.5 ± 9.7	0.008
Myoglobin24 hours after the test [ng/ml]	84.4 ± 24.0	27.7 ± 9.1	<0.001
LDH 24 hours after the test [U/L]	230.8 ± 73.9	151 ± 27.4	0.002
Zonulin 24 hours after the test [ng/ml]	40.4 ± 9.7	20.5 ± 8.5	<0.001

T1DM – patients with type 1 diabetes, LDH - lactate dehydrogenase

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Assessment of factors influencing changes in blood lactate levels in children and adolescents with type 1 diabetes during a football tournament (GoalDiab Study)

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Background and aims: Type 1 diabetes (T1D) is a chronic autoimmune disease characterised by complete deficiency of insulin. In the course of physical activity, muscles produce lactic acid which is a product of glucose metabolism. Changes in blood lactate levels are an important source of information about the intensity of physical exercise. Characterising physical exercise and adjusting insulin dosage accordingly is an important element of diabetes education for patients with T1D. The objective of the study was to assess the intensity of physical exercise and changes in lactate levels in children and adolescents with T1D during a football tournament, and to analyse factors influencing these changes by age group.

Materials and methods: We enrolled 141 participants out of 189 players with T1D taking part in the First Polish Football Championship for Children and Adolescents with Type 1 Diabetes, the results of 70 of whom were analysed: 7 girls (10%) and 63 boys (90%), aged 12.0 (11.0-13.7) years, treated with intensive functional insulin therapy, playing in two age categories: 10-13 years and 14-17 years. Lactate levels were measured in the capillary blood before and after each match using the Lactate Scout (EKF-Diagnostics - Manufacturer SensLab GmbH, Leipzig, Germany). OBLA (Onset Blood Lactate Accumulation) was used to assess the anaerobic threshold.

Results: Glycated haemoglobin level (HbA1c) was 7.3 (7.0-8.2)%. Sixty-one subjects (87%) used a personal insulin pump, and nine (13%) used insulin pens. The median lactate level was 1.8 mmol/l before matches; it increased to 4.4 mmol/l after matches ($p < 0.001$). The increase in lactate levels was higher in the older age category (4.3 [3.0-10.2] vs 1.8 [1.0-3.5]; p=0.001) and was not dependent on gender (3.2 [1.4-3.6] vs 2.1 [1.1-4.3], p=0.597), on personal insulin pump vs insulin pen use (3.0 [1.2-4.3] vs 1.5 [1.0-2.5], p=0.145) or training in a sports club (1.4 [0.6-3.2] vs 3.0 [1.4-4.3], p=0.084). A positive correlation was noted between increased lactate levels and age (Rs=0.253, p=0.034). During matches, 61% of the participants exceeded lactate levels ≥ 4 mmol/l (OBLA threshold). In univariate logistic regression analysis age was a significant predictor of OBLA threshold exceedance (OR=1.45 [1.08-1.95]). This correlation is independent of HbA1c, gender, treatment method and training in a sports club vs recreational play.

Conclusion: Physical exercise intensity levels in adolescents with type 1 diabetes during football matches in the course of the football tournament were found to be mixed aerobic-anaerobic. Increases in lactate levels after physical exercise were greater in the older subjects and did not depend on gender, method of insulin administration or training in a sports club vs recreational play.

Supported by: Novo Nordisk, Medtronic, and Ascensia were sponsors of the First Polish Football Championship for Children and Adolescents with Type 1 Diabetes. The sponsors were not involved in study design; the collection, analysis or interpretation of data; the writing of the report.

Disclosure: **J. Flotynska:** Grants; Polish Diabetes. Other; Novo Nordisk, Medtronic, Ascensia.

SO 15 Novel methods to study metabolism in diabetes

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Applying a microphysiological 3D human liver-islet microtissue platform to study drug-drug interaction

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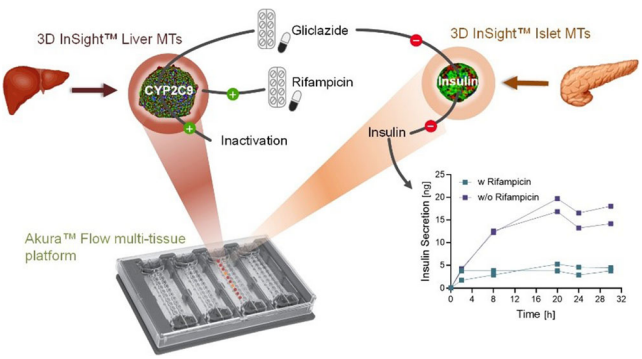
Background and aims: The conventional cell culture models lack the complex multi-organ interactions and dynamic drug processing in the human body, that is crucial to accurate disease modeling and predictive drug testing *in vitro*. We developed a microphysiological 3D human liver-islet microtissue platform, that enables direct organ crosstalk. As a proof-of-concept study, we recapitulated a well-known drug-drug interaction (DDI) phenomenon; the elevated risk of life-threatening hyperglycemia in type 2 diabetic-patients that are co-administered with antidiabetic drug gliclazide and antibiotic rifampicin. This adverse DDI is caused by rifampicin-enhanced activity of drug metabolizing CYP450 enzymes which results in increased gliclazide clearance rate. Lower gliclazide plasma levels lead to decreased plasma insulin concentrations and thus increased blood glucose levels in the patients.

Materials and methods: 3D human islet microtissues (hIsMTs) and 3D human liver microtissues (hLiMTs) were either cultured alone or together in a microfluidic multi-tissue assay plate. The plate consists of 10 compartments, which are interconnected by a straight channel with a medium reservoir on each side. Repeated tilting of the plate leads to gravity-driven flow from the upper reservoir along the channel to the lower reservoir and thereby enables continuous perfusion and interconnection of the MTs. The DDI was characterized by determine gliclazide clearance in hLiMT cultures and comparing insulin secretion in liver-islet co-cultures in the absence or presence of rifampicin.

Results: Rifampicin co-treatment led to an increase in gliclazide clearance by the hLiMTs (percentage of remaining compound: 34.7 % and 53.0 % respectively at 96 h in the presence and absence of rifampicin; $p < 0.0001$).Gliclazide significantly increased insulin secretion from hIsMTs (4-fold; $p < 0.001$). In the presence of rifampicin, insulin secretion from hIsMTs was markedly reduced (75% down, $p < 0.001$), and was comparable to the level of insulin secretion without gliclazide.

Conclusion: Based on the proof-of-concept study, we have paved the way for further application of the liver-islet microphysiological platform in the fields of metabolic disease progression, drug efficacy and toxicity testing, DDI and drug clearance assays.



Disclosure: **L. Hoelting:** None.

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Real-time metabolomics of glucose response in pancreatic islet cells resolved using infrared microspectroscopic imaging

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Background and aims: One of the recognised targets of type 2 diabetes, glucose metabolism in pancreatic islet cells can be interrogated in a static population-averaged manner, via mass-spectrometry. Several metabolites, such as ATP, NAD(P)H, FADH can be visualised in a dynamic cell-specific way. As many key glucose metabolites have distinct vibrational spectra, we utilised infra-red microspectroscopy to probe the dynamics of glucose metabolism in individual cells within intact pancreatic islets.

Materials and methods: Isolated mouse or human islets were immobilised in a double-dome ZnS optical chamber and imaged on Hyperion 3000 microscope system (Bruker optics) using a 36× reverse Cassegrain reflective objective (NA = 0.5, air) and a matching condenser. The spectral data was obtained via a Vertex 80v FTIR spectrometer. Spectra of pure metabolites were used as principal component controls.

Results: Overall, the metabolite levels increased in response to the elevation of glucose from 3 mM to 17mM. for human islets, glucose stimulus resulted in an increased intracellular concentration of MgATP (4.1±1.6-fold, n=130, $p<0.05$), creatine phosphate (3.8±1.5-fold, n=130, $p<0.05$), malate (3.7±0.9-fold, n=130, $p<0.05$), fructose-1,6-bisphosphate (2.5 ±0.8-fold, n=130, $p<0.05$), pyruvate (2.1±0.9-fold, n=130, $p<0.05$) and glutamate (1.9±0.7-fold, n=130, $p<0.05$). The response was heterogeneous across the cell population. The responses to glucose among the metabolites clearly clustered into two groups, mitochondrial and glycolytic. Interestingly, ATP and creatine phosphate clustered to the glycolytic group whereas pyruvate - to the mitochondrial. Likewise, the k-means clustering on the cell population demonstrated the presence of two clear cell clusters, "mitochondrial" and "glycolytic".

Conclusion: A proof of concept study for the single-cell IR metabolomic imaging, our work points out important destinations/metabolic sinks for the glucose signal in pancreatic islet cells. Pancreatic islets, in our hands, showed two clear groups of cells, "mitochondrial" and "glycolytic". Lastly, we believe creatine phosphate may have a role in glycolysis in the islet cells.

Supported by: diamond Light source

Disclosure: **A. Tarasov:** None.

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Single-cell optogenetics and cell-tracking reveal a functional hierarchy connecting leader and follower beta cells in vivo

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Background and aims: Previous studies suggested the presence of functional heterogeneity among the islet's β-cells. Specifically, Hub β-cells were shown to regulate the calcium waves in isolated islets. Likewise, recently leader or first-responder β-cells were identified in vivo, which guide the calcium response of the rest of β-cells to glucose. Here, we applied single-cell optogenetics to further characterize the potential of individual β-cells to control the islet's calcium dynamics in vivo.

Materials and methods: We employed fast confocal calcium imaging (6 Hz), 2-photon laser ablations, cell tracking and single-cell optogenetics. Particularly, we generated new zebrafish transgenic lines with β-cell-specific expression of the red calcium indicator K-GECO1, the photo-convertible protein mEOS2b, the inhibitory Hallorhodopsin NpHR, and the activating Channelrhodopsin CheRiff.

Results: Using two different GCAMP reporter lines: Tg(ins:GCaMP6s) and Tg(neuroD1:GCaMP6f) we reveal the presence of leader and follower cells. Moreover, there was a positive correlation between the time of response of each cell in the islet and their distance to the leader β-cell ($R2=0.6 \pm 0.27$). Using laser ablation of leader cells, we found that 12h post ablation, the islet's glucose responsiveness recovers, however the patterns of calcium wave-initiation remain erratic. We also utilized the inhibitory light-gated chloride pump NpHR to reversibly inhibit β-cells. We found that leader-cell hyperpolarization led to the strongest pan-islet inhibition of calcium influx in comparison to both no-inhibition and follower-cell inhibition. Conversely, we used the light-driven cation pump CheRiff to depolarize single-cells and investigate if artificially depolarizing a leader cell can co-activate follower cells. We found that the leader cells are capable to propagate calcium influx across most of the β-cells even in the absent of glucose stimulation, whereas follower cells in the islet have a limited capability for co-activation. Finally, to determine whether the leader cells represent a temporally stable subpopulation, we employed the green-to-red photo-convertible protein mEOS2b. After identifying in situ the first responder cell, we specifically photolabelled the leader cell's nucleus and tracked its calcium responses over time. We found that in 80% of the cases (n=5) the photo-labelled cell was again the same leader cell as previously identified. This suggest that leader cells tend to be stable for at least 24h.

Conclusion: Our data show that β-cells coordinate their function through temporally defined leader cells. The leader β-cells possess a disproportionate control over the islet's calcium dynamics and present a rather stable functional state over time in vivo.

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Disclosure: **L. Delgadillo Silva:** None.

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Estimated glucose disposal rate, non-alcoholic fatty liver disease and micro- and macrovascular complications in type 1 diabetes: Towards a new predictor?

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Background and aims: Insulin resistance is increasingly prevalent in patients with type 1 diabetes (T1D). Insulin resistance is associated with cardiovascular complications and non-alcoholic fatty liver disease (NAFLD), but its assessment is challenging in T1D due to the technical difficulties of the euglycaemic clamp technique. The estimated glucose disposal rate (eGDR) is a validated alternative to quantify insulin resistance in T1D, but its role in clinical practice is still largely unexplored. This study aims to explore the association between eGDR and micro- and macrovascular complications and NAFLD, compared to body mass index (BMI) or HbA1c alone.

Materials and methods: Individuals with confirmed T1D were included in this cross-sectional study. BMI was categorised into normal weight, overweight and obesity. eGDR was categorised in four groups (≥ 8, 6-7.9, 4-5.9 and < 4 mg/kg/min). NAFLD was determined by ultrasound

combined with elastometry (Saverymuttu grade ≥ 1 and controlled attenuation parameter ≥ 248 dB/m). Multiple logistic regression was used to identify associations with vascular complications and NAFLD, after adjusting for relevant confounders.

Results: A total of 510 individuals were eligible. NAFLD was present in 21.8% of cases. Median age was 48 [32-59] years with diabetes duration of 27 [15-36] years. An eGDR < 4 was present in 7.8 % of cases, obesity prevalence was 19.1 %. Odds ratios (OR) for nephropathy and NAFLD in obese compared with normal weight individuals were 2.19 (95 % CI: 1.13-4.10; p = 0.020) and 10.32 (95 % CI: 5.73-18.60; p < 0.001). While the association with retinopathy seemed absent (OR 1.21 [95 % CI: 0.67-2.19; p = 0.538]), the association with macrovascular disease just barely failed to reach statistical significance (OR 2.47 [95 % CI: 0.92-6.65; p = 0.074]). Comparing individuals with eGDR ≥ 8 mg/kg/min, equaling high insulin sensitivity, and < 4 mg/kg/min indicating insulin resistance, showed significant OR for nephropathy, NAFLD and macrovascular disease of 9.96 (95 % CI: 3.85-25.77; p < 0.001), 8.88 (95 % CI: 3.87-20.38; p < 0.001) and 6.06 (95 % CI: 1.60-22.73; p = 0.007), respectively. The association with retinopathy barely failed to reach clinical and statistical significance (OR 2.11 [95 % CI: 0.91-4.93; p = 0.083].

Conclusion: Obesity, insulin resistance and NAFLD are prevalent in T1D and diabetes complications are not only related to BMI or metabolic control. Insulin resistance is associated with the presence of NAFLD and micro- and macrovascular complications.

Clinical Trial Registration Number: NCT04664036

Disclosure: **J. Mertens:** None.

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Development and evaluation of a glucagon sensitivity test

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Background and aims: Glucagon regulates hepatic glucose production and increased levels of glucagon (hyperglucagonemia) contribute to diabetes. Glucagon may have an equally important role in regulating amino acid (AA) levels that in turn control glucagon secretion. This physiological feedback system is known as the liver-alpha cell axis. Hepatic steatosis may uncouple glucagon's effect on AA metabolism causing impaired actions of glucagon (resistance) on AA metabolism but not glucose production, thereby creating a diabetogenic circle. In order to quantify glucagon's effect on AA metabolism, we developed and evaluated a glucagon sensitivity test. We hypothesized that the effects of exogenous and endogenous glucagon on AA levels would be impaired in individuals with obesity and hepatic steatosis compared to lean controls.

Materials and methods: The test consists of two study days: Day 1 includes an intravenous (i.v.) bolus-infusion of glucagon (200 µg) and thereby evaluates the effect of exogenous glucagon on amino acid disappearance. Day 2 includes a 45-min i.v. infusion of mixed AAs (330 mg/min/kg body weight) to evaluate the liver-alpha cell axis (sensitivity of endogenous glucagon signaling). Both study days were performed following an overnight fast. Liver fat was measured using magnetic resonance imaging. Preliminary data from six individuals without diabetes (HbA1c < 48mmol/mol) including three lean controls (CON) (mean ± SD; Age: 32 ± 7 years, liver fat: 4.1 ± 1 %, BMI; 22 ± 2 kg/m^2) and three individuals with obesity (OBE) (47 ± 12 years, 12 ± 6 %, 30 ± 4 kg/m^2) are presented.

Results: A glucagon bolus reduced AA levels 29% less in OBE compared to CON ($dAUC_{0\text{-}120min}$; 41 ± 6 vs 29 ± 10 mmol/L x min) at fasted state. AA levels increased 33% more in OBE compared to CON during an AA infusion ($iAUC_{0\text{-}45min}$; 118 ± 28 vs 89 ± 12 mmol/L x min). Glucose levels were similar between CON and OBE at fasted state (5 ± 0.4 vs 5 ± 0.1 mmol/L) and during the bolus-infusion of glucagon ($tAUC_{0\text{-}120}$; 590 ± 47 vs 570 ± 38 mmol/L x min).

Conclusion: We conclude that glucagon sensitivity towards AA metabolism may be evaluated by a bolus-infusion of glucagon and an AA infusion. Hepatic steatosis may impair the liver-alpha cell axis by selective impairment of glucagon signaling (glucagon resistance) towards AA metabolism but not glucose.

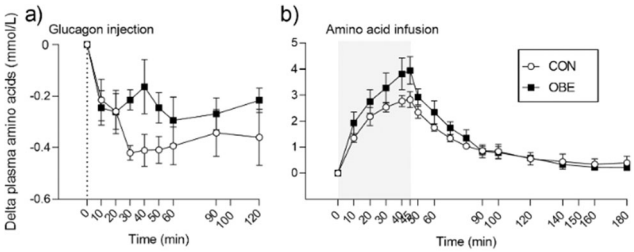

Figure 1. Plasma concentrations of amino acids in lean controls (CON) and individuals with obesity (OBE) after an overnight fast following a) an intravenous injection of 200 µg glucagon (GlucaGen Hypokit Novo Nordisk at 0 min) and b) an amino acid infusion (Vamin Electrolyte Free 14 g/L; 330 mg/min/kg body weight from 0-45 min, grey scattered area). Data are presented as mean ± SEM.

Supported by: Novo Nordisk Foundation (116113)
Disclosure: **S.A.S. Kjeldsen:** None.

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In-vivo determination of NAD metabolites in skeletal muscle, using phosphorus magnetic resonance spectroscopy

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Background and aims: Nicotinamide adenosine dinucleotide (NAD) plays an important role in energy metabolism. NAD+ forms a redox couple with NADH and a high concentration of NAD^+ as well as a high NAD^+/NADH ratio are strongly associated with metabolic health. In contrast, decreased NAD^+ bioavailability is reported in both ageing and obese humans as well as in diabetic mice. Currently, assessment of NAD metabolites in muscle relies on (invasive) biopsies. Determination of NAD metabolites using standard ^{31}P-MRS is not possible in muscle, since the resonance of α-ATP is overlapping and thereby masking the NAD resonances. Here, we developed a non-invasive phosphorus magnetic resonance spectroscopy (^{31}P-MRS) technique to determine NAD metabolites *in vivo*. The newly developed MRS sequence was validated *in vivo* by measuring the NAD^+/H levels in resting state and during ischemia, as it is well known that ischemia decrease NAD^+/NADH levels.

Materials and methods: We applied a homonuclear BIRD (HB) filter to suppress the α-ATP resonance, allowing quantification of NAD^+/NADH (*figure 1*). For validation, we included 8 young healthy lean participants and NAD^+/NADH were measured in the lower leg in rest and during eight minutes of ischemia using this new ^{31}P-MRS technique. Ischemia was induced by inflating an upper leg cuff to a pressure of 50 mmHg above systolic blood pressure.

Results: The HB filter succeeded in suppressing the α-ATP resonance by 85% and allowed quantification of NAD^+/NADH levels. Validation measurements with acute ischemia revealed an acute reduction in NAD^+ signal intensity (from 93 ± 4 to 78 ± 4 [AU], $p<0.05$) and tended to reduce the ratio NAD^+/NADH (from 3.41 ± 0.71 to 2.21 ± 0.29, $p=0.08$), in line with previous research in muscle biopsies.

Conclusion: We developed a state-of-the-art ^{31}P-MRS technique enabling non-invasive quantification of NAD metabolites. Applying this technique during ischemia, we could detect the expected changes in NAD+ and NADH, showing that physiological changes in NAD metabolites can be detected by dedicated ^{31}P-MRS. This provides new non-invasive means for future metabolic research on the relevance of NAD metabolism in type 2 diabetes.

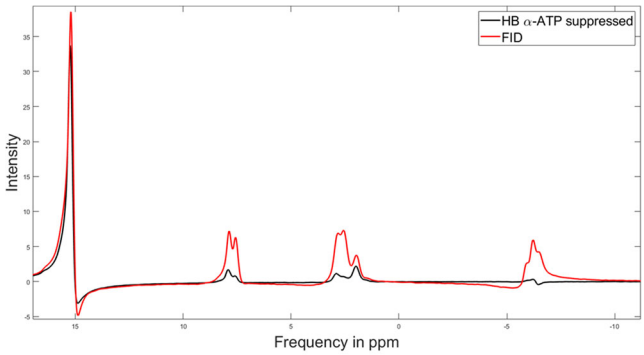

Figure 1: MRS FID acquisitions with (black line) and without (red line) homonuclear BIRD filter of an ATP + NAD phantom. α-ATP is at approximately 3.0 ppm and NAD at 2.7 ppm in the acquired spectra. An 85% reduction of the α-ATP resonance by the homonuclear BIRD filter can be observed. Signal loss due to T_2 effects of the filter reduces NAD amplitude by ca. 30%

Clinical Trial Registration Number: NL8888
Supported by: ERC starting grant (grant no. 759161 'MRS in diabetes')
Disclosure: **Y.M.H. Bruls:** None.

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The extracellular vesicles from myotubes improved insulin-stimulated glucose uptake in adipocytes by regulating AMPK pathway and Glut4 expression

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Background and aims: Extracellular vesicles (EVs) such as exosomes and microvesicles have been indicated the possibility of signal transmitters in intercellular network and biomarkers, because they include DNA, miRNAs and proteins. We have reported that the myotubes' EVs induced by mechanical stretch improved insulin-stimulated glucose uptake in adipocytes and this mechanism was insulin-independent signaling. However, this mechanism is still unknown. Therefore, this study aimed to investigate the functions of EVs derived from mechanical stretched myotubes in vitro.

Materials and methods: C2C12 myoblasts were grown on an elastic silicone chamber and induced differentiation. Differentiated C2C12 myotubes were stimulated by cyclic uniaxial stretch (10% of initial length, 10 cycle/min) for 5 hours. EVs were isolated from supernatants with Mag-capture isolation kit and co-incubated with 3T3L1 adipocytes before insulin stimulation. The cells were stimulated with 10 nM insulin for 10 minutes and then glucose uptake experiment was performed. The amount of 2-deoxyglucose uptake was measured by enzymatic assay. And the signaling pathway was examined by western-blotting.

Results: We confirmed both EVs from basal and stretch condition were incorporated into 3T3L1 adipocytes by confocal microscopy. EVs from mechanical stretch statistically increased insulin-stimulated glucose uptake by 22% and Akt phosphorylation in 3T3L1 adipocytes. And glucose transporter 4 (Glut4) expression was also elevated in 3T3L1 adipocytes co-incubated with stretch-induced EVs. Next, we checked the small RNA including in stretched-induced EVs. Some of siRNAs, which has been reported in association with Glut4 expression, were changed between basal and stretched EVs. Furthermore, phosphorylated AMPK was dramatically increased in stretch EVs.

Conclusion: These results suggest that mechanical-stretch induced extracellular vesicles from muscle cells have a potential for improvement insulin sensitivity of adipocytes by upregulating of Glut4 and AMPK signaling pathway. Therefore, muscle EVs is a one of candidate target for treatment of diabetes.

Supported by: KAKENHI

Disclosure: **T. Saito:** None.

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Insulin resistance and type 2 diabetes studied using a new combination of microphysiological systems and mathematical modelling

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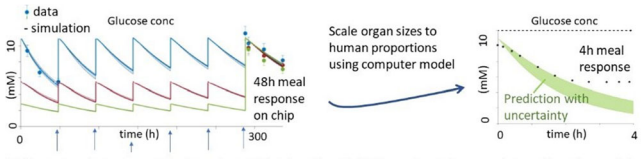
Background and aims: Glucose homeostasis lies at the heart of the type 2 diabetes (T2D) and is thus important. Current *in vivo* T2D research is primarily based on rodents, which is problematic, since genetically modified animals only display some T2D phenotypes and have a poor translatability to humans. It is therefore important to develop better non-animal alternatives such as microphysiological systems (MPS). We have previously developed the first multi-organ MPS for glucose homeostasis: 3D liver spheroids and pancreatic islets from human donors. However, i) the mechanisms in this system are still not fully understood, and ii) timescales and volumes are different from those in humans. To tackle these two issues, we here present and validate a new combined experimental-mathematical modelling approach.

Materials and methods: We have trained and validated a new mathematical model on data from new targeted MPS experiments from 7 human donors, in chips with single organs and liver-pancreas crosstalk. Conditions include hyper- (Fig A, blue), normo (red) and hypo-glycemia (green), which induces different degrees of glucose control and insulin resistance, as seen e.g. by altered insulin levels in glucose tolerance tests, and by media- and cell sample-analyses, done either at the beginning, middle, and/or at the end of a two 2-week experiment.

Results: The developed mathematical model (-) can describe glucose and insulin data (error bar) on both short (hours) and long (2 weeks) timescales (Fig A). This is validated by non-rejection of a chi-square test ($p>0.05$), which means that we describe all variation in data except for measurement noise. The model can describe both estimation data, and correctly predict new independent validation data, not used for model training. The thus validated model can also infer non-measured mechanistic variables such as β-cell mass and liver insulin resistance, which is useful since it allows us to follow and understand what the model believes happens in the chips. Finally, we use the computer model to go beyond current experimental limitations, by scaling the volumes in the MPS to human sizes, and thereby also both explaining and removing previously observed differences in timescales (Fig B).

Conclusion: We have presented the first combination of MPS and mathematical modelling, which is a competitive alternative to rodents, both regarding the ability to dissect realistic mechanisms concerning insulin resistance and T2D, and regarding the ability to translate results to humans.



(A) Comparison between model and experimental data from the chip (B) Comparison between scaled model and human data.

Supported by: VR-M (2018-03319)

Disclosure: **G. Cedersund:** None.

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Deletion of the mammalian Indy homologue (Slc13a5) improves hepatic insulin sensitivity through vagal nerve signalling

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Background and aims: INDY (I'm Not Dead Yet) is a plasma membrane citrate transporter and is highly expressed in liver and brain. In mammals, whole body deletion of the coding gene (*mIndy*, *Slc13a5*) increased energy expenditure and protected mice from diet-induced obesity and insulin resistance. Generation of neuronal *mIndy*-KO (NINKO) mice revealed improved insulin sensitivity (IS) in NINKO mice, mediated through better hepatic IS. Gene expression studies in wildtype C57Bl/6 mice revealed high expression of *mIndy* in the hypothalamus (HTM), which is the main brain area regulating hepatic glucose metabolism. One mechanisms is the regulation through AMPK. Reduced hypothalamic AMPK phosphorylation is known to reduce hepatic glucose output, probably mediated via vagal nerve signaling. Therefore, the aim of this study was to investigate if hepatic glucose production in NINKO mice is regulated by *mIndy* expression in the HTM through AMPK phosphorylation and vagal nerve signaling.

Materials and methods: Hyperinsulinemic-euglycemic clamp studies were performed to assess IS after hepatic vagotomy of NINKO mice. NestinCre$^+$ controls were sham denervated. *Ex vivo* brain slices of C57Bl/6 mice and the neuronal hypothalamic cell line CLU183 were acutely stimulated with 1 mM sodium citrate to investigate the effect of citrate on the brain and neurons.

Results: Acute stimulation of brain slices and CLU183 cells with citrate decreased cellular respiration (-19.7%, $p\leq0.01$), ATP production (-37.7%, $p\leq0.01$) and increased AMPK phosphorylation (+24.7%, $p\leq0.001$). In line with that, AMPK phosphorylation in the HTM of NINKO mice was significantly reduced (-68.1%, $p\leq0.05$). With hepatic branch vagotomy, no differences in hepatic IS could be observed anymore (suppression of basal hepatic glucose output (%); NestinCre$^+$: 100.0±27.2, NINKO: 99.4±8.8).

Conclusion: These data suggest that neuronal *mIndy* is a critical regulator of glucose homeostasis in mammals, probably regulated via AMPK phosphorylation in the HTM and vagal nerve signaling. Further studies will address the exact mechanisms involved in the effect.

Disclosure: **A. Kurzbach:** None.

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Insulin sensitivity and beta cell function in IGT and treatment-naive patients with type 2 diabetes of different ethnicities: a pooled analysis from clinical studies

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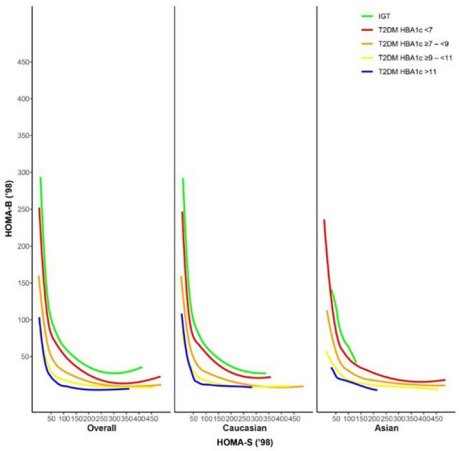
Background and aims: The hyperbolic nature of the relationship between insulin sensitivity and β-cell functionality has been previously established. We aimed to describe the interaction between β-cell activity and insulin sensitivity in early stages of type 2 diabetes mellitus (T2DM) by extracting metabolic data from a pool of 17 globally conducted clinical studies in participants with either impaired glucose tolerance (IGT) (n=981) or treatment-naïve, newly, but not necessarily timely diagnosed T2DM (n=7409).

Materials and methods: Data were extracted only from pre-treatment baseline visits. The homeostatic model (revised version '98) was used to calculate the β-cell activity (HOMA-B) and insulin sensitivity (HOMA-S). Lowess smoothing was used for line fitting. Given the heterogeneous data source, only medians are provided without any formal statistical testing.

Results: In the overall pooled population (n=8390), median HbA1c at baseline was 7.5% (IGT 5.8%; T2DM 7.7%), median HOMA-B 49.2% (IGT 88.9%, T2DM 43.6%), median HOMA-S 58.0% (IGT 61.7%, T2DM 57.2%) and median BMI 29.8 kg/m^2 (IGT 30.3 and T2DM 29.7). Study population included 5015 Caucasian, 1543 East-Asian, 641 Hispanic, 389 South-Asian, 322 Black, and 390 individuals from other ethnicities. Median HOMA-B was consistently higher in IGT (Caucasian 86.4%, East-Asian 106.0%, Hispanic 122.0%, South-Asian 95.1%, Black 108.0%) than in T2DM (Caucasian 48.1%, East-Asian 34.2%, Hispanic 33.5%, South-Asian 33.4%, Black 46.6%). Median HOMA-S values were more evenly distributed among those with IGT (Caucasian 62.3%, East-Asian 56.6%, Hispanic 61.7%, South-Asian 61.9%, Black 53.0%) than T2DM (Caucasian 51.0%, East-Asian 93.8%, Hispanic 55.8%, South-Asian 63.8%, Black 51.3 %). Despite the limitation of using cross-sectional data derived from different clinical studies, visual inspection of the overall population shows progressive deterioration of the relationship between insulin sensitivity and β-cell activity with the elevating HbA1c level. At 50% HOMA-S, estimated HOMA-B was 110% in IGT, while in T2DM with different HbA1c cut-off values of <7%, 7-9%, 9-11% and >11%, the estimates were 85%, 50%, 40% and 27%, respectively. The same progressive deterioration pattern is detectable in the most represented ethnicities (Caucasian and Asian; Figure).

Conclusion: The activity of the β-cell is increasingly blunted with diagnostic values of HbA1c levels >7%. Median β-cell activity was comparable among different ethnicities in patients with IGT whereas in those with T2DM, East- and South-Asians had higher β-cell activity compared with Caucasians, Hispanics and Blacks.

Figure: β-cell activity (HOMA-B) at 50% insulin sensitivity (HOMA-S) among patients with IGT and T2DM



HbA1c, glycated haemoglobin; HOMA-B, homeostatic model assessment of β-cell activity; HOMA-S, homeostatic model assessment of insulin sensitivity; IGT, impaired glucose tolerance; T2DM, type 2 diabetes mellitus.

Disclosure: **S. Del Prato:** None.

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Metabolic effects on aortic valves in static and pulsatile 3D cultivation environments

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Background and aims: Type 2 diabetes mellitus (T2DM) is associated with a high risk for secondary cardiac and vascular diseases. Nevertheless, little is known about the influence of a disturbed metabolic state on molecular changes of heart valves. This study is designed to analyze the impact of hyperglycemia and hyperinsulinemia on degenerative processes in intact aortic valves (AV) exposed to a static versus pulsatile 3D environment.

Materials and methods: Static cultivation of AV tissue was performed with passive biomechanical stress acting on AV leaflets fixed on silicone rings. For pulsatile cultivation, AV were cultivated in their physiological aortic root anatomy in a software-governed bioreactor system with controlled pulsatile flow as well as physiological pH-value, temperature and oxygen saturation. In both models, ovine AV were cultivated for seven days under diabetic conditions using DMEM culture medium with 100 mg/dl or with 450 mg/dl glucose (HG) without or with additional insulin (100 nM; HI). Fibrotic changes of AV leaflets were estimated by light transmission analysis as well as H&E staining. Data was collected in independent experiments with five to six replicates and p-values < 0.05 were considered as statistically significant.

Results: AV samples of pulsatile cultivation showed a significantly stronger fibrosis compared to static cultivation, both, in terms of density of the tissue and in thickening of the spongiosa. In contrast, diabetic cultivation had no impact on fibrosis. However, diabetic treatment affected mRNA expression of the proteoglycans biglycan and decorin, which have been shown to be involved in AV degeneration. In addition, mRNA expression changes were observed under HG and HI in an environment-dependent manner. While a significant downregulation of α smooth muscle actin (*αSMA*) was observed in static samples, dynamic flow conditions led to reduced mRNA expression of transforming growth factor β (*TGFβ*). Western blot analysis revealed a significant increase of protein kinase B (AKT) phosphorylation after acute insulin stimulation, which was lost in AV under HI. The downstream signaling pathway proteins glycogen synthase kinase 3 α/β (GSK3α/β) and forkhead box protein O1 (FOXO1) as well as the kinases extracellular signal-regulated kinase 1/2 (ERK1/2) of the mitogen-activated protein kinase (MAPK) pathway also showed altered phosphorylation levels due to the diabetic treatment, but to a lesser extent in comparison to AKT.

Conclusion: AV tissue is highly sensitive to insulin. Chronic hyperinsulinemia induces insulin resistance in AV. Furthermore, diabetic conditions interrelate with processes of AV degeneration, whereby alterations depend on the cultivation environment. Taken together, the presented data provide an elaborated model to study the impact of T2DM on AV homeostasis.

Supported by: the German Research Foundation (DFG).

Disclosure: **J.I. Selig:** Grants; DFG.

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Cardiac insulin resistance does not predict mortality or morbidity in ischaemic heart failure

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Background and aims: The connection between peripheral insulin resistance (IR) and coronary artery disease are well-established. Both are major risk factors for the development of ischemic heart disease potentially leading to heart failure (HF) and death. However, the impact of *cardiac* IR on overall survival and morbidity is still debated. Furthermore, it is unknown whether cardiac IR is associated to left ventricular ejection fraction (EF) improvement after coronary intervention. Therefore, we aimed to test if cardiac IR predicts mortality, major cardiovascular events (MACE), and improvement in EF in patients with ischemic HF scheduled for cardiac viability testing before revascularization.

Materials and methods: In this retrospective study, we included 131 patients with a clinical diagnosis of ischemic HF (114 (87%) male, 33 (25%) with diabetes) referred for viability testing with Rubidium-82 and dynamic ^{18}F-Fluorodeoxyglucose positron emission tomography combined with computed tomography prior to a potential revascularization procedure. Cardiac IR was assessed by myocardial glucose uptake (MGU) in a remote (non-scarred) area of the left ventricle during a hyperinsulinemic-euglycemic clamp (1 mIE/kg/min). The study population was followed from the time of the PET/CT scan until death or end of study June 1st, 2020. Survival analyses and incidence rate estimates were performed on the study population. Receiver operator curve analyses were performed on patients (44 (34%)) who underwent a revascularization procedure.

Results: MGU did not predict the risk of death (HR: 1.0 (0.97-1.0)) or MACE. MGU did not predict improvement in EF in patients undergoing coronary revascularization (AUC: 0.3376). Instead, overt diabetes and reduced coronary flow reserve predicted overall survival. MGU correlated with skeletal muscle glucose uptake ($p<0.001$) and whole-body glucose uptake (M-value) ($p<0.001$) in individual without diabetes, whereas no association was observed for individuals with diabetes.

Conclusion: Cardiac IR is not a predictor of mortality, MACE or EF improvement after coronary revascularization in individuals with ischemic heart failure.

Disclosure: **T.V. Luong:** None.

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The association of insulin resistance with early predictors of cardiovascular, renal and neurologic complications in patients with type 1 diabetes

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Background and aims: Insulin resistance (IR), measured by the estimated glucose disposal rate (eGDR), is associated with a higher degree of type 1 diabetes (T1D) complications. We wanted to examine the relationship between eGDR and early surrogate markers of micro- and macrovascular disease (CVD), in patients with T1D.

Materials and methods: A total of 172 consecutive patients with T1D without a history of cardiovascular,renal and eye complications were included. Patients underwent anthropometric evaluation and laboratory measurement of metabolic parameters (Hb1Ac, lipid profile, renal function tests), and eGDR was calculated as an IR measure.Pulse wave velocity (PWV) and global longitudinal strain (GLS) measurements were used as CVD surrogates. Through heart rate variability(HRV), subclinical autonomic neuropathy was evaluated at rest and dynamically by measuring total power (TP) and expiration to inspiration index (EI), respectively. Early nephropathy was assessed based on the measurement of urinary albumin to creatinine ratio (ACR). None of the patients had retinopathy.

Results: Our population sample included more female patients (62.2%) with a mean age of 34 years (±12.3) and a mean disease duration of 15.8 years (± 9.6), a mean BMI value of 24.6 kg/m^2 (±4.7), an HbA1C of 7.6% (±1.4) and mean eGDR (lower values indicate higher insulin resistance) of 9.2 (±1.9). Lower eGDR values were significantly correlated ($p<0.1$) with higher BMI values, worse glycemic control, and higher basic and prandial insulin needs.Higher IR (Table 1) was significantly associated with worsening of age-adjusted PWV and GLS, and age-adjusted HRV at rest (TP) but not after breathing stimulation (EI). No association was shown with ACR values.The assoatiaton of eGDR with PVW, GLS and TP remained significant even after after adjustment for duration of diabetes.

Conclusion: Insulin resistance in patients with T1D, measured by eGDR, correlates independent of diabetes duration with early CVD surrogate markers and autonomic neuropathy at rest, but not with dynamically evaluated neuropathy and neither with early markers of nephropathy.

Table 1: eGDR association with early markers of diabetic complications

		aa PWV	GLS	aa TP	aa EI	ACR
eGDR	EXP(B)	0.794	1.424	0.679	0.747	1.374
	P value	0.028	<0.001	<0.001	0.7	0.4
	Number of patients	149	144	154	156	151

Abbreviations: eGDR, estimated glucose disposal rate; aa PWV, Pulse wave velocity, GLS,global longitudinal strain; aa TP, age-adjusted total power; aa EI, age-adjusted expiration to inspiration index; ACR, albumin to creatinine ratio;

Disclosure: **A. Barmpagianni:** None.

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Human skeletal muscle THY1$^+$fibro-adipogenic progenitors are associated with muscle degeneration in type 2 diabetic patients

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Background and aims: Type 2 diabetes mellitus (T2DM) is associated with impaired skeletal muscle function and degeneration of the skeletal muscle microenvironment. However, the cellular origin and mechanisms underlying the degeneration are not well described, in particular in human skeletal muscle.

Materials and methods: Fluorescence activated cell sorting (FACS), Whole-tissue, FACS isolated and single-cell RNA sequencing, immunohistochemistry, Bioenergetics, Western blotting, qPCR and primary cell culture.

Results: Here we show that skeletal muscles of T2DM patients exhibit degenerative remodeling of the extracellular matrix. In addition, we found increased adipocyte accumulation as evidenced by Perilipin-1 protein expression in T2DM muscle. To delineate the origin of fibro-fatty infiltration we performed single-cell RNA sequencing and FACS and found a unique population of fibro-adipogenic progenitors (FAPs) that are highly efficient in generating fibroblasts and adipocytes. We identified Platelet-derived growth factor (PDGF) signaling as key regulator of human FAP biology, as it promotes proliferation and collagen production, whereas

adipogenic differentiation was reduced. This change in cellular phenotype was accompanied by a metabolic shift towards glycolytic lactate fermentation, likely to support collagen formation. From our single-cell RNA-seq data we found several FAP subpopulations, with one marked by expression of THY1 (CD90) - the FAP^{CD90+}. This subpopulation of FAPs showed a PDGF-mimetic phenotype, with high proliferative activity and clonogenicity, increased production of extracellular matrix and enhanced glycolysis. This was accompanied by an increased activity of PDGF-signaling in the FAP^{CD90+} compared to FAP^{CD90-}. Moreover, the FAP^{CD90+} were non-responsive to further PDGF-AA treatment, in contrast to FAP^{CD90-}, suggesting saturation of PDGF-AA signaling in FAP^{CD90+}. When examining skeletal muscle from T2DM patients we found an increased presence of FAP^{CD90+} cells compared to non-diabetic controls. No other mononuclear cell population displayed any changes. Furthermore, the FAP^{CD90+} from T2DM skeletal muscle displayed an increased ability to enter the cell-cycle.

Conclusion: These data identify PDGF-driven conversion of a subpopulation of FAPs as a key event in the pathogenic accumulation of extracellular matrix in T2DM muscle.

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SO 17 Novel aspects of beta cell and insulin secretion

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Proinsulin insulin in situ localisation defects are associated to UPR response and loss of beta cell phenotype in islets of type 2 diabetic and glucose intolerant living donors

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Background and aims: Our preliminary results showed that in pancreatic islets of impaired glucose tolerant (IGT) and type 2 diabetic (T2D) patients, proinsulin (PI) intracellular localization was altered. Moreover, the insulin-proinsulin (PI-INS) colocalization coefficient as well as PI levels and PI/INS ratio gradually increased from NGT to IGT and T2D pancreatic islets and were related to the loss of glucose tolerance and impaired β-cell function. The molecular mechanisms driving such alterations are not fully characterized neither their occurrence were correlated with patients' metabolic profile. To fill in this gap, we investigated the correlation between altered PI expression and localization, and phenotypic/functional changes of β-cells during metabolic stress in extensively clinical characterized IGT and T2D individuals, compared to NGT.

Materials and methods: We analyzed microdissected pancreatic islets of n=4 NGT, n=7 IGT and n=4 T2D patients subjected to partial pancreatectomy (PP) and metabolically profiled. We evaluated the expression of ER stress genes and β-cell mature phenotype-related genes. Given the high heterogeneity among pancreatic islets we also performed an individual islets analysis on two frozen serial pancreatic sections for each donor. We analyzed n=88 individual islets from n=3 NGT, n=3 IGT and n=3 T2D patients. On the first section we performed an INS-PI double immunofluorescence staining in order to evaluate the *in-situ* expression levels of INS and PI. After image analysis we mapped individual islets in the whole section. Subsequently, on the second serial section, we microdissected the same single islets previously mapped for gene expression analysis.

Results: We observed that PDIA1 (Protein Disulfide Isomerase-1), GRP78 (Glucose Regulated Protein-78) and XBP1 (Splicing X-Box Binding Protein-1) genes, involved in unfolded protein response (UPR) were significantly upregulated in pancreatic islets of IGT and T2D patients vs NGT ($p<0.05$) and were positively correlated with *in-situ* PI/INS ratio ($r=0.6$; $p=0.01$) and PI-INS colocalization ($r=0.5$, $p<0.05$), with *in-vivo* measurements of glucose intolerance ($r=0.6$-0.8; $p<0.001$) and β cell functional reduction ($r=-0.6$; $p=0.04$). Individual islets phenotyping approach revealed a progressively increased heterogeneity from NGT to IGT and T2D patients. Of note, *in-situ* PI/INS ratio and PI-INS colocalization were positively correlated with the expression of UPR genes ($r=0.3$; $p<0.01$) and negatively with those associated to β cell identity ($r=-0.2$; $p<0.01$).

Conclusion: Our data demonstrated that β-cells PI localization alterations reflected metabolic and molecular defects in IGT and T2D patients. Furthermore, individual islet phenotyping analysis, even though revealing a high heterogeneity among pancreatic islets, uncovered the association of PI-INS intracellular localization alterations with increased ER stress and loss of β-cell phenotype during T2D metabolic alterations.

Disclosure: **N. Brusco:** None.

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Pancreatic and gut hormone responses to mixed meal test differentiate pancreatic cancer associated diabetes from type 2 diabetes

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Background and aims: The early identification of pancreatogenic diabetes and its distinction from the more prevalent type 2 diabetes mellitus (T2DM) are clinically significant. However, there is no validated method to differentiate this diabetes subtype at the present. This study is aiming to examine the pancreatic and gut hormones responses to a mixed meal tolerance test (MMTT) in the individuals with pancreatic cancer (PaC) in comparison with T2DM.

Materials and methods: A total of 44 participants were recruited into the study, which is consisted of 10 healthy controls, 12 pancreatic cancer patients with normal blood glucose (PaCNG), 12 pancreatic cancer associated diabetes mellitus (PaCDM) and 10 newly developed type 2 diabetes mellitus (T2DM). All participants (after fasting for at least 10 h) underwent MMTT. Blood samples were collected at 0, 15, 30, 60 and 120 min to measure insulin, C-peptide, glucagon, pancreatic polypeptide (PP), glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP). Indices of insulin sensitivity (HOMA2 %S, HOMA2 IR) and insulin secretion (HOMA2 %β, insulinogenic index t_{30} and t_{120}, AUC insulin:glucose ratio) were calculated. Increases in hormone levels from baseline, as well as the insulin secretion and senstivity differences were compared among all groups.

Results: Insulin and C-peptide responses to MMTT were blunted in PaCDM patients compared to T2DM. The AUC of insulin were comparatively lower in PaCDM; between-group differences were observed at the fasting (197.15 ± 16.59 pg/mL to 537.96 ± 118.69 pg/mL; $P = 0.040$) and 15 min (523.94 ± 81.15 pg/mL to 1182.51 ± 219.35 pg/mL; $P = 0.036$) time-points. No statistical differences among groups were found for glucagon. The mean peak PP concentration after MMTT in PaCDM group (466.67 ± 79.05 pg/mL) was higher than control group (258.54 ± 31.36 pg/mL, $P = 0.034$), but not statistically different to T2DM patients (452.34 ± 62.96 pg/mL, $P = 0.892$). The fasting concentration of GLP-1 was lower in PaCDM patients than T2DM (97.04±9.79 pg/mL to 199.69 ±29.80 pg/mL, $P = 0.020$), and the postprandial concentration of GIP was prolonged in PaCDM patient. PaCDM patients had lower insulin secretion capacity but better insulin sensitivity compared to T2DM patients.

Conclusion: PaCDM patients tend to present with reduced early phase insulin secretory capacity, lower β-cell function and better insulin resistance compared to T2DM patients. There is no difference in glucagon and PP responses to the MMTT. PaCDM patients have lower fasting GLP-1 concentration, but prolonged postprandial GIP concentrations than T2DM patients. The above findings may help with early screening for sporadic PaC in new-onset diabetes cohort. Confirmation in larger studies is still needed.

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The nutrient sensor mTORC1 regulates insulin secretion by modulating beta cell autophagy

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Background and aims: Autophagy is a lysosomal degradation process aimed to provide energy under conditions of nutrient deprivation. mTORC1 inhibits autophagy via phosphorylation of ULK1 and nuclear exclusion of TFEB, a master regulator of lysosome biogenesis. The dynamic regulation of autophagy in β-cells by the cycles of fasting-feeding has not been studied, and its effect on insulin secretion is unknown. We studied the role of mTORC1-autophagy crosstalk in insulin secretion in the post-absorptive state and following short-term and prolonged administration of nutrients.

Materials and methods: β-Cell mTORC1 activity was assessed in fasting and in response to glucose and/or amino acid (AAs) administration, in vivo (by oral gavage) or ex vivo, by immunostaining of pancreatic sections or dispersed islets for pS6. For genetic inhibition of mTORC1 and/or autophagy we generated mice with induced β-cell specific knockout (KO) of *Raptor* and heterozygous deletion of *Atg7*, respectively. Autophagy was measured by immunostaining for LC3, electron microscopy and by Western blotting for ULK1 phosphorylation. TFEB nuclear localization was assessed by immunostaining, and expression of TFEB-regulated genes was quantified by qPCR. Insulin secretion was assessed by static incubations and analyzed by ELISA.

Results: We found that in β-cells mTORC1 is inhibited while fasting, and is rapidly stimulated by nutrients in vitro and in vivo, most notably by leucine and glucose. Stimulation of mTORC1 by nutrients inhibited ULK1 and induced nuclear exclusion of the transcription factor TFEB with subsequent inhibition of genes that regulate autophagy and lysosome function, including Cathepsin D (*CtsD*), H^+/Cl^- exchanger, transporter 7 (*Clcn7*) and *WIPI1* (Atg18 homolog); this resulted in time-dependent inhibition of autophagy when β-cells are continuously exposed to nutrients. Inhibition of autophagy by *Atg7* KO or pharmacologically using chloroquine increased insulin secretion. Inhibition of mTORC1 by *Raptor* KO mimicked the effects of fasting and stimulated autophagy while inhibiting glucose- and amino acid-stimulated insulin secretion, whereas inhibition of autophagy under these conditions in heterozygous *Atg7* KO mice rescued insulin secretion.

Conclusion: mTORC1 regulates insulin secretion through modulation of autophagy under different nutritional situations. In the fasting state, autophagy is regulated in an mTORC1 dependent manner and its stimulation is required to keep insulin levels low, thereby preventing hypoglycemia. When stimulation of autophagy is sustained, it inhibits nutrient-stimulated insulin secretion. Reciprocally, stimulation of mTORC1 by elevated branched-chain amino acids, which is common in obesity, may promote hyperinsulinemia by inhibiting autophagy.

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EPDR1, a novel human batokine regulating glucose-stimulated insulin secretion in beta cells

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Background and aims: The biological function of ependymin-related protein 1 (EPDR1) remains to be resolved. Its crystal structure suggests a role in lipid transport or degradation. Recently, EPDR1 was described as a secreted batokine in a proteomics-based study in human brown adipose tissue (hBAT). EPDR1 was required for the norepinephrine (NE)-stimulated mitochondrial proton leak respiration and its silencing altered the expression of multiple mitochondrial proteins in hBAT. Interestingly, we have observed that human islets and INS1 832/13 β cells also express

EPDR1. However, so far, no study has been conducted to investigate the role of EPDR1 in β cell function.

Materials and methods: EPDR1 mRNA levels in human islets from non-diabetic (ND) and type 2 diabetes (T2D) subjects were assessed. INS-1 (832/13) cells were transfected with scramble (control) and EPDR1 siRNAs (EPDR1-KD) and glucose-stimulated insulin secretion (GSIS), mitochondrial activity parameters and glucose-derived metabolites were measured by ELISA, confocal microscopy and GC-MS metabolomics, respectively.

Results: *EPDR1* mRNA levels in human islets correlated positively with hyperglycemia (HbA1c) (r=0.381, p=0.001), body mass index (BMI) (r=0.372, p<0.001) and GSIS (r=0.262, p=0.038). Remarkably, *EPDR1* mRNA levels were upregulated in islets from T2D donors (p=0.047) and correlated positively with insulin secretion (r=0.61, p=0.007). *In vitro* studies show that EPDR1 increased metabolism-dependent GSIS (p=0.0004) and was required for the glucose-stimulated increases in cytosolic ATP:ADP ratio. Metabolomics studies in EPDR1-deficient INS1 832/13 β cells showed alteration of TCA-cycle metabolites, further supporting an impact on mitochondrial function.

Conclusion: The data obtained so far suggest an important role of EPDR1 in regulation of β cell function. We conclude that EPDR1 expression in β cells may increase under metabolic overload (hyperglycemia/obesity) to alleviate mitochondria from metabolic stress. This would preserve their function and, in turn, help to maintain β cell glucose-responsiveness.

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Disclosure: **L.R. Cataldo:** None.

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The Nova1-Bim axis in pancreatic beta cells does not alter glucose homeostasis in obesity and multiple low-dose streptozotocin-induced diabetes

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Background and aims: The loss of functional pancreatic β-cell mass is an important hallmark of both type 1 and type 2 diabetes. The RNA-binding protein Neuro-oncological ventral antigens 1 (NOVA1) is expressed in human and rodent pancreatic β-cells. Previous *in vitro* studies indicated that NOVA1 is necessary for glucose-stimulated insulin secretion and its deficiency prevented cytokine-induced apoptosis. Moreover, Bcl-2 Interacting Mediator of cell death (BIM), a proapoptotic protein, is differentially spliced and potentiates apoptosis in NOVA1-deficient β-cells. The role of NOVA1-BIM in β-cell function under metabolic stress *in vivo* remains elusive. Collectively, we aim to test the hypothesis that β-cell-specific deletion of *Nova1* or *Bim* will impair β-cell function and survival under metabolic stress.

Materials and methods: We generated 2 independent mouse models by Cre-Lox technology lacking *Nova1* (βNova1$^{-/-}$) or *Bim* (βBim$^{-/-}$) in β-cells. We used the Insulin-1 promoter to express the Cre recombinase specifically in β-cells. To test the impact of *Nova1* or *Bim* deletion on β-cell function, mice were subjected to high-fat diet-induced insulin resistance or multiple low-dose of streptozotocin-induced diabetes. Intraperitoneal glucose and insulin tolerance tests, pancreatic insulin content, and body composition were measured.

Results: We confirmed *Nova1* or *Bim* deletion in isolated pancreatic islets from βNova1$^{-/-}$ and βBim$^{-/-}$ mice, respectively. 9-10 weeks old β-cell-specific knockout and littermate control (fl/fl) mice were fed a high-fat diet (60% fat) for 12-14 weeks (βNova1$^{-/-}$, n=6-7; βBim$^{-/-}$, n=13-18). We measured body composition, glucose and insulin tolerance test, and pancreatic insulin content *in vivo*. All these parameters were indistinguishable between control and βNova1$^{-/-}$ or βBim$^{-/-}$ mice on high-fat diet. Moreover, β-cell-specific Nova1 or Bim deficiency failed to affect diabetes development in response to multiple low-dose of streptozotocin-induced diabetes, evidenced by unaltered blood glucose levels and pancreatic insulin content (βNova1$^{-/-}$, n=5-11; βBim$^{-/-}$, n=6). Interestingly, 6 weeks after streptozotocin treatment βNova1$^{-/-}$ improved glucose tolerance compared to control mice, (n=5-11; p<0.0001).

Conclusion: Our *in vivo* data show that Nova1 deletion in β-cells does not adversely impact glucose homeostasis in mice but moderately improves glucose tolerance in response to multiple low-dose streptozotocin-induced diabetes. BIM deletion in β-cells does not affect glucose homeostasis in obesity or diabetes development. Together these data argue against an *in vivo* role for the Nova1-Bim axis in β-cells.

Disclosure: **M.K. Brahma:** None.

SO 18 It must be my hormones

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Evaluation of the 26RFa/GPR103 peptidergic system in a mouse model of insulinopenia

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Background and aims: The neuropeptide 26RFa also referred to as QRFP (for pyroglutamilated RFamide peptide) is the latest member of the RFamide peptide family discovered. It has been identified as the ligand of the human orphan G-protein-coupled-receptor GPR103. Initialy described as a modulated food intake peptide, recent studies have shown that 26RFa is also able to regulate glycemia. This peptide, expressed by the gut and by the pancreatic β cells, acts as an incretin. Although it is known that in high fat mice the antihyperglycemic effect of 26RFa is markely blunted, until now, no data concerning the 26RFa/GPR103 system are available in insulinopenia mouse model. Therefore, the aim of this study was to evaluate the 26RFa/GPR103 system in a streptozotocin (STZ) mouse model.

Materials and methods: 30 mice were treated with a single injection of 150 mg/kg of streptozotocin. After 7 days, metabolic tests were performed after an intraperitonal injection of 500 µg/kg of 26RFa or 5mg/kg of a GPR103 antagonist (25E). After one month, mice were euthanized and tissues were collected. Plasma levels of 26RFa were measured by using a radioimmunoassay, and the expression of 26RFa/GPR103 was determined in muscle and duodenum samples by RT-qPCR. All animal studies has been carried out along the "Principles of laboratory animal care" (NIH Publication no. 85-23, revised 1985) and approved by the Normandy Regional Ethics Committee.

Results: As expected, 7 days after treatment, STZ injected mice exhibited significant higher fasting glycemia (before : 1.47 ± 0.04 g/L vs after 4.55 ± 0.20 g/L ; $p<0.0001$) associated with significant insulinopenia (before : 9.68 ± 0.97 µUI/mL vs after : 3.07 ± 0.53 µUI/mL ; $p<0.0001$) while the weight remained significantly unchanged (before : 27.78 ± 0.38 g vs after 27.47 ± 0.34 g ; $p=0.07$). 26RFa plasma levels were significantly lower in STZ treated mice compared to control mice (215.5 ± 34.73 pg/mL vs 597.3 ± 142.2 pg/mL respectivly ; $p=0.03$). In STZ mice, fasting hyperglycemia was not significantly improved by injection of 26RFa. Similarly, peripheral injection of 26RFa during an IPGTT or OGTT has no effect on glucose induced hyperglycemia ($p=0.9$ and $p=0.6$ respectively). Moreover, insulin sensitivity measured during an ITT was not significantly modified by a 26RFa injection ($p=0.5$). A GPR103 antagonist injection in STZ mice did not significantly alter glucose homeostasis during all metabolic tests. qPCR experiment revealed a trend to a down-regulation of the 26RFa receptor in muscle while no significant difference of the expression levels was found in the duodenum samples ($p=0.9$).

Conclusion: Altogether, the present findings reveal a total loss of the incretin effect of 26RFa in the insulinopenia mouse model, strenghtening the previously described link between 26RFa/GPR103 system and insulin secretion. Moreover, low 26RFa plasma levels found in STZ mice support a role of β cells in 26RFa production/secretion.

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Disclosure: **M. Le Solliec:** None.

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Urolithin B protects pancreatic beta cells against IAPP proteotoxicity: a potential strategy for dietary interventions in diabetes

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Background and aims: Diabetes is an epidemic with frightening numbers worldwide. Despite the advancement in therapeutics, the disease is still associated with an important reduction of life expectancy and life quality. Therefore, new strategies are needed to overcome this personal, social, and economic burden. A commonly overseen aspect in diabetes onset and progression is the aggregation of Islet Amyloid PolyPeptide (IAPP), or amylin, in pancreatic beta (B)-cells. In fact, IAPP deposition is a histopathological hallmark of the disease. Dietary (poly)phenols (PP), and their low molecular weight metabolites, have been associated with the inhibition of IAPP aggregation, representing a pool of compounds with great potential.

Materials and methods: A library of PP metabolites, predicted to be found circulating in humans after PP-rich food consumption, was tested in docking studies with IAPP (NMR structure 2L86 from Protein Data Base) using Auto Dock Vina. The best hit was tested in cell-free systems using synthetic IAPP by means of Thioflavin-T assays, Transmission Electronic Microscopy (TEM) and Filter Trap Assays. Metabolite-mediated protection was assessed in INS-1 B-cells challenged with toxic IAPP aggregates by means of viability tests, Glucose Stimulated Insulin Secretion assays and flow cytometry. The molecular targets of protection were determined by high throughput RNA sequence analysis.

Results: *In* silico studies were carried out to identify potential PP metabolites interacting with IAPP. The simulations pointed out ellagitannin metabolites, the Urolithins (Uro), as the best performing molecules, particularly UroB. First, the ability of UroB to interfere with the kinetics of IAPP fibril formation was validated in cell-free assays. Most importantly, UroB was shown to modulate the size and morphology of IAPP fibrils. *In vitro*, UroB protected against IAPP-induced cytotoxicity majorly by mechanisms associated with the modulation of calcium signaling pathways as revealed by transcriptomic analysis. Consistent with the central role of calcium signaling in insulin secretion, UroB increased insulin secretion in response to hyperglycemia. In addition, UroB attenuated oxidative stress in response to hyperglycemia and hyperlipidemia.

Conclusion: UroB interacts with IAPP protecting B-cells against IAPP proteotoxicity by mechanisms including the modulation of calcium signaling pathways, insulin secretion and cell antioxidant responses. Being UroB a bioavailable metabolite resulting from the consumption of ellagitannin-rich foods, this study may open new venues for the exploration of dietary strategies contributing to diabetes control.

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Disclosure: **A.F. Raimundo:** None.

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Postprandial dynamics of proglucagon cleavage products and their relation to metabolic health

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Background and aims: The proglucagon cleavage products glucagon, glucagon 1-61, glicentin, and glucagon-like peptide (GLP)-1 all originate from the same preproglucagon gene product. In a previous study, we found >20% stable or increasing glucagon levels during an oral glucose tolerance test from fasting to 2 hours post-challenge. Unexpectedly, this non-suppression of glucagon secretion was associated with a metabolically healthier phenotype with lower liver fat and glycemia. Since the radioimmunoassay (RIA) used in this study shows cross-reactivity to other proglucagon cleavage products it is possible, that the observed positive effects on metabolism are at least partly due to other cross-reacting incretins. Therefore, in this study we aimed to systematically assess proglucagon cleavage products and their relation to metabolic health.

Materials and methods: Oral glucose tolerance test samples (0, 30, 60 and 120 minutes) were randomly selected from an extensively phenotyped prediabetes cohort. Highly sensitive ELISAs were used to quantify glucagon (0.8 % cross-reactivity with glicentin and <0.3 % with GLP-1), glicentin and GLP-1. Glucagon 1-61 was measured with a prototype ELISA from Mercodia (0.5 % cross-reactivity with glicentin and < 0.2 % with glucagon respectively). Relative change of RIA-measured glucagon was used to define individuals as "glucagon suppressors" and "non-suppressors". A linear mixed model was applied to evaluate differences in the measured analytes between the groups.

Results: In 36 % of the participants, glucagon concentrations quantified with RIA were non-suppressed at 2 hours after oral glucose load. Non-suppressors showed lower fasting glucagon levels compared to suppressors ($p=0.011$). Similar to RIA measurements, ELISA-measured fasting glucagon was lower in non-suppressors ($p<0.001$). Variance in RIA-measured glucagon was mostly explained by ELISA-measured glucagon (93%) and to a small extent by glicentin (5%). Glucagon 1-61 and glicentin kinetics were different between suppressors and non-suppressors (glucagon 1-61: $p=0.004$, glicentin: $p=0.002$) with higher concentrations of both hormones in non-suppressors. Insulin, C-peptide and free fatty acids were comparable between groups. Plasma glucose concentrations were lower in non-suppressors ($p=0.047$). Non-suppressors were leaner ($p=0.03$) and had lower waist circumference ($p=0.04$). Despite comparable liver fat content and insulin sensitivity ($p\geq0.3$), they had lower 2-hour post-challenge glucose concentrations ($p=0.01$).

Conclusion: Glucagon 1-61 and glicentin partially account for RIA-derived glucagon measurements due to cross-reactivity of the assay. However, this contribution is small, since the investigated proglucagon cleavage products contribute less than 10% to the variation in RIA measured glucagon. Altered glucagon levels together with higher post-challenge incretin levels in the non-suppressor group are associated with significantly lower 2-hour glucose as well as lower BMI. Since both features are strongly linked to lower risk for cardiovascular events, cancer incidence, and reduced mortality, higher post-challenge proglucagon cleavage products appear to be indicative of metabolic health.

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Effects of irisin on human pancreatic islets from type 2 diabetic subjects

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Background and aims: Irisin is a hormone secreted by skeletal muscle following physical activity and excess of saturated fatty acids, able to improve metabolic homeostasis and promote energy expenditure. In a previous study, we have demonstrated that, in beta-cells, irisin promotes proliferation and glucose-stimulated insulin secretion (GSIS), increases insulin content levels, and reduces lipotoxicity-induced apoptosis. The aim of this study was to evaluate the effects of irisin on the function and survival of pancreatic islets isolated from patients with type 2 diabetes mellitus (T2DM), characterized by reduced ability to secrete insulin and high levels of apoptosis, and to investigate the molecular mechanisms underlying this action.

Materials and methods: Pancreatic islets isolated from T2DM patients (n=9) and non-diabetic subjects (ND) (n=6), as well as INS-1E cells, were exposed to 100 nM irisin for different times. GSIS and insulin content levels were measured by ELISA assays; apoptosis was evaluated by the measurement of cytoplasmic oligosomes concentration and TUNEL assay; finally, the activation of the main mediators of beta-cell intracellular signalling was evaluated by immunoblotting (AKT, CREB and PKA) or fluorimetric assay (cytoplasmic calcium level).

Results: Irisin increased GSIS approximately 2-fold ($p<0.05$) in both ND and T2DM islets. Notably, in T2DM islets, characterized by reduced GSIS, this was restored to the levels of ND islets. In addition, irisin increased insulin content (1.6-fold in ND, 1.9-fold in T2DM, $p<0.05$) and significantly decreased apoptosis levels in T2DM islets. Moreover, irisin was able to activate AKT and CREB under conditions of high glucose in the ND islets, while, in the T2DM islets, AKT or CREB activation was not detected. Similarly, in INS1-E cells exposed to glucotoxic diabetogenic milieu, irisin was not able to promote AKT and CREB phosphorylation. Interestingly, glucotoxicity blunted the glucose-evoked increase in cytoplasmic calcium levels, while irisin restored it to basal levels.

Conclusion: In conclusion, the myokine irisin is able to increase the secretory function and survival of human pancreatic islets and to improve the functional defects typical of the T2DM islets. The mechanisms underlying the secretagogue effects of irisin in the T2DM islets do not apparently involve AKT and CREB activation. On the other hand, irisin could act directly on insulin granules exocytosis machinery, since it is able to restore the physiologic cytoplasmic calcium influx in response to acute increases in glucose concentrations.

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Disclosure: **N. Marrano:** None.

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Association between bone biomarkers Osteoactivin and Osteoprotegerin with plasma levels of irisin and meteorin-like protein in people with type 2 diabetes and obesity

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Background and aims: In our previous studies, we have shown that plasma levels of Meteorin-like protein (METRNL) and irisin were significantly higher in T2D people compared to non-diabetic individuals which were further exacerbated with obesity. Osteoporosis is a known

complication linked to obesity and T2D. Knowing that irisin is involved in bone remodeling, our objective was to investigate the association of irisin and METRNL with various bone markers in individuals with obesity and T2D in our population.

Materials and methods: A total of 228 individuals were enrolled in this study, including 124 non-diabetic (73 non-obese and 51 obese) and 104 T2D (38 non-obese and 66 obese). Plasma level of METRNL and irisin were assessed using ELISA. Multiplex assay was used to assess the level of various bone markers namely, Osteoactivin, Syndecan, Osteoprotegerin (OPG) and Secreted Protein Acidic And Cysteine Rich (SPARC).

Results: Plasma level of METRNL (1272.34 ± 24.87 pg/ml) and irisin (634.93 ± 21.06 pg/ml) were significantly higher in T2D people compared to non-diabetic individuals (1210 ± 20.29 pg/ml , P = 0.05 and 550.86 ± 14.74pg/ml ,P ≤ 0.001 respectively). This was further exacerbated with obesity. In the current study, we observed that the plasma level of bone markers Osteoactivin (20.614 ± 0.58ng/ml), Syndecan (2075.57 ± 97.93 pg/ml), OPG (989.55 ± 33.08 pg/ml) and SPARC (1118.75 ± 108.29 ng/ml) were significantly higher in T2D individuals compared to Non-diabetics (16.591 ± 0.29ng/ml, 1748.32 ± 63.11 pg/ml, 726.76 ± 17.39 pg/ml and 810.86 ± 49.72 ng/ml respectively) (P-values ≤0.01). When we classified the population on the basis of obesity, we observed a significant increase in the level of Osteoactivin (19.128 ± 0.44 ng/ml) and OPG (890.43 ± 28.28 pg/ml) in obese when compared to non-obese individuals (16.93 ± 0.4ng/ml and 752.87 ± 20.9 pg/ml respectively) (P-values ≤0.01). Correlation analysis on the non-diabetic population (age adjusted) showed a strong positive association of irisin with Osteoactivin (r=0.32, P ≤ 0.001) and OPG (r =0.21, P = 0.01). On the other hand, METRNL level showed a strong positive association with Osteoactivin (r=0.2, P=0.01).

Conclusion: Our data showed an increase in plasma levels of irisin, METRNL and bone markers (Osteoactivin and OPG) in obesity and T2D. We also observed a strong positive correlation of these myokines with Osteoactivin that is known to play a key role as a bone remodeling factor. Therefore, we suggest that the association seen through our study between these adipomyokines and the bone markers may indicate a dysregulation in the functional interaction between these molecules that could play a possible role in the development of bone related complications associated with obesity and T2D.

Supported by: Kuwait Foundation for the Advancement of Sciences

Disclosure: **P. Cherian:** None.

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Post prandial glucagon metabolism in humans with and without type 1 diabetes

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Background and aims: Inappropriately elevated postprandial (PP) glucagon concentrations contribute to hyperglycemia in Type 1 Diabetes (T1D). To define the underlying mechanisms, we applied isotope dilution methods to estimate PP glucagon turnover.

Materials and methods: We infused $^{13}C^{15}N$ glucagon tracer in 17 non-diabetic (ND) and 17 T1D subjects during a mixed meal (75 gm carb, 15% fat, 35% protein; 8 kcal/kg). T1D subjects administered prandial insulin dose based on their customary insulin: carb ratio and correction factor at the start of the meal. Data from 15 healthy (age 28 ± 7 yrs, BMI 24.6±2.7 kg/m^2, fasting plasma glucose 4.8±0.2 mM, HbA1c 5.0±0.2%) and 5, C-peptide negative T1D (age 37 ± 13 yrs, BMI 26.9 ± 2.4 kg/m^2, fasting plasma glucose 10.2 ± 3.3 mM, HbA1c 7.4 ± 1.2%) subjects analyzed thus far are shown. Arterialized venous samples were obtained periodically for measurements of $^{13}C^{15}N$ glucagon (tandem mass spectrometry), glucagon (Mercodia), and glucose (YSI) concentrations throughout the study. Systemic glucagon turnover was calculated by non-steady-state, single compartment equation after determining volume of distribution of glucagon.

Results: Integrated PP glucose (811 ± 530.2 vs. 298 ± 191.4 mM/3 hrs: T1D vs. ND; p<0.02) and glucagon concentrations were higher (2.1 ± 0.9 vs. -0.2 ± 2.0 ng/ml/3 hrs; T1D vs. ND; p<0.01) in T1D subjects during the first 3 hours after meal ingestion. Integrated rates of glucagon appearance (4.0 ± 6.6 vs. -2.5 ± 2.0 ng/kg/3 hrs: T1D vs. ND; p=0.15; Fig 1: upper panel) and disappearance (7.8 ± 5.4 vs. 1.3 ± 20.6 ng/kg/3 hrs: T1D vs. ND; p=0.38) were numerically but not statistically higher in T1D than healthy subjects during 0-180 min after meal. However, integrated glucagon clearance during 0-180 minutes was lower (-3.5 ± 4.4 vs. 0.9 ± 1.9 ml/kg/3 hrs: T1D vs. ND; p<0.001; Fig 1: lower panel) in T1D subjects.

Conclusion: These data suggest that lower glucagon clearance contribute to elevated PP glucagon concentrations during the first three hours after meal ingestion in T1D. Data analyses of remaining study subjects will further clarify the contributions of rates of glucagon appearance and disappearance to differences in PP glucagon concentrations in T1D.

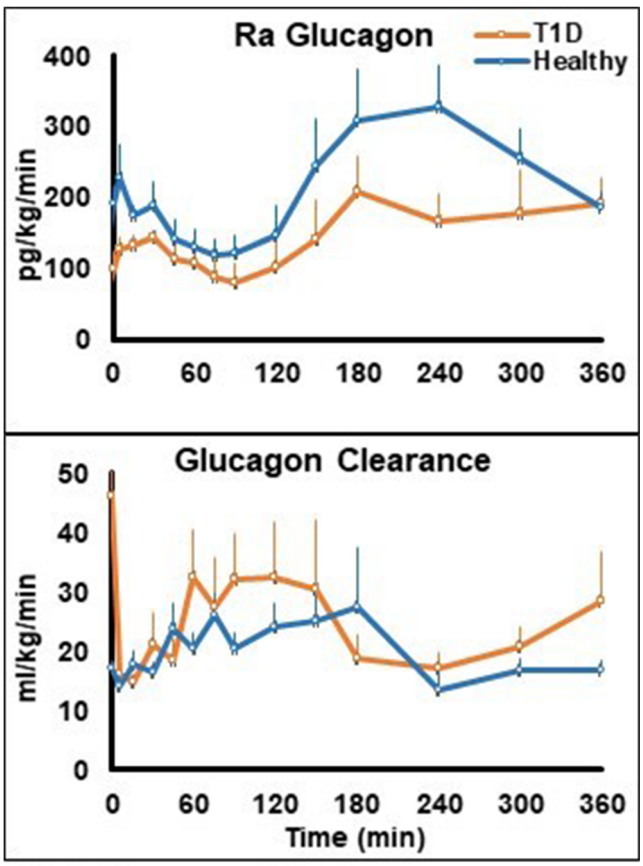

Fig 1: Upper panel: Ra Glucagon
Lower panel: Glucagon Clearance

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Disclosure: **R. Ruchi:** None.

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Tmem117 in vasopressin neurons is a novel regulator of counterregulatory response to hypoglycaemia

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Background and aims: The counterregulatory response (CRR) to hypoglycemia, is an essential survival function. Glucose responsive neurons of the central nervous system (CNS) play a crucial role in the initiation of the CRR, but the molecular mechanisms underlying this coordinated response remain largely unexplored. With the current study we aimed to identify novel hypothalamic regulators of hypoglycemia-induced glucagon (GCG) secretion.

Materials and methods: We performed a genetic screen for insulin-induced glucagon secretion in a panel of 36 BXD recombinant inbred mouse strains and, separately, characterized their hypothalamic transcriptome by RNA sequencing. Mice with a cell type-specific gene inactivation were used in physiological studies to characterize the function of the identified gene.

Results: By combining cQTL for hypoglycemia-induced GCG secretion and eQTL data, we identified *Tmem117* as a potential regulator of GCG secretion. Immunofluorescence microscopy analysis (IF) on brain slices showed Tmem117 to be expressed in vasopressin (AVP) magnocellular neurons of the hypothalamus. c-Fos IF revealed that these AVP neurons were activated by insulin-induced hypoglycemia. Ex vivo slice electrophysiological recordings from AVP neurons of the supraoptic nucleus (SON) revealed that a subpopulation is activated by hypoglycemia (GI neurons). In line with these observations, plasma levels of copeptin (CPP) (a surrogate for AVP) were elevated 1 hour after insulin injection (sal: 38.5±7, ins: 89.7±5.3; pg/ml; p<0.001; 2way-ANOVA RM). To determine the role of Tmem117 in AVP neurons, we generated *Tmem117*$^{flx/flx}$ mice and induced *Tmem117* gene inactivation by injection in the posterior pituitary of an adenovirus expressing cre recombinase under the control of the AVP promoter (cKO). This led to higher hypoglycemia-induced CPP secretion and higher plasma GCG levels [CPP (WT: 89.7±5.3, cKO: 121.4±13.5); GCG (WT: 124±19, cKO: 185.8±21); pg/ml; p<0.01; 2way-ANOVA RM]. When studied over the longer term, cKO mice exhibited progressive loss of these neurons (WT: 286.7±15.4, cKO: 208.7±12.3; p<0.05; t-test). In line with this observation, the phenotype was also time dependent. Repeating the insulin induced hypoglycemia model 1 month after Tmem117 deletion revealed no difference between WT and cKO mice in CPP and GCG levels [CPP (WT: 55.4±14.1, cKO: 83.2±5.7); GCG (WT: 81.3±10.9, cKO: 119.5 ±18.9); pg/ml; p>0.05; 2way-ANOVA RM]. Interestingly, the described secretory phenotype was observed in male mice and only during the proestrus phase in females, suggesting a possible implication of sex hormones in the underlying molecular mechanism.

Conclusion: Our genetic screen identifies Tmem117 as a novel regulator of hypoglycemia-induced CGC secretion, expressed in AVP magnocellular neurons. The secretory activity of AVP neurons is increased by hypoglycemia and inactivation of Tmem117 augments hypoglycemia-induced copeptin secretion and plasma glucagon levels. This effect is observed in male mice and only during the proestrus phase in female mice. Tmem117 inactivation leads to cell death over time. Overall, Tmem117 is a novel regulator of CRR that affects AVP secretion and neuronal survival, and its action appears to be sexually dimorphic.

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Disclosure: **S. Gaspari:** None.

SO 19 Gastro-entero pancreatic factors

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Importance of endogenous GLP-1 and GIP for postprandial glucose tolerance after Roux-en-Y gastric bypass and sleeve gastrectomy surgery

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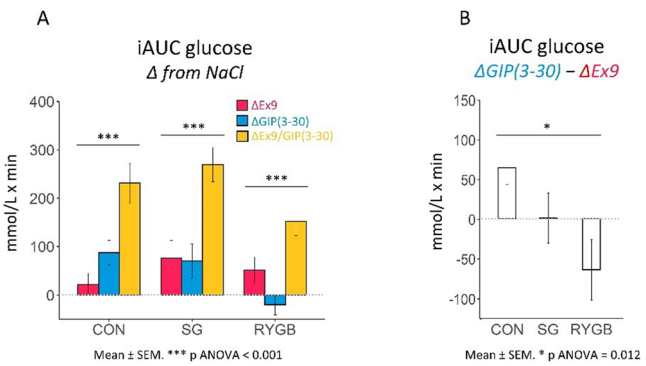
Background and aims: Roux-en-Y gastric bypass (RYGB) dramatically increases postprandial GLP-1 concentrations whereas postprandial GIP concentrations are modestly enhanced after sleeve gastrectomy (SG). We hypothesized that GLP-1 is more important after RYGB and GIP more important after SG, and we investigated effects of single and combined GLP-1 and GIP receptor antagonism on postprandial glucose tolerance after these procedures.

Materials and methods: Normal glucose-tolerant and weight stable unoperated (CON, n=12), SG-operated (n=11), and RYGB-operated (n=12) participants were matched on age, sex, and BMI. Participants underwent four liquid mixed meal tests in random order during continuous infusions of saline, GLP-1 receptor antagonist exendin(9-39)NH_2 (Ex9), GIP receptor antagonist GIP(3-30)NH_2 (GIP(3-30)), or combined Ex9 and GIP(3-30).

Results: Incremental AUC of glucose concentrations increased (Δ from saline) more during combined compared with single incretin hormone receptor antagonism in all three groups: *CON* (ΔEx9: 22 [−27;71] (mean [95%CI]) mmol·L^{-1}·min, ΔGIP(3-30): 87 [31;144], ΔEx9/GIP(3-30): 232 [141;322], p ANOVA < 0.001); *SG* (ΔEx9: 76 [−6;158], ΔGIP(3-30): 71 [−8;149], ΔEx9/GIP(3-30): 270 [190;349], p ANOVA < 0.001); *RYGB* (ΔEx9: 51 [−9;111], ΔGIP(3-30): −20 [−66;26], ΔEx9/GIP(3-30): 152 [87;217], p ANOVA < 0.001) (figure 1A). The relative effect of GIP versus GLP-1 receptor antagonism (ΔGIP(3-30) − ΔEx9) on incremental AUC of glucose concentrations was greatest in CON and lowest in RYGB (*CON*: 65 [20;110], *SG*: −5 [−82;71], *RYGB*: −71 [−149;6]) (figure 1B).

Conclusion: The relative importance of endogenous GIP versus GLP-1 for postprandial glucose tolerance is reduced after RYGB due to the combination of numerically greater effect of GLP-1 and reduced effect of GIP compared with CON. After SG, there was a smaller and non-significant reduction in the relative importance of GIP versus GLP-1 as a result of numerically greater effect of GLP-1 and maintained effect of GIP.



Clinical Trial Registration Number: NCT03950245

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Disclosure: **M. Hindsø:** Grants; Novo Nordic Foundation Excellence Project Grant (NNF18 OC0032330).

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Characterisation of biological activity and antidiabetic efficacy of a novel neurotensin/xenin fusion peptide in high fat fed mice

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Background and aims: Neurotensin (NT) and xenin (XN) have been shown to possess notable antidiabetic potential, primarily through augmentation of the bioactivity of the incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), respectively. In the current study, biologically active fragment peptides of NT and XN, namely acetyl-NT(8-13) and XN-8-Gln, were fused together to create Ac-NT/XN-8-Gln. Following assessment of enzymatic stability, effects of Ac-NT/XN-8-Gln on *in vitro* insulin secretion and beta-cell proliferation were studied. In addition, sub-chronic antidiabetic efficacy of Ac-NT/XN-8-Gln alone, and in combination with exendin-4, was assessed in high fat fed (HFF) mice.

Materials and methods: Ac-NT/XN-8-Gln was incubated with murine plasma (0-4 h) to assess *in vitro* enzyme stability. BRIN-BD11 beta-cells (n=8) were used to evaluate the acute (20 min) effects of Ac-NT/XN-8-Gln (10^{-12} - 10^{-6} M) on insulin release. Actions of Ac-NT/XN-8-Gln (10^{-8} and 10^{-6} M) on beta-cell proliferation, by Ki-67 antibody staining, were also examined in BRIN BD11 cells (n=3). To assess antidiabetic benefits, HFF mice (n=8) were employed and received twice daily (09:30 and 17:00 h) injections of saline vehicle, Ac-NT/XN-8-Gln, exendin-4 or a combination of both peptides (all at 25 nmol/kg bw) for 32 days. Energy intake, body weight, blood glucose and plasma insulin concentrations were measured at regular intervals. Intraperitoneal glucose tolerance (18 mmol/kg), and insulin sensitivity (10 U/kg) tests were conducted at the end of the study. Terminal analyses included assessment of HbA1c and plasma lipid status as well as examination of pancreatic insulin content and islet architecture.

Results: Ac-NT/XN-8-Gln remained fully intact following incubation in murine plasma for 4 h. The fusion peptide enhanced (P<0.001) insulin secretion from BRIN-BD11 beta-cells, as well as augmenting (P<0.001) GIP-induced insulin secretion. Ac-NT/XN-8-Gln also increased (P<0.01) proliferation of BRIN BD11 beta-cells. Twice daily treatment of HFF mice with Ac-NT/XN-8-Gln for 32 days resulted in a sustained improvement in glycaemic control and circulating insulin concentrations, with these beneficial effects significantly (P<0.05 to P<0.001) enhanced by combined exendin-4 treatment. This was also reflected by reduced (P<0.01) HbA1c levels on day 32 in the combined treatment group. Exendin-4 reduced (P<0.05) body weight and food intake, with combined therapy provoking the greatest (P<0.001) reduction in body fat mass. Following an oral glucose challenge, glucose levels were markedly decreased (P<0.05) in HFF mice treated with Ac-NT/XN-8-Gln in combination with exendin-4. The combined treatment group also presented with improved (P<0.05) peripheral insulin sensitivity in accordance with decreased (P<0.001) pancreatic insulin content. Both exendin-4 alone, and in combination Ac-NT/XN-8-Gln, reduced (P<0.01) plasma cholesterol, but only combined therapy decreased (P<0.05) circulating triacylglycerols. Interestingly, islet and beta cell areas were unaffected by Ac-NT/XN-8-Gln but were significantly (P<0.05 to P<0.01) increased by combined therapy with exendin-4.

Conclusion: These data reveal that Ac-NT/XN-8-Gln is a biologically active fusion peptide and displays prominent antidiabetic efficacy when administered together with exendin-4 in HFF mice.

Supported by: Invest NI, PoC funding

Disclosure: **N. Irwin:** None.

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Circulating dopamine is regulated by gut nutritional signals and acts in the adipose tissue to sensitise for GLP-1 action

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Background and aims: Previous studies from our group have shown that peripheral dopamine acts in the liver and white adipose tissue (WAT) to regulate insulin signalling, glucose uptake and lipid metabolism. Moreover, expression of dopamine receptors is decreased in the visceral adipose tissue of patients with insulin resistance and Bromocriptine (D2 agonist) treatment to diabetic obese rats restores WAT and liver dopaminergic signalling, while increasing lipid oxidation and insulin sensitivity. Given that dopamine was also observed to inhibit insulin secretion, we hypothesized that peripheral dopamine could be regulated by dietary factors in the gut and could modulate the gut nutrient-sensing mechanisms like GLP-1.

Materials and methods: Wistar rats (10-12 w.o.) were submitted to gavage (3ml) of different nutrients (glucose, starch, corn oil, L-arginine, albumin) and a liquid mixed meal (Fortimel, Nutricia) and blood samples were collected before and several time points later (15, 30 and 60') for plasma dopamine determination. Gut involvement in dopamine secretion and action was evaluated in diabetic Goto-Kakizaki (GK) rats (GKHCD) fed with a high-caloric diet (1-6 m.o.) rats submitted to sleeve gastrectomy at 4 m.o. A group of GKHCD rats were submitted to Bromocriptine treatment (last month) to determine GLP-1 receptor (GLP-1R) expression. The expression of dopamine and GLP-1 receptors was determined in visceral adipose tissue samples from obese patients.

Results: Plasma dopamine levels were increased after the consumption of a mixed meal. When studying isolated nutrients, only glucose and starch led to a postprandial rise of plasma dopamine, being more delayed in response to starch. Gut remodelling after sleeve gastrectomy also resulted in higher plasma dopamine levels in response to a mixed meal. In the same rats, the expression of dopamine receptors and tyrosine hydroxylase was also increased in the WAT. In turn, in animals treated with Bromocriptine, GLP-1R was upregulated in the white and brown adipose tissue, while no changes were observed in pancreas, liver, skeletal muscle and hypothalamus. In the visceral adipose tissue of obese patients, GLP-1R and dopamine receptors expression was correlated, suggesting the crosstalk between the molecular pathways (ρ=0.373, p<0.001 vs D1DR; (ρ=0.269, p<0.01 vs D4DR).

Conclusion: Peripheral dopamine secretion and signalling are regulated by meal nutrients and gut remodelling. Dopamine upregulates GLP-1R signalling in adipose tissue, which may be involved in the modulation of adipose tissue metabolic function and may be a new therapeutic strategy for obesity and related pathologies.

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Disclosure: **P. Matafome:** None.

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Pro-neurotensin levels and the prediction of cardiovascular risk in individuals with type 1 diabetes: a longitudinal study with 10 year follow-up

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Background and aims: Neurotensin (NT) is a gut hormone that promotes lipids absorption and controls appetite. Elevated circulating pro-NT, the stable precursor of NT, is associated with cardiovascular (CV) disease, metabolic syndrome (MS) and type 2 diabetes. Features of MS and insulin resistance are reported also in type 1 diabetes, with detrimental impact on the overall CV risk profile. This study aimed to evaluate plasma pro-NT in type 1 diabetes and tested whether its levels are associated with and/or predictive of CV risk factors and overall risk profile.

Materials and methods: For this longitudinal retrospective study, we analyzed data from forty-one individuals with type 1 diabetes referring to our Diabetes outpatient clinics. Plasma pro-NT levels were measured by a chemiluminometric immunoassay and tested also in thirty-four age-sex- BMI-comparable healthy individuals. The insulin sensitivity was calculated by using the estimated Insulin Sensitivity (eIS) formula (eIS = exp (4.1075 − 0.01299 [waist circumference, cm] − 1.05819 [insulin dose, daily units per kg] − 0.00354 [triglycerides, mg/dL] − 0.00802 [diastolic blood pressure, mm Hg]).

Results: Pro-NT did not differ significantly between patients and controls (median [IQR] pro-NT: 156.3 [96.6-198.2] vs. 179.4 [139.7-230.7] pmol/L respectively, p= 0.26). In type 1 diabetes subjects, greater circulating pro-NT associated with poor glycemic control at baseline as indicated by greater fasting blood glucose and HbA1c levels (r= 0.29, p= 0.05 and r= 0.4, p= 0.02, respectively), and predicted increased waist circumference (r= 0.37, p= 0.01), reduced insulin sensitivity (r= -0.77, p< 0.001) and the development of dyslipidemia (r= 0.72, p<0.001) and hypertension (r= 0.53, p<0.001) at 10-year follow-up, resulting in increased CV risk. High pro-NT predicted the development of very high CV risk, according to ESC criteria, after 10 years with an adjusted OR=11 (95%C.I.: 1.4-94.5; p= 0.029).

Conclusion: In type 1 diabetes individuals, elevated pro-NT levels predict the development of cardiometabolic risk factors, comprising visceral adiposity, dyslipidemia and hypertension at 10- year follow-up. Pro-NT represents a novel predictor/marker of CV risk in adults with type 1 diabetes.

Supported by: Sapienza University, Rome, Italy

Disclosure: **I. Barchetta:** None.

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The feature of the gut microbiota in patients with newly diagnosed type 2 diabetes

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Background and aims: Gut microbiota (GM) is a complex of bacteria that colonize the gastrointestinal tract. Changes in the composition of GM leads to the onset of type 2 diabetes mellitus (T2DM). Thus, a decrease of bacteria metabolizing cellulose to butyrate (Roseburia, Faecalibacterium, Coprococcus, Eubacterium, Akkermansia) increases the risk of T2DM, as the intestinal barrier function decreases and the risk of development of systemic inflammation increases, the intestinal gluconeogenesis does not arise, the synthesis of incretin hormones and insulin decreases.The decrease of Bacteroides leads to a decrease of synthesis of incretin hormones, as the production of indole is decreased by Bacteroides. The decrease of Bifidobacterium and some strains of Lactobacillus lead to the development of insulin resistance because Bifidobacterium improves GLUT-4 translocation, and some strains of Lactobacillus increase the expression of GLUT-4 in adipocytes and myocytes. The decrease of Akkermansia and Christensenella is associated with the development of obesity, which is a risk factor for the development of T2DM. In this research, we have investigated the dynamics of the decrease of these bacteria in patients with newly diagnosed T2DM.

Materials and methods: 50 patients with newly diagnosed T2DM have done a genetic study of the human intestinal metagenome - 16S rRNA sequencing.

Results: Of the 50 patients 46% (n = 23) were men, 54% (n = 27) were women. The average age of the patients was 54.8 ± 11.7 years (95% CI 51.4 - 58.1). The median of fasting blood glucose was 12.01 mmol / l [8.5; 18.2]. The average level of glycated hemoglobin (HbA1c) was 9.0 ± 2.0% (95% CI 8.4 - 9.6). The most common types of bacteria among patients with T2DM were Firmicutes 58.9 ± 12.1% (95% CI 55.4 - 62.3), Bacteroidetes 30.6 ± 13.2% (95% CI 26.9 - 34, 4), Proteobacteria 2.81% [1.52; 4.13], Actinobacteria 1.08% [0.44; 3.02], Verrukomicrobia 0.85% [0.14; 3.36]. In 92.0% of patients, the Firmicutes / Bacteroidetes ratio > 1, Me 1.82 [1.2; 3.0]. There was a significant decrease in the alpha diversity index compared to the norm (more than 7): 5.7 ± 0.7 (95% CI 5.5 - 5.9), p <0.05. A correlation analysis was carried out, which showed that with an increase of the level of Bacteroidetes, the alpha diversity index decreased (r = -0.37, p <0.05), with an increase of Firmicutes, it increased (r = 0.48, p <0.05). Most patients were detected the decrease of the number of genera of bacteria preventing the development of T2DM: in 94.0% of patients was a decrease in Coprococcus, 84.0% - Akkermansia, 70.0% - Bacteroides, 58.0% - Faecalibacterium, 52.0% - Biffidobacterium , 42.0% Roseburia, 38.6% Lactobacterium, 30.0% Eubacterium. Also, patients with T2DM have been detected a decrease in the number of bacteria preventing the development of obesity: 98.0% of patients were the decrease in Christensenella. Moreover, an inverse correlation was established between the level of fasting glucose and Faecalibacterium (r = -0.33, p <0.05). Also, it was found that people with a higher level of HbA1c had a decrease in the level of Faecalibacterium (r = -0.31, p <0.05), while people with a higher body mass index had an increase in the level of Roseburia (r = 0.42, p <0.05). In addition, a direct relationship was found between Faecalibacterium and Roseburia (r = 0.40, p <0.05).

Conclusion: The predominance of Fermicutes over Bacteroides, a decrease of the alpha-diversity index, a decrease of bacteria preventing the onset of T2DM are observed in patients with newly diagnosed T2DM.

Disclosure: **K.G. Lobanova:** None.

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Altered hormonal milieu in pancreatic islets and intestine accompany alterations of metabolism in high-fat fed Wistar rats

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Background and aims: Classical gut hormones glucose-dependent-insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1) and Peptide YY (PYY) regulate metabolism by controlling feeding behavior and glucose homeostasis. During obesity, attenuated feedback signals from the pancreas and small intestine contribute to changes of circulating fasting and post-prandial gut hormones. It is important to elucidate how this hormone expression differs in obesity, leading to insulin resistance and type 2 diabetes. In the present study, we have evaluated how prolonged consumption of high-fat diet influences islet and intestinal morphology together with the differential expression of the key entero-pancreatic hormones.

Materials and methods: Female Wistar rats were maintained on a high-fat diet for 20 weeks with measurement of body weight and blood glucose at regular intervals. Control animals were fed a normal chow diet. Excised pancreas and intestine tissues were used for detailed immunohistochemical analysis. Area and number of positively stained cells for insulin, glucagon, PYY, GLP-1 and GIP were calculated using ImageJ software.

Results: Prolonged high-fat diet significantly ($p<0.05$ to $p<0.01$) increased islet area, beta cell area and alpha cell area in female Wistar rats. Significant ($p<0.001$) increases in percentage of islet beta cells was observed in the high-fat group compared to controls. In contrast, relative proportion of alpha cells was decreased significantly ($p<0.001$) in the high-fat group compared with controls. PYY and delta cell areas exhibited significant ($p<0.001$) increases under high fat diet regime. Although, the numbers of large islets were higher in the high-fat group, no significant difference was observed. Co-localization of islet PYY with somatostatin and glucagon showed no significant difference in high-fat rats compared to control. Similarly, no change in GLP-1 co-expression in glucagon expressing alpha cells was seen. On quantifying beta cell apoptosis and proliferation, significant ($p<0.05$) increases of TUNEL positive beta cells in high-fat group was observed compared to controls, with no change in beta cell proliferation frequency. Alterations in intestinal morphology were observed in high fat fed rats as villi length in the ileum significantly ($p<0.001$) decreased compared with controls. Significant ($p<0.001$) decreases in the number of GLP-1 positive cells were also observed in the high fat fed group. The significant decrease in GLP-1 expression was consistent in the ileal crypt ($p<0.01$) and villi ($p<0.01$) of high-fat fed rats. In contrast, expression of PYY and GIP in the ileum remain unchanged in the high fat group.

Conclusion: High fat feeding induces prominent changes in the morphology and endocrine cellular composition of the pancreatic islets and small intestine. These changes undoubtedly contribute to manifestation of dietary-induced insulin resistance and their role in reversal of remission following bariatric surgery awaits elucidation.

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Disclosure: **A. Sridhar:** None.

SO 20 Carbohydrate and protein metabolism

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The postprandial methylglyoxal formation during an oral glucose tolerance test is derived from exogenous glucose

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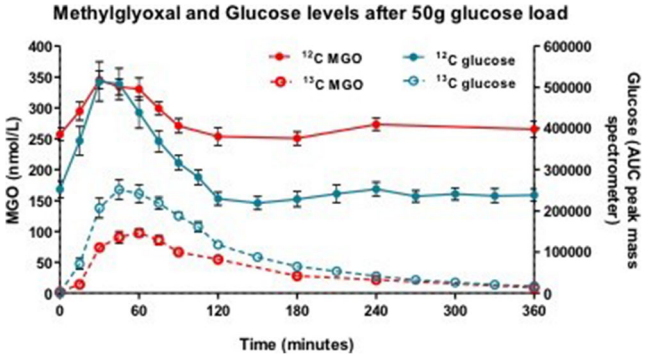
Background and aims: Methylglyoxal (MGO), a reactive dicarbonyl compound and a major precursor in the formation of advanced glycation endproducts, is mainly formed as a byproduct of glucose during glycolysis. Excess formation and accumulation formation and accumulation of MGO are linked to diabetes and its vascular complications. We have previously shown that plasma MGO concentrations rapidly increased both during an oral glucose tolerance test (OGTT) or a mixed meal test, with a higher increase in individuals with type 2 diabetes. Spikes of MGO are believed to be a major contributor for vascular complications. However, the exact source of MGO is unknown. The aim of the study was therefore to investigate whether postprandial MGO formation directly originates from exogenous glucose.

Materials and methods: We performed a stable isotope labeled OGTT in 12 healthy males (age 25 yrs (range 21-30 yrs), BMI 22.5 kg/m2 (range 19.2-24.7 kg/m2). The treatment was a solution of 50 g glucose of which 2% is universally labeled D(+)^{13}C glucose and 98% unlabelled ^{12}C glucose. Blood samples were taken every 15 min for first two hours and every half an hour for the next four hours after the intake of glucose. Concentrations of MGO and glucose in plasma during OGTT (6 h) were measured at eleven time-points with ultra-performance liquid chromatography-tandem mass spectrometry. ^{13}C MGO data were corrected for enrichment (*50) and for a difference in mass spectrometry response factor between ^{13}C MGO and ^{12}C MGO.

Results: During the first 180 min of the OGTT, plasma ^{12}C glucose levels increased rapidly and reached a peak at 30 min, after which levels started to decline with the lowest level at 180 min post-load. After 180 min, plasma concentration of ^{12}C glucose showed a slight increase. The plasma ^{13}C glucose curve showed the same pattern as for ^{12}C glucose, but the increase after 180 min was not observed, probably reflecting formation of ^{12}C glucose in the second phase as a compensation for insulin-mediated glucose lowering below baseline levels. During the first 180 min of the OGTT, plasma ^{12}C MGO and ^{13}C MGO followed the same pattern with a rapid increase with a peak at 45 min, after which levels started to decline, with the lowest level at 180 min post-load. After 180 min post-load, ^{12}C MGO, but not ^{13}C MGO, showed a clear increase. We calculated that newly formed MGO in the early phase of the OGTT is completely derived from exogenous glucose, while the increase of MGO after 180 min is not.

Conclusion: These data show that the rapidly increase in levels of plasma MGO during an OGTT arise from exogenous glucose, while the increase of MGO after 180 min originates from an endogenous substrate. Where exactly MGO is produced in the postprandial phase is subject of further research.



Disclosure: **X. Zhang:** None.

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Glucose suicide mechanisms induced by methylglyoxal

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Background and aims: Methylglyoxal (MG) is a highly reactive dicarbonyl compound derived mainly from glucose metabolism. MG has been deeply linked to the pathogenesis of diabetic complications. Hyperglycemia-associated accumulation of MG resulting from glucose overflow results in a marked production of advanced glycation end products (AGEs). These AGEs impair the cellular redox balance and modulate several cell death pathways. Therefore, this present study aimed to evaluate the influence of MG on the glucose uptake and cell death response in hyperglycemic cells stably overexpression of GLUT4 after short or long-term cultivation in different glucose concentrations.

Materials and methods: H9C2 cells stably overexpressing GLUT4 (H9C2GLUT4) and wide type cells (H9C2) were incubated with 5, 20 or 30mM of glucose either for 3 or 6 months. Afterwards, cells were treated with 25µM MG for 24h or 100nM insulin for 30min or cotreated with both treatments. Following these interventions, H9C2GLUT4 and H9C2 cells were co-incubated with 2-NBD-Glucose (NBDG) for 0, 5, 20 and 60 minutes, in order to measure glucose uptake. In parallel, further samples were used for quantifying the level of apoptosis by using Annexin V staining and necrosis by using Propidium Iodide labeling. Nonparametric t-tests were used to analyse for significance, $p<0.05$ was regarded statistically significant.

Results: After insulin treatment, the uptake of glucose increased 2-fold in H9C2GLUT4 when compared to H9C2. This occurs, particularly, after 20 minutes stimulation in cells exposed to 20mM: 31% *vs* 17%, $p<0.01$ after 3 months; 27% *vs* 8%, $p<0.05$ after 6 months or 30mM: 43% *vs* 15%, $p<0.001$ after 3 months. Similarly, MG treatment increased the uptake of glucose in all the different concentrations: 5mM: 30% *vs* 13%, $p<0.01$ after 20 min; 52% *vs* 22%, $p<0.05$ after 60 min, 20mM: 54% *vs* 44%, $p<0.01$ after 60 min and 30 mM: 16% *vs* 12%, $p<0.01$ after 5 min; 50% *vs* 39%, $p<0.05$ after 60 min over 3 months and 6 months exposition. Co-treatment with MG and insulin lowered the glucose uptake in H9C2GLUT4 stimulated with 5mM: 20% *vs* 4%, $p<0.0001$ after 5min. However, at same concentrations, but longer time, the glucose uptake increased 14% *vs* 26%, $p<0.0001$ after 20 min and 12% *vs* 34%, $p<0.01$ after 60min. On the other hand, in higher glucose concentrations (20 and 30mM), the co-treatment was just able to significantly increase the glucose uptake after 5min with NBDG incubation: 4% vs 9% $p<0.001$ and 19% vs 13% $p<0.001$ over 3 months and 6 months exposition. Following the measurements, it was also found that H9C2GLUT4 cells stimulated with 20mM for 3 months had half fold less apoptosis events in groups treated or co-treated with MG ($p<0.01$ and $p<0.05$). However, H9C2GLUT4 cells stimulated with 30mM glucose had 3-fold ($p<0.01$ and $p<0.05$) more apoptosis events after co-treatment or treatment with insulin. On the other hand, insulin-only treatment increased the necrosis in H9C2GLUT4 cells stimulated with 20mM after 3 months (2-fold, $p<0.05$). Interestingly, in a similar period of time the overall treatment effectively reduced the necrosis in H9C2GLUT4 cells stimulated with 30mM.

Conclusion: Regardless of intervention time, MG treatment effectively hampers insulin mediated glucose uptake and also was able to activate a glucose suicide mechanism in hyperglycemic cells with overexpression of GLUT4 by increasing glucose influx. However, a co-treatment with insulin failed to worse these aforementioned effects, indicating that MG possibly is involved in insulin mediated glucose uptake.

Disclosure: **B. Stratmann:** None.

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Anti-hyperglycaemic effect of alpha-cyclodextrin after oral glucose loading via GLP-1 dependent- and independent-pathways

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Background and aims: We previously reported that administration of miglitol, an alpha-glucosidase inhibitor with maltose triggered glucagon-like peptide-1 (GLP-1) secretion, leading to suppression of the rise in blood glucose. In addition, short-chain fatty acid (SCFA) produced by microbiota was acutely increased in response to a single ingestion of maltose plus miglitol. Dietary fiber fermentation by microbiota is known to raise SCFA levels, contributing to host glycemic control. Therefore, we tested whether alpha-cyclodextrin (alpha-CD), a soluble dietary fiber derived from corn starch, affects blood glucose levels in response to its single administration.

Materials and methods: We performed oral glucose (2g/kg) tolerance test together with or without different doses of alpha-CD (1g/kg and 2g/kg). We evaluated the effect of alpha-CD by measuring blood glucose and GLP-1 levels in the portal vein. We also used Kir6.2 knockout mice (*Kir6.2*$^{-/-}$) to evaluate if the effect of alpha-CD on blood glucose is mediated by increasing insulin secretion.

Results: The rise in blood glucose was significantly suppressed by oral administration of glucose (2g/kg) plus alpha-CD (2g/kg). Interestingly, the suppressive effect was much stronger when alpha-CD was administered 30 min prior to glucose administration. Nevertheless, alpha-CD did not affect glucose-induced insulin secretion. In addition, glucose-lowering effect of alpha-CD was more robust in *Kir6.2*$^{-/-}$ mice that lack glucose-stimulated insulin secretion. GLP-1 secretion in portal vein was significantly increased by 2g/kg alpha-CD (3.78 ±0.45 pmol/l in vehicle; 30.89 ±4.05 pmol/l in alpha-CD, $p<0.001$), but not by a lower (1g/kg) dose, even though 1g/kg alpha-CD apparently suppressed the rise in blood glucose levels. We found that alpha-CD administration makes the intestinal contents turbid and that alpha-CD lowered blood glucose levels in the systemic circulation, but not in the portal vein. Conjugation of alpha-CD (1g/kg) with lecithin cancelled their blood glucose reduction in both wild-type and *Kir6.2*$^{-/-}$mice. Expression of the genes related to cholesterol synthesis was significantly increased in duodenum and jejunum at 30 min after alpha-CD administration.

Conclusion: A high dose of alpha-CD (2g/kg) stimulated GLP-1 secretion and delayed intestinal transit. By contrast, alpha-CD at a low (1g/kg) dose did not increase GLP-1 secretion, but it significantly suppressed blood glucose levels most likely via the inclusion of lecithin into its cylindrical space, leading to metabolic alteration of cholesterol in gut lumen and that of glucose in liver.

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Disclosure: **E. Lee:** None.

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Mechanisms of improvement in glucose tolerance and insulin sensitivity after complex carbohydrate meal in type 2 diabetes

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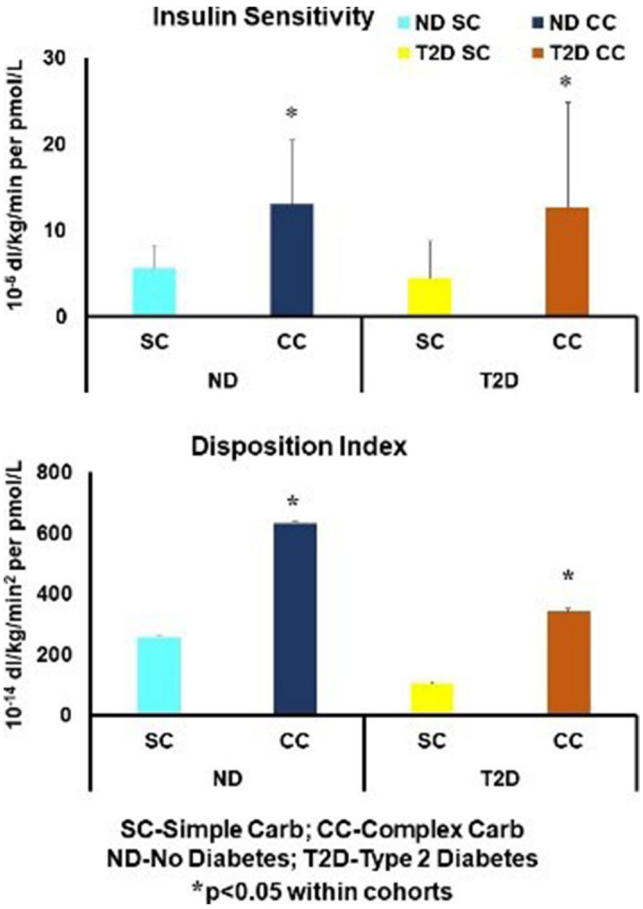
Background and aims: We have recently established a method utilizing the natural abundance of [^{13}C] polysaccharide enabling measurement of enrichment of [^{13}C] glucose in healthy humans. We now compare complex carb (CC) meal with a single tracer infusion of [6,6-$^{2}H_2$] glucose at -120 min to the established simple carb (SC) triple tracer protocol with infusion of [6,6-$^{2}H_2$] glucose at -120 min and [2-^{13}C] glucose at 0 min with meal ingested at time 0.

Materials and methods: We have completed study in 7 individuals with Type 2 diabetes (T2D: 2F, age = 65±7 yr; BMI = 33±3 kg/m^2; FFM = 61±12 kg; HbA1c 51±6 mM/mol) and 6 anthropometrically matched controls without diabetes (ND: 3F, age 56±10 yrs; BMI 30 ±4 kg/m^2; FFM = 56±10 kg). Healthy subjects underwent a 75 gm OGTT to rule out diabetes. Subjects ingested either sorghum (natural enriched ^{13}C) or glucose in the form of Jello labelled with ^{13}C mixed meals containing 75 gm carbs and matched protein and fat. We measured plasma glucose, insulin and C-peptide concentrations and used the oral glucose and C-peptide minimal model to assess insulin sensitivity (S_I), beta-cell responsivity to glucose (Φ) and disposition index (DI) in simple versus complex carbohydrate meals. Rates of meal glucose appearance (MRa), glucose disappearance (Rd) and endogenous glucose production (EGP) were calculated using the dual isotope tracer-tracee method.

Results: Fasting and post-prandial glucose excursions (iAUC) were significantly lower after CC ingestion compared to SC in both cohorts ($p<0.03$). MRa, and Rd were significantly lower during CC than SC meal in both groups ($p<0.001$) while EGP tended to be similar regardless of the type of meal consumed. Insulin sensitivity index (S_I) was significantly higher following CC than SC meals ($p<0.03$) in both cohorts with the magnitude of the difference being greater in ND. Insulin secretion index (Φ) was similar between SC and CC meals for both groups. Disposition Index (DI) was significantly higher during CC vs SC meals in both groups ($p<0.03$).

Conclusion: These data indicate that SI and DI are higher in people with and without type 2 diabetes when they eat a CC meal. We present a sophisticated methodology utilizing a single isotope infusion coupled with a preferred CC meal to measure carbohydrate turnover. We conclude that CC meal result in lower glucose excursions with improved meal tolerance and beta cell function, which is desirable in patients with type 2 diabetes.



Supported by: NIH RO1 DK 029953 to RB, NIH DK 085516 to AB from National Institute of Health

Disclosure: **D. Romeres:** None.

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Metabolite secretome profiling of human pancreatic islets and rodent beta cells under glucolipotoxicity reveals key mitochondrial metabolism rerouting

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Background and aims: Progressive decline of pancreatic beta cell function is central to the pathogenesis of type 2 diabetes (T2D). Here we revisit a paradox of beta cell: how an excess of energetic substrate and specially glucose and lipid, lead to beta-cell defect of energy which is critical for insulin secretion? Intracellular metabolite homeostasis modifications are key markers of pancreatic islet remodeling, but how cells exchange within its environment remains unknown.

Materials and methods: Using ^{1}H nuclear magnetic resonance, we quantified metabolite secretome from human islets and INS-1E β-pancreatic rodent cell line exposed during 48 hours to glucotoxicity (25 mM glucose) and/or lipotoxicity (0.1 mM palmitate) and performed biochemical analyses.

Results: Taking together, these analysis reveal drastic remodeling of key metabolic hubs tightly associated with mitochondrial dehydrogenase dysregulation. Ours results highlight metabolic mitochondrial alteration

with a clear effect on citrate pathway in both human islets and INS-1E cells in glucotoxity conditions associated with a decrease of insulin secretion. However, instead of being secreted out of the cell as in islets, citrate is rerouting toward lipogenesis in this cell line.

Conclusion: The regulation of the pyruvate and alpha-ketoglutarate dehydrogenase seems to be at the heart of the functional mitochondrial alteration and could explain the lack of human β-pancreatic cell insulin secretion in T2D. Moreover, our resources enable investigations of mechanism targets in T2D. It also paves the way to use metabolite secretome as biomarker to select good pancreatic islet batches for graft in diabetic patients.

Disclosure: **J. Perrier:** None.

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Proteome dynamics of extracellular vesicles in plasma following an acute bout of exercise

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Background and aims: Extracellular vesicles (EVs) are a heterogeneous population of small lipid-bound vesicles that take part in intercellular communication by transporting functional proteins, nucleic acids, and lipids to recipient cells. EVs are involved in many biological processes and play important roles in the development of several physiological and pathophysiological conditions including diabetes. Exercise is known counteract these pathophysiological conditions by facilitating beneficial effects on peripheral tissues. Acting as important mediators of intercellular communication, EVs may also play important role in mediating these effects. In this study, we aim to map the circulating plasma protein-cargo of EVs following an acute bout of exercise using high-resolution liquid chromatography mass spectrometry (LC-MSMS). This may lead to discovery of novel exercise-induced insulin sensitizing peptides and proteins.

Materials and methods: Twenty-five healthy normal-weight male volunteers undertook a 45 min acute exercise bike intervention. Blood plasma samples were collected at just prior to, and immediately after cessation of the exercise intervention as well as 30 min, 1 hour and 3 hours into recovery. The last blood samples were collected the next day, 24 hours after the exercise intervention. Plasma samples were pooled from each timepoint and EVs were isolated using size exclusion chromatography (SEC). The EV isolation protocol was evaluated using transmission electron microscopy (TEM) and protein cargo analysis was performed using mass spectrometry. EV isolation and proteomics analysis was performed in the triplicates.

Results: TEM images showed successful isolation of intact rounds vesicles in size ranges ~30-200 nm. Mass spectrometry-based EV proteome analysis revealed known protein EV-markers in each of the EV samples. Lipoproteins were also identified, but albumin was absent. In total, more than 1200 proteins were identified and subsequent filtering led to the quantification of 834 proteins. ANOVA analysis revealed that 478 proteins were significantly changed in the plasma EV proteome during the time course of the intervention.

Conclusion: These data reveal the dynamic nature of EVs following exercise. Several novel candidate exerkines packed in EVs and released into circulation were identified. Some of these proteins are likely to mediate cross talk between different tissue types and putatively facilitate important metabolic functions. The area of exercise induced EV-cargo is still under-explored and future experiments directed at discovering biological properties of EVs and their cargo are currently in process.

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Disclosure: **L. Peijs:** None.

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Amino acid- and keto acid-induced changes of the ATP/ADP ratio in alpha cells in comparison with beta cells

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Background and aims: While the infusion of glucose decreases glucagon secretion, infusion of amino acids stimulates glucagon secretion. As with the response to glucose, the question is whether the secretory response to amino acids and keto acids is an intrinsic function of the alpha cell or dominated by exogenous signals. Here we have studied the effect of amino- and keto acids on the adenine nucleotide levels in isolated alpha- and beta-cells using the fluorescent indicator PercevalHR.

Materials and methods: To obtain single alpha-cells, islets of NMRI mice were incubated with alloxan for 20 min before dissociation in Ca^{2+}-free solution. The same procedure without alloxan treatment was used to isolate beta cells. Alpha cells and beta cells were adenovirally transduced with PercevalHR, the fluorescence was excited at 400 nm and 490 nm, and the 490/400 nm ratio was the semiquantitative measure of the ATP/ADP ratio. Additionally, ATP and ADP were measured by luciferase luminometry. The cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured using Fura2.

Results: Alpha- and beta-cells were compared using the same perifusion protocol. After the initial perifusion with 5 mM glucose, the concentration was lowered to 1 mM for 10 min. Thereafter, glucose was raised to 30 mM for 10 min or non-glucidic nutrients were added for the same duration. These were either 10 mM *a*-ketoisocaproic acid (KIC), 10 mM glutamine or 10 mM glutamine plus 10 mM BCH (a non-metabolizable leucine analogue). In the presence of 5 mM glucose the PercevalHR ratio was 4.9 in beta-cells and 6.0 in alpha-cells. Following the lowering of glucose to 1 mM the ratio decreased to 3.4 in beta cells, but decreased only slightly, to 5.7 in alpha cells. The ratio values as reported by PercevalHR corresponded to the ATP/ADP ratios as measured by the luciferase method. Interestingly, the slight decrease in alpha cells was preceded by a moderate, but significant transient increase. 10 mM KIC increased the PercevalHR ratio with similar kinetics and similar strength as 30 mM glucose in beta-cells but was virtually ineffective in alpha-cells, where 30 mM glucose has a slight, but significant increasing effect. 10 mM glutamine was without effect in alpha- and beta-cells. The additional presence of BCH however, led to a slow but marked increase in beta cells but did not modify the inefficiency of glutamine in alpha cells. 10 mM arginine was as inefficient in alpha-cells as it was in beta-cells, comparable to KCl depolarization. Alpha- and beta-cells showed closely similar increases of $[Ca^{2+}]_i$ in response to 40 mM KCl and to the mitochondrial inhibitor azide, but only beta cells reflected the increased levels of nutrients.

Conclusion: The present data show clear differences between the oxidative phosphorylation of alpha- and beta-cells. These differences exist not only with respect to glucose but also with respect to the tested keto- and amino acids, which were more effective to raise the ATP/ADP ratio in beta-cells than in alpha-cells. So far, the glucagonotropic effect of amino acids cannot be ascribed to a metabolism-mediated signal recognition in alpha-cells.

Supported by: DDG
Disclosure: **D. Brüning:** None.

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Gender specific alterations in glucose homeostasis driven by glyoxalase 1: New road towards type 2 diabetes?

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Background and aims: The glyoxalase system, consisting of Glyoxalase 1 (Glo1) and Glyoxalase 2 (Glo2), is the main detoxification system of dicarbonyl species, primarily methylglyoxal (MG). MG is formed during glycolysis and is the major precursor of advanced glycation end products (AGEs) and therefore a risk factor for late diabetic complications. Preliminary results suggest that MG could be also involved in the onset of type 2 diabetes (T2D), which would place Glo1 as an even more important defense mechanism. The aim of this study is to elucidate the role of Glo1 in the onset of T2D by generating a Glo1 knockout ($Glo1^{-/-}$) mice model.

Materials and methods: $Glo1^{-/-}$ was established *in vivo* using CRISPR-Cas9 technique. $Glo1^{-/-}$ and WT mice (n = 79) were kept under different feeding conditions for 12 months (normal fat diet - NFD; high fat diet - HFD). Bodyweight and blood glucose were recorded over time. Glucose (ipGTT) and insulin tolerance tests (ipITT) were performed and metabolic cages were used to study renal function. After sacrifice, blood was collected to measure HbA1c and major organs were stored and preserved for further analysis. Proteins involved in the insulin signaling pathway (ISP) were quantified by western blot in liver extracts. Pancreas, liver and kidney samples underwent H&E stainings to study morphology changes.

Results: *In vivo,* the bodyweight and blood sugar values of $Glo1^{-/-}$ female mice under NFD conditions were significantly higher ($p < 0.05$) than in control mice. These differences were neither seen in male mice nor under a HFD. Data from ipGTT and ipITT suggested an altered response in both $Glo1^{-/-}$ females and males and under HFD conditions. However, these effects were only seen in mice older than 30 weeks. The ISP screening in the liver showed a significantly decreased phosphorylation of the cytoplasmic (Akt, GSK3β, mTOR, p70S6k, ERK1/2) and nuclear (Akt2, ERK1/2) mediators in $Glo1^{-/-}$ male mice and in both genders under a HFD ($p < 0.05$). This effect was mildly pronounced in $Glo1^{-/-}$ female mice under NFD conditions. The endocrine pancreas showed slightly differences regarding islet size or distribution. Albumin/Creatinine ratio and Creatinine Clearance were not significantly altered in any of the groups, as well as HbA1c levels at point of sacrificing.

Conclusion: The decrease in ISP phosphorylation found in $Glo1^{-/-}$ male mice and under HFD conditions correlates with the impaired response showed in the ipGTT and ipITT, suggesting an insulin resistance induced by the deletion of Glo1. This is in line with previous studies in fly and fish. Differences were found between genders with males showing a more pronounced decrease in the ISP. In contrast, females showed a T2D-like phenotype with increased bodyweight and blood sugar. This suggests differences in the underlying mechanism between genders in response to the loss of Glo1. Furthermore, this study shows a potential relation between Glo1 and aging, as these results were only seen when mice were older than 30 weeks. Given this background, further studies should focus on the gender specificity of Glo1 as a tool to prevent not only late diabetic complications but the onset of a disturbed ISP and consequently glucose homeostasis.

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Disclosure: **M. Campos:** None.

SO 21 Metabolic control during and after pregnancy

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Effects of vitamin D3 supplementation in obese pregnant women on maternal and fetal lipid metabolism

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Background and aims: Prior studies have observed associations between vitamin D and lipid metabolism in pregnancy. Vitamin D deficiency was associated with adverse lipid profiles. Thus we aimed to investigate the effects of vitamin D supplementation in obese pregnant women throughout pregnancy on maternal and fetal lipid metabolism**.**

Materials and methods: In the DALI Vitamin D trial 154 obese pregnant women with BMI >29kg/m² were randomized (stratified by site) to receive either Vitamin D3 (n=79, 1600IU/day) or placebo (n=75). Pregnant women were included before 20 weeks of gestation with a singleton pregnancy, absence of diagnosis of pre-existing diabetes or GDM (IADPSG criteria) and BMI >= 29kg/m². Lipid parameters (triglycerides, LDL-C, HDL-C, FFA, 3BHB) were analyzed at <20, 24-28, 35-37 gestational weeks and at birth in cord blood. ANCOVA was performed to analyze the effects of vitamin D supplementation on lipid parameters.

Results: Women were included at 15.2 (± 2.7) wks, mean age 32.5 (± 5.3) years, BMI 33.5 (± 4.3) kg/m². Eightyfive percent were of Caucasian origin. At 24-28 and at 35-37 weeks gestation (wks) the vitamin D group had comparable levels of triglycerides, HDL-C, FFA and 3BHB, but significantly increased vitamin D levels compared to placebo (25-OH-Vit.D3 (µg/L): 24-28 wks: 46.65±14.08 vs 31.48±15.70, 35-37wks: 48.29±15.56, p<0.001 all). At 24-28 weeks LDL-C levels were significantly higher in the vitamin D group compared with placebo (3.99±0.96 vs 3.55±0.83, p<0.01) and showed a trend for higher levels at 35-37 wks (4.06±1.01 vs 3.68±0.93, p=0.06). After adjustment for baseline LDL-C levels, no significant differences were observed at 24-28 wks (mean difference 0.198 (-0.032;0.428) mmol/l, p=0.09) and 35-37 wks (0.169 (-0.140;0.478), p=0.28). After adjustment for baseline levels, 25-OH-D3 was significantly higher in the vitamin D group at both time points. Gestational age at birth, weight at birth and fetal lipid parameters were not significantly different between both treatment arms, besides significantly higher 25OH-D3 levels in cord blood in the vitamin D group (29.69±8.83 vs. 20.40±8.42, p<0.01).

Conclusion: Vitamin D supplementation with 1600IU vitamin D3 per day significantly increases 25OH-D3 levels in obese pregnant women and fetal cord blood. However, these changes did not have any effects on maternal or fetal lipid metabolism.

Clinical Trial Registration Number: ISRCTN70595832

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Disclosure: **J. Harreiter:** None.

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Improvements in diabetes related outcomes for women attending peripheral centres: the ATLANTIC DIP experience

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Background and aims: Over the past 15 years, the ATLANTIC DIP network have engaged in a process of audit and research with the aim of improving outcomes for women with diabetes in pregnancy. In this study we compare the outcomes of pregnancies affected by pre-gestational diabetes mellitus (PGDM) over 2 time points - 2006-2007 and 2016-2018.

Materials and methods: In this study we compare the outcomes of pregnancies affected by PGDM across 2 time points 2006-2007 and 2016-2018. We collected data on demographic status, pregnancy preparedness, glycaemic control, services provided, teratogenic medication use, birth outcomes, congenital malformations and neonatal intensive care unit (NICU) admission. We further analysed pregnancy outcomes against glycaemic control and maternal body mass index to assess for significant changes across time points

Results: In 2007 we recorded 104 pregnancies (80/24 to women with type 1/2 diabetes respectively); 148 were recorded in 2018 (101/47 in women with T1/T2 DM respectively). There was a significant increase in the number of pregnancies affected by T2DM (23% vs 31.7%, $p<0.05$). The mean age and duration of disease in women with T1DM is unchanged at 33 and 14 years respectively. The mean age of women with T2DM has decreased from 36 to 34 years and average disease duration went from 4-5 years (NS). The rate of obesity (body mass index $\geq$30kg/m^2) rose from 18 to 34% ($p<0.05$). Mean pre-pregnancy HbA1c has reduced from 61.7 to 58.4mmol/mol (NS). Pre-pregnancy clinic attendance did not change; folic acid usage increased from 43%-55% (ns). The rate of livebirths increased from 76% to 90% ($p<0.05$) and the congenital malformation rate decreased from 25/1000 to 20/1000 births (NS). Caesarean section rates(42 vs 62%, $p<0.05$) and the number of infants born large for gestational age (26% vs 57%, $p<0.05$). We postulate that this is related to the rise in maternal obesity however this did not reach statistical significance (OR for BMI $\geq$30kg/m^2 1.1 (95% CI 0.5-2.3, p 0.79).We further compared the outcomes between the larger central and smaller peripheral units (table 1).

Conclusion: In summary central units maintained their outcomes and progress was noted in peripheral units where rates of livebirth and neonatal intensive care unit admissions improved. The most pressing changes noted in this study were rising rates of maternal obesity and babies born LGA.

Table 1

	Central 2018	Central 2007	P value	Peripheral 2018	Peripheral 2007	P VALUE
N	71	31		77	73	
Livebirth	62 (87%)	25 (81%)	NS	71 (92%)	54 (74%)	<0.05
Miscarriage	8 (11%)	6 (19%)	NS	6 (8.4%)	17 (23%)	<0.05
Stillbirth	0	0		0	2 (4%)	NS
Congenital Malformation	2 (3.2%)	0	NS	1 (1%)	2 (4%)	NS
SGA	0	0		0	4 (7%)	<0.05
LGA	36 (58%)	5 (20%)	<0.05	39 (55%)	16 (30%)	<0.05
NICU	24 (39%)*	5 (20%)*	NS	42 (59%)	45 (83%)	<0.05
PPC	37 (52%)*	20(65%)*	NS	17 (22%)	10 (14%)	NS

LGA – large for gestational age; birth weight above the 90th centile for gestation
SGA – small for gestational age; birth weight below the 10th centile for gestation
NICU – neonatal intensive care unit admission
PPC – pre-pregnancy clinic
*depicts a statistically significant difference between peripheral and central unit outcomes from the same time period

Disclosure: **C. Newman:** None.

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Prediabetic values of OGTT results and HbA_{1c} postpartum as predictors of later development of diabetes in 101 Danish women with previous gestational diabetes

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Background and aims: As women with Gestational Diabetes Mellitus (GDM) have increased risk of subsequent overt diabetes, postpartum assessment of glucose metabolism and long term follow-up are important tools to identify subjects at risk. Aim of this study was to investigate results from postpartum OGTT and HbA1c measurements as predictors for development of diabetes in women with previous GDM.

Materials and methods: Women with previous GDM, who delivered at Odense University Hospital during 1995-2010 and attended routine postpartum examination within 1 year after delivery were invited for a follow-up assessment in 2011-14. Postpartum examination included HbA1c and a 2-hour 75 g OGTT with measurement of fasting (FCG) and 2-h (2hCG) whole blood capillary glucose. Follow-up assessment included a repeated OGTT with measurement of venous fasting plasma glucose (FPG), 2-h plasma glucose (2hPG) and HbA1c and was carried out in 108 women who did not have diabetes at postpartum examination. Data of 101 women (94%) were eligible for the present analyses. GDM was defined by current Danish criteria based on 3rd trimester OGTT results (FCG $\geq$6.1 mmol/L and/or 2hCG $\geq$9.0 mmol/L); diabetes was defined as diabetes diagnosed prior to follow-up or one or more of the following criteria at follow-up: FPG $\geq$7.0 mmol/L, 2hPG $\geq$11.1 mmol/L, HbA1c $\geq$48 mmol/mol.

Results: Postpartum examination was performed 13.6 weeks after delivery (IQR 12.0-17.6) and follow-up was performed 7.7 years after delivery (IQR 6.0-10.2) at which 21 women (20.8%) had diabetes. Compared to women without diabetes prior to or at follow-up, women with subsequent diabetes tended to have higher prevalence of prediabetic FCG (5.6-6.0 mmol/L): 14.3% vs. 5.0%; prediabetic 2hCG (7.8-11.0 mmol/L): 28.6% vs. 23.8%; and prediabetic HbA1c (42-47 mmol/mol): 9.5% vs. 2.5%, at postpartum examination. However, this did not reach statistical significance (p-values 0.16, 0.77 and 0.19, respectively). ROC analyses showed areas under ROC curves [95% CI] for FCG, 2hCG and HbA1c at postpartum examination of 0.57 [0.42, 0.73], 0.55 [0.40, 0.70] and 0.61 [0.46, 0.75] respectively, whereas a ROC comparison of the three tests found no significant difference (p=0.85). Crude hazard ratio [95% CI] of subsequent diabetes in women with prediabetic vs. normal HbA1c was 6.76 [1.50-30.46], whereas no significant hazard ratio was found regarding prediabetic vs. normal FCG and 2hCG (1.40 [0.39-5.06] and 1.52 [0.58-4.00], respectively). Adjusting for selected confounders did not alter associations. Crude hazard ratios [95% CI] of subsequent diabetes per 1 unit increase in FCG, 2hCG and HbA1c at postpartum examination were 0.98 [0.40, 2.39], 1.45 [1.08, 1.96] and 1.11 [1.01, 1.22] respectively, thus reaching statistical significance for 2hCG and HbA1c; the associations remained significant after adjusting for selected confounders.

Conclusion: This study indicates potential superiority of HbA1c as compared to FCG and 2hCG at postpartum examination in predicting overt diabetes after 7.7 years in women with previous GDM. The results should be tested in larger populations.

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Disclosure: **M.H. Christensen:** None.

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Is HbA_{1c} associated with materno-fetal complications in type 1 pregnancies?

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Background and aims: During organogenesis, the developing fetus is particularly susceptible to maternal glucose excursions, and periconception hyperglycaemia. HbA_{1c} is strongly associated with increased risk for congenital anomaly. Large population are required to confirm the associations between HbA1c and other adverse pregnancy outcomes. Our objective was to clarify whether HbA1c or its variation has a predictive role in preventing other materno-foetal complications.

Materials and methods: We conducted a retrospective observational study at the University Hospital in Lille (1997-2019). The clinical and biological data concerning characteristics before pregnancy, maternal and fetal complications were analyzed. HbA1c was measured monthly. The composite criterion was defined by at least one complication among: prematurity, preeclampsia, macrosomia, IUGR and cesarean section. Interest metabolic balance groups have been created to define the period during which HbA1c was a better predictor: Group I as optimal balance throughout pregnancy (HbA1c <6.5 % (48 mmol/mol) 1st trimester and HbA1c <6 % (42 mmol/mol) 3rd trimester) considered as reference, Group II as early imbalance but progressive correction (HbA1c> 6.5 % 1st trimester and HbA1c <6 % 3rd trimester), Group III as a late glycemic imbalance (HbA1c <6.5 % 1st trimester and HbA1c> 6 % 3rd trimester) and Group IV as persistent glycemic imbalance (HbA1c> 6.5 % 1st trimester and HbA1c> 6 % 3rd trimester).The results were expressed in odds ratios (OR) and their 95 % confidence intervals (CI).

Results: 648 pregnancies out of 1587 involved women with T1D. Women were 30.2 ± 4.09 years old and had a BMI of 23.6 kg/m2. The duration of diabetes was on average about 14 years (4; 20). HbA1c before pregnancy was 7.2 % (6.5; 8.1). HbA1c decreased to 6.7 % (6.1, 7.4) in the first trimester, to 6.3 % (5.8; 6.9) in the 2nd trimester and 6.4 % (5.9; 6.9) in the 3rd trimester. Childbirth was 38 (37; 38.2) weeks of amenorrhea without preterm delivery in 76.1 % of pregnancies and preeclampsia in 7.2 %. The average birth weight was 3484 ± 675.6 grams with 56 % LGA and 4.5 % SGA. For an 0.1 % increase in HbA1c during the first trimester and during the 3rd trimester, the composite criterion was, respectively, 1.04 (1.02-1.06) times higher and 1.07 (1.04 -1.1) times higher during pregnancy. For a 0.1 % increase in HbA1c in the first trimester, an association with LGA, prematurity, cesarean section was found. In the third trimester, only LGA and prematurity were associated with HbA1c. Group II (HbA1c> 6.5 % in the first trimester and <6 % in the third trimester) was associated with the composite criterion (2.81 (1.01-7.86)) and the presence of an LGA (2.2 (1.01-4.78)). In contrast, the composite criterion and the LGA were not associated with Group III (HbA1c <6.5 % in the first trimester and> 6 % in the third trimester).

Conclusion: HbA1c is associated with maternal-fetal complications during the first and the 3rd trimester. Regarding maternal-fetal complications, despite the optimization of the metabolic balance during the 3rd trimester, onthese patients with imbalance during the 1st trimester, the risk of LGA persists in comparison with patients who are balanced throughout pregnancy. This risk is, however, lower than that of women with persistent glycemic imbalance. Furthermore, it is not excluded that there are other mechanisms: overweight, lipid abnormalities or immune mechanisms to explain the persistent risk of complications.

Disclosure: **M. Lemaitre:** None.

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Duration of breast-feeding associates with anti-atherogenic lipid profile in women

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Background and aims: Few studies investigated favorable effects of lactation on anthropometric and metabolic parameters as well as reduced risk of diabetes and cardio-metabolic disease later in life in women. We investigated the medium-term effects of lactation duration on anthropometric parameters and glucose and lipid metabolism in women with and without history of gestational diabetes mellitus (hGDM) from the ongoing PREG study.

Materials and methods: Duration of exclusive and mixed breastfeeding was assessed in 112 women (63 hGDM) retrospectively through questionnaire for index pregnancy. All subjects had a 5-point OGTT with 75 g glucose 417d [IQR 374-715] after delivery. Framingham risk score (FRS) and indices for insulin sensitivity and secretion were calculated from anthropometric data and plasma concentrations of lipids, glucose and insulin, respectively. Body fat distribution and liver fat content was assessed with MRI and MR spectroscopy, respectively. The effect of lactation duration was assessed using linear regression adjusted for BMI, age, follow up time, physical activity and parity.

Results: 93.8% of the women reported breastfeeding. The average duration of total lactation was 11 months [7.5-14.5]. A longer lactation period was negatively associated with waist circumference, triglyceride levels, LDL/HDL-Ratio and FRS and positively with HDL (all $p \leq 0.05$). Only in hGDM the duration of lactation was associated with lower waist circumference ($p=0.049$), and a trend for lower subcutaneous adipose tissue ($p=0.063$) and liver fat ($p=0.082$) was observed. Lactation duration was negatively associated with liver fat and FRS (both $p=0.052$) in women with BMI ≥30 kg/m^2 (n=46) at the end of pregnancy. A positive association with insulin sensitivity ($p=0.048$) and disposition index ($p=0.057$) was observed only in the high BMI group.

Conclusion: Our findings suggest that longer duration of lactation could exert anti-atherogenic effects and may reduce risk of cardiovascular disease. Especially women with higher metabolic risk (GDM, overweight or obesity) could benefit and should be advised to prolonged breastfeeding.

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Disclosure: **D. Löffler:** None.

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Postpartum oral glucose tolerance test in women with prior gestational diabetes: Does breastfeeding affects the results?

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Background and aims: Most clinical guidelines include an oral glucose tolerance test (OGTT) in the postpartum re-evaluation of women with gestational diabetes (GDM). The impact of breastfeeding (BF) on the results is of concern, with observational studies suggesting that BF during OGTT may reduce 2h plasma glucose by □0.33 mmol/L. We aimed to evaluate whether BF during OGTT affects glucose and insulin outcomes (main outcome: 2h plasma glucose).

Materials and methods: A randomized crossover trial was conducted in 20 women with prior GDM. Each woman was scheduled to receive two OGTTs in the first trimester postpartum (women should breastfeed the infant during one of the OGTTs and avoid BF in the other; random order). Venous samples for measurement of glucose and insulin were drawn at 0, 30, 60 and 120 min. Statistics: Chi square, Student's T test for paired data or General linear model (GLM) for repeated measures; significance set at $p < 0.05$; results expressed as mean ± SE.

Results: In the OGTTs performed WITH BF, the rates of impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and IFG/IGTs were 10%, 20% and 25% respectively compared with 0%, 20% and 20% for the OGTTs WITHOUT BF. Concordance rate for IFG/IGT was 75%, ns. The GLM showed global differences between OGTT WITH vs WITHOUT BF for glucose ($p = 0.015$) and insulin concentrations ($p = 0.05$); for the interaction with time in the case of glucose ($p = 0.001$) and for individual time points of glucose and insulin as indicated in the table. The glucose Δ from baseline to peak value (4.44 ± 0.26 vs 3.95 ± 0.24 mmol/L, $p<0.03$) and the ensuing □ from peak to 120 min (-2.77 ± 0.32 vs -1.89 ± 0.26 mmol/L, $p < 0.01$) were larger in the OGTT with BF. Similarly, insulin Δ from baseline to peak value (67.07 ± 5.71 vs 58.13 ± 5.92 mU/L, $p < 0.03$) and the ensuing □ from peak to 120 min (-16.39 ± 4.09 vs -5.68 ± 2.81 mU/L), $p < 0.03$) were also larger in the OGTT with BF. The distribution of glucose and insulin concentrations in the OGTT with BF were skewed to the right without outliers.

Conclusion: BF during the OGTT resulted in similar 2h glucose concentrations and categorization of glycemic status. However, glucose and insulin differed during the first hour of the test with more marked Δ and □ in the OGTT WITH BF. Thus, in women included in this study, BF affected OGTT results but without an impact on the categorization of glycemic status

	OGTT WITH BF	OGTT WITHOUT BF	P
Glucose 0′ (mmol/L)	5.03 (0.01)	4.84 (0.07)	0.04
Glucose 30′ (mmol/L)	8.35 (0.31)	7.78 (0.31)	0.01
Glucose 60′ (mmol/L)	8.99 (0.40)	8.06 (0.40)	0.01
Glucose 120′ (mmol/L)	6.70 (0.30)	6.90 (0.26)	0.51
Glucose peak (mmol/L)	9.47 (0.27)	8.79 (0.27)	0.01
Insulin 0′ (mU/L)	8.58 (0.93)	6.32 (1.10)	0.03
Insulin 30′ (mU/L)	43.08 (3.42)	38.67 (4.21)	0.19
Insulin 60′ (mU/L)	69.34 (6.82)	56.87 (7.73)	0.03
Insulin 120′ (mU/L)	61.41 (6.56)	59.67 (6.92)	0.74
Insulin peak (mU/L)	74.98 (5.86)	61.63 (6.48)	0.01

Supported by: SJD Research Foundation

Disclosure: **G. Monroy:** None.

SO 22 News from the drug pipeline

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Liraglutide decreases plasma PCSK9 in patients with type 2 diabetes not treated with statins

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Background and aims: Dyslipidemia observed in type 2 diabetes (T2D) plays an important role in the increased cardiovascular risk in T2D patients. It includes abnormal metabolism of LDL lipoproteins. Our group has recently shown that liraglutide increases LDL catabolism in patients with T2D and that it reduces the expression of PCSK9 (a major inhibitor of LDL-receptor expression at the cell membrane surface) in vitro and mice. This prompted us to study the effect of liraglutide on plasma PCSK9 level in patients with T2D.

Materials and methods: Because statins are known to modify PCSK9 expression, we studied prospectively 51 patients with T2D not treated with statins. Plasma PCSK9 and plasma lipids were measured before and 6 months after the initiation of a treatment with liraglutide at a dose of 1.2 mg/day. Plasma PCSK9 was assessed by a high-sensitivity quantitative sandwich enzyme immunoassay.

Results: Six months after initiation of liraglutide treatment, significant reductions in the means of HbA1c (6.9±1.3 vs. 9.6±2.3 %, $p<0.0001$), body weight (99.8±19.5 vs. 104.5 ±19.4 kg, $p<0.0001$), fasting triglycerides (1.71±0.75 vs. 2.18±1.07 Mmol/L, $p=0.003$) and LDL-cholesterol (2.44±0.73 vs. 2.65±0.72 Mmol/L, $p=0.012$) were observed. Plasma PCSK9 was significantly reduced by liraglutide treatment (214.97 ±56.37 vs. 244.52±99.25 ng/ml, $p=0.024$). Reduction of plasma PCSK9 was very significant in patients with baseline HbA1c <10 % (n=33; -44.97±88.16 ng/ml), when it was not observed in patients with baseline HbA1c ≥10 % (+5.20±55.19 ng/ml). In multivariate analysis, baseline HbA1c was an independent factor associated with plasma PCSK9 reduction.

Conclusion: Treatment with liraglutide induces a significant reduction of plasma PCSK9 in patients with T2D not treated with statins. This is in line with the acceleration of LDL catabolism that has been observed with liraglutide. However, this decrease in plasma PCSK9 is significantly influenced by baseline HbA1c and is not observed in patients with poorly controlled T2D.

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Disclosure: **B. Vergès:** Grants; grant from Novo Nordisk.

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Effects of simvastatin on human subcutaneous adipose tissue metabolism

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Background and aims: Inhibitors of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, known collectively as statins, are widely prescribed drugs that lower blood lipids and control hypercholesterolemia. Despite reducing the risks of cardiovascular events and overall mortality in the patients, statins are associated with an increased risk of

new-onset type 2 diabetes (T2D) in patients. The effects of statins on human adipose tissue (AT) have been scarcely studied. Therefore, our work focused on investigating the effects of simvastatin on human AT metabolism and mRNA expression of adipokines, pro-inflammatory cytokines, and genes involved in regulation of mitochondrial function.

Materials and methods: Subcutaneous AT obtained by needle biopsies from 16 healthy volunteers (4M/12F) was pre-incubated without (control) or with simvastatin (25-250 nM) or its active metabolite simvastatin hydroxyl-acid (SA) (8-30 nM) for 24 h. Basal and insulin-stimulated D-[U-^{14}C]-glucose uptake and lipolysis were measured in isolated mature adipocytes. The effects of these drugs on AT mRNA expression of cytokines, adipokines, and genes regulating mitochondrial function were assessed. The effects of statins on adipocyte differentiation was also measured. Additionally, association studies between the AT expression of *HMGCR* gene and other genes were performed using transcriptomics data from a cohort of 19 healthy + 12 T2D statin-free subjects matched for sex, BMI, and age. Significant genes were included in gene set enrichment analysis. The effect of statin treatment on the index of systemic insulin sensitivity, Matsuda index, was measured in an independent cohort of 113 patients, of whom 20 were on statin treatment.

Results: Simvastatin and SA reduced insulin-stimulated glucose uptake in adipocytes by 10% at supra-therapeutic concentrations ($p<0.05$) and nominally reduced the expression of *SLC2A4* (GLUT4) by about 20%. The drugs had no significant effect on adipocyte lipolysis or the expression of genes regulating mitochondrial functions. However, the drugs nominally increased the mRNA expression of pro-inflammatory cytokine *IL1B* by 60%. The drugs did not affect adipocyte differentiation. The association studies indicated that *HMGCR* expression in adipose tissue is significantly correlated with various metabolic and mitochondrial enriched terms, including insulin signalling and insulin resistance (positive correlation), and citrate cycle, and oxidative phosphorylation (negative correlation). Clinical data showed that statin treatment reduced plasma LDL in the patients. However, no significant association was observed between statin treatment and Matsuda index after adjustment for subjects' age, sex, BMI, HbA1c levels, and diabetes or prediabetes status.

Conclusion: Our results show that *HMGCR* in the AT appears to be associated with different metabolic pathways in adipose tissue, including insulin signalling. However, simvastatin and SA at therapeutic concentrations do not directly affect adipocyte glucose uptake and lipolysis. This suggests that the diabetogenic effects of clinical statin use do not appear to involve adipose tissue and that other organs, such as liver, muscle, pancreas or brain, might be of greater interest.

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Disclosure: **A. Sarsenbayeva:** None.

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Exogenous secretin decreases ad libitum food intake and exhibits a biphasic effect on supraclavicular brown adipose tissue activity in healthy men

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Background and aims: Secretin is best known for its effect on pancreatic exocrine secretion. It also has osmoregulatory effects and was recently shown to provide a postprandial satiety signal in mice. This effect was proposed to be mediated by secretin-induced activation of brown adipose tissue (BAT) and subsequent hypothalamic registration of a rise in body temperature. We investigated the effects of a 5-hour infusion of secretin on food intake, BAT activity, resting energy expenditure (REE), postprandial plasma glucose and lipids, and gallbladder motility as well as haemodynamics in healthy young males.

Materials and methods: In a randomised, double-blind, placebo-controlled, crossover study, 25 healthy young males aged (mean±SD) 25.7±6.1 years with a BMI of 23.4±1.8 kg/m^2 underwent two 5-hour i.v. infusions of secretin (1 pmol/kg/min) or placebo, with an interposed 8-week washout period. After 30 minutes of infusion, a standardised liquid mixed meal was ingested (603 kcal). Prior to and with regular intervals during the 5-hour infusions, we assessed REE (by indirect calorimetry) and supraclavicular BAT activity (by thermal imaging). Furthermore, blood samples were drawn, ultrasonography of the gallbladder performed and haemodynamics measured, before and during the infusion period. Before terminating the infusions, ad libitum food intake (primary outcome) was assessed.

Results: Steady-state plasma concentrations of secretin amounted to 113 ±2 pmol/l, i.e. ~10-fold higher than physiological levels. Compared to placebo, secretin decreased ad libitum food intake by [mean±SEM] 173 ±88 kcal (p = 0.039). Within the first 15 minutes of infusion, secretin decreased supraclavicular temperature by 0.10±0.02°C (p <0.001), but after 75-90 minutes and 240-255 minutes it increased supraclavicular temperature by 0.05±0.01 (p <0.001) and 0.08±0.01 (p <0.001), respectively, compared to placebo. Secretin did not affect REE. Postprandial plasma triglyceride excursions were higher during secretin than placebo infusions (baseline-subtracted AUC: 111±49 vs. 83±36 pmol × min/l [p = 0.012]), whereas excursions of glucose, glycerol and free fatty acids, respectively, were similar. Compared to placebo, secretin reduced maximum gallbladder ejection fraction by 32±27% (p <0.001) and increased mean systolic and diastolic blood pressure by 5.3±4.5 (p <0.039) and 2.2 ±3.2 mmHg (p <0.001), respectively, and increased heart rate by 5.5±3.6 beats/min (p <0.001). Mild to moderate gastrointestinal adverse events were more frequent during the secretin infusion compared to placebo infusion.

Conclusion: A 5-hour infusion of secretin in healthy young males decreased ad libitum food intake and exhibited a biphasic effect on supraclavicular BAT activity. Secretin infusion had no effect on fasting and postprandial glucose levels, but it reduced postprandial gallbladder contraction, while increasing plasma triglycerides, blood pressure, and heart rate.

Clinical Trial Registration Number: NCT04613700

Disclosure: **M.J. Bentzen:** None.

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Targeting G protein-coupled receptor 110 for the treatment of obesity and its related metabolic diseases

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Background and aims: Obesity is a major chronic disease giving rise to numerous metabolic complications, including type 2 diabetes, cardiovascular diseases and nonalcoholic fatty liver diseases. GPR110 is an orphan receptor which is involved in neuronal development and hepatocarcinogenesis. However, the role of GPR110 in metabolic diseases remains unexplored. Therefore, the aim of this study is to investigate the metabolic functions of GPR110 and the underlying mechanisms.

Materials and methods: Global GPR110 knockout (KO) mice were generated and subjected to standard chow (STC) or high-fat diet (HFD) feeding. Body weight and body composition of mice was measured. Glucose tolerance and insulin sensitivity was examined by glucose tolerance test, insulin tolerance test and pyruvate tolerance test. Basal serum lipid profiles were checked using biochemical methods. Serum FGF21 levels were measured by ELISA. Morphological analyses of liver and white adipose tissue were conducted by H&E staining.

Results: GPR110 KO mice gained significantly less weight during HFD feeding, but similar weight during STC feeding as compared to their wild-type (WT) littermates. This was attributed to lower fat mass in the GPR110 KO mice upon HFD feeding. Consistently, the GPR110 KO mice were more resistant to HFD-induced glucose intolerance and insulin resistance when compared to the WT mice. Diet-induced fatty liver and adipose expansion were also markedly reduced in GPR110 KO mice. Notably, both hepatic expression and the circulating level of FGF21 in GPR110 KO mice were much higher than that in their WT littermates.

Conclusion: These findings collectively suggest the involvement of GPR110 in the pathogenesis of obesity which is potentially mediated through the actions of FGF21.

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Disclosure: **Z. Huang:** None.

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Exendin-4 treatment improves adipose tissue microcirculation in obese rats through AMPK -eNOS pathways

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Background and aims: Obese adipose tissue microcirculation dysfunction may be the pathophysiologic link between endothelial dysfunction and insulin resistance. Glucagon-like peptide 1 (GLP-1) can dilate blood vessels and stimulate endothelial cell proliferation. We aimed to investigate the effect of exendin-4, a GLP-1 receptor agonist on microcirculation and insulin resistance in adipose tissue of diet-induced obese (DIO) rats.

Materials and methods: Wistar rats were randomly distributed into two initial groups fed with a standard chow diet (NC) or a high-fat diet (HFD) within 12 weeks, and then intraperitoneally injected with or without exendin-4 (24nmol/kg) for 4 weeks. After 4-week exenatide administration, intraperitoneal glucose tolerance test and insulin tolerance test, and abdominal subcutaneous adipose tissue microcirculation perfusion were measured by contrast-enhanced ultrasound at baseline and 1-hour post- intraperitoneal glucose challenge. Microvascular density was measured by immunochemistry staining. AMPK-VEGF/VEGFR2 -eNOS signaling pathways were determined by western blot.

Results: HFD mice showed significantly increased body weight, fat mass and ratio of fat mass to body weight and impaired glucose tolerance and insulin sensitivity. After a 4-week exenatide treatment, HFD rats reduced body weight, body fat mass, and ratio of fat mass to body weight and improved glucose tolerance and insulin sensitivity. HFD rats had significantly reduced adipose tissue microvascular blood flow at 1-hour post-intraperitoneal glucose challenge. HFD rats with exendin-4 treatment significantly improved adipose tissue microvascular blood flow. Exendin-4 also increased microvascular density in adipose tissue in HFD rats. Exendin-4 treatment significantly related to the phosphorylation of AMPK-eNOS and enhanced proteins of VEGF/VEGFR2 expression.

Conclusion: Our study demonstrates that exendin-4 may improve adipose tissue microcirculation and mitigate insulin resistance by regulating AMPK-VEGF/VEGFR2 -eNOS signaling pathway from HFD induced obese rats.

Supported by: Science and Technology Program of Sichuan (2019YFS0300)

Disclosure: **L. Han:** None.

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Potential effect of a novel combination of GLP-1RA (efpeglenatide) and long-acting glucagon analogue (HM15136) in animal models of metabolic disorder

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Background and aims: In addition to glycemic control, GLP-1RA efficacy is established in obesity. However, more efforts are still required to close efficacy gap with bariatric surgery. Recent studies demonstrate the favorable effect of glucagon (GCG) receptor activation on energy expenditure and hepatic lipid metabolism. So, combination of GCG with GLP-1RA not only enhances body weight loss (BWL) barely achieved by current therapy, but also expands its therapeutic utility to various metabolic disorders. Previously, we confirmed the long-acting GCG properties of HM15136 from animal to human. In addition, GLP-1 class effect of efpeglenatide (Efpe), once-weekly GLP-1RA, was confirmed in clinical studies. Thus, to develop a novel combination therapy, potential effects of Efpe and HM15136 combination (COMBO) were investigated in animal models of metabolic disorders.

Materials and methods: For BWL efficacy evaluation, either Efpe mono or Efpe and HM15136 COMBO were subcutaneously administered to DIO mice. Acylated GLP-1/GIP co-agonist was used as comparative control. After 4 weeks treatment, changes in BW, fat mass and blood lipid profile were analyzed. To investigate the potential COMBO effects on obese and T2DM, Efpe mono, HM15136 mono and their COMBO were subcutaneously administered to DIO/STZ rats, and changes in BW and HbA1c were determined after 4 weeks treatment.

Results: In DIO mice, 4 weeks treatment of Efpe and HM15136 COMBO showed more potent BWL than Efpe mono treatment. Of note, greater BWL was also confirmed for COMBO treatment compared to acylated GLP-1/GIP co-agonist (-18.5, -55.9, -44.9% *vs.* vehicle for Efpe, COMBO, GLP-1/GIP). Similar results were observed when measuring fat mass (-19.5%, -83.8%, -77.2% *vs.* vehicle for Efpe, COMBO, GLP-1/GIP) and blood lipid profiles (total cholesterol: -41.8%, -82.0%, -63.2% *vs.* vehicle for Efpe, COMBO, GLP-1/GIP) in DIO mice. To further expand therapeutic benefits of this novel COMBO beyond obesity, additional study was performed in DIO/STZ rats, and COMBO treatment consistently showed more BWL than Efpe (-5.1, -12.1% *vs.* vehicle for Efpe, COMBO) along with numerically more HbA1c reduction (-1.0, -1.5% *vs.* vehicle for Efpe mono, COMBO).

Conclusion: HM15136 might be a novel COMBO partner for Efpe, providing enhanced BWL and favorable metabolic profiles. These results warrants further evaluation for therapeutic potential of this novel COMBO in various metabolic disorders including T2DM, dyslipidemia and NASH.

Disclosure: **S. Lee:** None.

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Chronic effects of an antifibrotic agent pirfenidone on insulin sensitivity and cardiac function in high-fat diet induced insulin-resistant mice

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Background and aims: The net accumulation of extracellular matrix (ECM) is associated with fibrosis in several tissues and can cause organ dysfunction. In mice, insulin resistance (IR) is strongly associated with increased deposition of ECM components. Pharmacological inhibition of ECM deposition by hyaluronidase attenuated IR and cardiac dysfunction in diet-induced obese mice. Pirfenidone is an orally active small molecule with antifibrotic properties. The present study investigated the effects of Pirfenidone on insulin sensitivity and cardiac function in high-fat-fed mice.

Materials and methods: Male mice fed a high-fat (HF) diet for 12 weeks received twice-daily oral gavage of either vehicle (0.25% Carboxymethyl cellulose) or Pirfenidone (125 mg/kg body weight) for 21 days. After the treatment, insulin sensitivity was measured by hyperinsulinaemic-euglycaemic clamp and left ventricular dynamics was determined by Pressure-Volume (PV) loop analysis (Transonic) using PV conductance catheter in closed-chest preparation. Immunohistochemical analysis was performed to assess changes in collagen expression in the left ventricle (LV) of the heart.

Results: Pirfenidone treatment resulted in a 41% decrease in collagen expression in the LV ($P<0.01$). Body weight & body composition remained unchanged. Pirfenidone treated HF-fed mice displayed decreased fasting blood glucose (13.18 ± 0.47 vs. 11.67 ± 0.49 mM, $P<0.05$) without appreciably altered fasting insulin. Pirfenidone treated mice also showed improved glucose tolerance, while the insulin tolerance remained unchanged. During the hyperinsulinaemic-euglycaemic clamp, Pirfenidone treated HF-fed mice displayed higher glucose infusion rate (GIR) compared to vehicle controls (17.37 ± 2.44 vs. 31.60 ± 6.04 mg/kg/min, $P<0.05$), suggesting an improved insulin sensitivity. PV loop analysis revealed a significant decrease in pulse pressure by Pirfenidone treatment (32.76 ± 0.65 vs. 27.08 ± 0.97 mmHg, $P<0.001$), suggesting improved vascular compliance. Pirfenidone also reversed HF-diet induced increase in the absolute value of the minimum rate of pressure change (dp/dt_{min}) in the LV (9011 ± 216.4 vs. 7488 ± 463.4 mmHg/s, $P<0.01$). In contrast, other systolic and diastolic parameters, including end-systolic pressure-volume relationship (ESPVR) and end-diastolic pressure-volume relationship (EDPVR), were not different among the groups.

Conclusion: Pirfenidone improved whole-body insulin sensitivity and cardiac performance in HF-fed obese mice. These beneficial metabolic effects were accompanied by decreased cardiac collagen deposition. These data suggest that the ECM remodelling may represent a potential target for the treatment of diabetes and its associated cardiovascular complications.

Supported by: British Heart Foundation

Disclosure: **V. Musale:** None.

SO 23 Fatty matters

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A microRNA cluster controls fat cell differentiation and adipose tissue expansion by regulating SNCG

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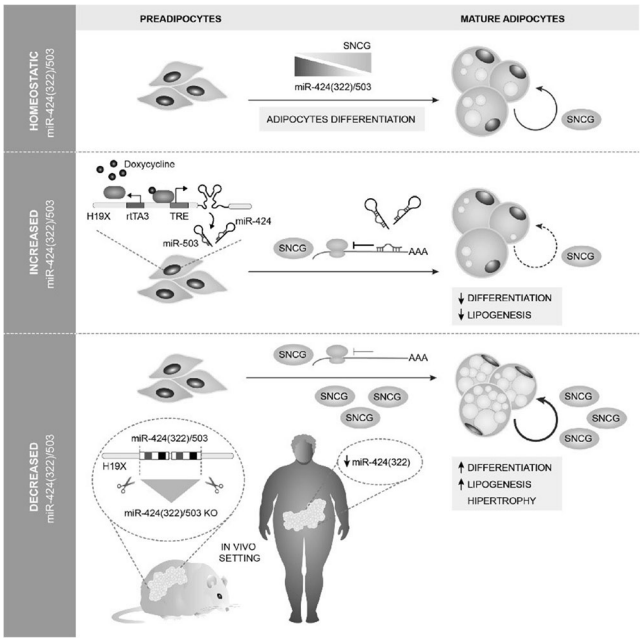
Background and aims: The H19X-encoded miR-424(322)/503 cluster regulates multiple cellular functions. By generating a knockout (KO) mouse model, the role in the mammary gland was first reported. In parallel to its regulatory activity in breast epithelium, intriguing observations made in our laboratory revealed additional alterations, mainly consisting on the significant enlargement of fat depots. Thus, we initiated a research line aimed at define the role of this set of miRNAs in adipogenesis and its putative implications in obesity.

Materials and methods: We used our KO mouse model exposed to normal chow (NC) and high-fat diet (HFD), engineered cell systems, and human fat samples to investigate the functional roles of adipose miR-424(322)/503 in obesity. Deep-sequencing transcriptomes unveiled mechanistic insights related to the regulatory activity of miR-424(322)/503 in adipocytes, while additional analyses confirmed its ability in orchestrating fat cell commitment by inhibiting γ-Synuclein (SNCG).

Results: Under HFD, increased size and body weight in KO male and female mice was consistent with a significant enlargement of adipose tissue. Together with expanded fat depots, KO females exhibited traits of impaired glucose tolerance under NC (but not upon HFD). We show that this miRNA cluster is negatively regulated during adipogenesis, which in turn allows the expression of genes of relevance in the development of obesity. Indeed, ablation of miR-424(322)/503 in mouse embryonic fibroblasts led to enhanced differentiation, while its transient doxycycline-inducible pTRIPz-mediated expression displayed impaired adipogenesis and de-differentiation of human adipocytes. Deep-transcriptomes revealed the impact on many canonical hallmarks, namely adipogenesis and fatty acid metabolism, while twenty-four common target genes were validated in complementary approaches. Mechanistically, we found that SNCG is a direct target of the miR-424(322)/503 to mediate metabolic functions in adipocytes. Accordingly, diminished adipose miR-424(322)/503 in KO mice and humans co-segregated with increased SNCG in fat and blood as mutually exclusive features of obesity, being normalized upon weight loss.

Conclusion: Our data unveil a previously unknown regulatory mechanism of fat mass expansion tightly controlled by the miR-424(322)/503 through SNCG.



Supported by: MTV3, PERIS, CERCA, ERDF, ESF, CIBEROBN, ISCIII

Disclosure: **F.J. Ortega:** None.

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Increased contribution of fructose to de novo synthesis of saturated over unsaturated fatty acids in mice fed high-sugar and high fat-high sugar diets

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Background and aims: Fructose is considered to promote non-alcoholic fatty liver disease (NAFLD) through its conversion to fatty acids in the liver and mouse models are widely used to study the lipogenic actions of fructose. Saturated fatty acids (SFA) are considered to be more lipotoxic than monounsaturated fatty acids (MUFA) such as oleate and palmitoleate. Previously, we showed that for healthy mice that received a single overnight supplement of high-fructose corn syrup-55 (HFCS-55), the fructose component contributed a higher proportion of acetyl-CoA to the synthesis of SFA compared to MUFA. In this study, we sought to determine whether this preference of fructose for SFA over MUFA synthesis was happening in mice that had previously been on a long term high-sugar or a high fat + high sugar diet.

Materials and methods: 12 male C57/BL6 mice fed with standard chow supplemented by HFCS-55 at 30% w/v in the drinking water for 18 weeks (HS) and 10 mice fed a high-fat diet for 18 weeks supplemented likewise by HFCS-55 (HFHS) were studied. During the final evening, the HFCS-55 fructose component was enriched with 20% [U-^{13}C]fructose and the mice were administered with deuterated water. The mice were allowed to feed naturally overnight and then sacrificed. Livers were freeze-clamped and triglycerides were isolated for ^{13}C and ^{2}H NMR spectroscopy. The contribution of the HFCS-55 fructose component to *de novo* synthesis of SFA and MUFA was estimated from the ^{13}C- and ^{2}H-NMR enrichment data. Data are presented as means ± standard error.

Results: For HS mice, fructose contributed 28 ± 4% of acetyl-CoA to the synthesis of SFA compared to 16 ± 3% to oleate synthesis (p = 0.054 vs SFA) and 18 ± 3% to palmitoleate synthesis (p = 0.118 vs SFA). For HFHS mice, fructose contributed 18 ± 3% of acetyl-CoA to the synthesis of SFA compared to 7 ± 2% to oleate (p = 0.002 vs. SFA) and 7 ± 2% to palmitoleate synthesis (p = 0.003 vs SFA).

Conclusion: For mice fed either standard chow or high-fat diets that were supplemented with HFCS-55, fructose contributed significantly more acetyl-CoA to SFA synthesis than to either oleate or palmitoleate synthesis thereby promoting a more lipotoxic lipid profile. For mice fed a normal chow diet supplemented with HFCS-55 there was only a tendency for higher contributions of fructose to SFA synthesis in comparison to oleate or palmitoleate.

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Disclosure: **J. Jones:** None.

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Purinergic receptor P2X7: a new target for the degradation of lipid droplets by lipophagy

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Background and aims: Defective autophagy is associated with the pathogenesis of nonalcoholic fatty liver disease (NAFLD) through unclear mechanisms. Purinergic receptor P2X7 (P2RX7) is an ATP-gated ion channel that belongs to the P2XR family. At present, there are many contradictory studies on the function of P2RX7 in NAFLD. We aimed to explore the role and mechanism of P2RX7 in NAFLD.

Materials and methods: The protein expressions level of P2RX7 in FFA treated HepaG2 cells, ob/ob and db/db mouse liver were detected by Western blot (WB). Specific P2RX7 agonist and inhibitor were used to evaluate the effects of P2RX7 on hepatocyte steatosis. Oil red O staining and electron microscopy were used to observe the accumulation of lipid droplets in hepatocytes. Furthermore, the possible mechanisms are then explored through relevant GEO data by bioinformatics analysis. Next, the autophagy flux was monitored by LC3 double-labeled adenovirus, and the dynamics of the lipid droplets and lysosomes were dynamically monitored by using the specific dye of lipid droplets and lysosomes. Finally, combined the expression of protein to verify the functions of autophagy and lysosome.

Results: P2RX7 protein expressions were significantly decreased in the liver of ob/ob and db/db mice. In vitro model of lipid overload, P2RX7 protein expressions were also reduced with the increase of free fatty acid concentration and intervention time. Oil red R staining and electron microscope observation showed that the size and number of lipid droplets decreased after treatment with P2RX7 agonist, while the number of lipid droplets increased after treatment with inhibitor. Bioinformatics prediction showed P2RX7 mainly affected the biological process of vacuolar acidification, and KEGG analysis showed that it was related to lysosome and autophagy. Western blotting assay showed that P2RX7 stimulation increased the phosphorylation of AMPK and ULK1, while inhibition of P2RX7 decreased the phosphorylation of AMPK and ULK1, suggesting that P2RX7 promoted autophagosome synthesis through the AMPK/ULK1 pathway and thus improved lipid accumulation in hepatocytes. In addition, activation of P2RX7 increased the expression of the lysosomal surface protein LAMP2. Confocal observation showed that activation of P2RX7 increased the expression of autolysosomes and lysosomes, suggesting that P2RX7 accelerated the process of lipophagy by promoting the entry of autophagosomes into lysosomes.

Conclusion: Our study reveals a novel protective effect of P2RX7 on regulation of lipid metabolism and improving abnormal accumulation of lipid droplets in the liver, which may be a potential therapeutic target for NAFLD.

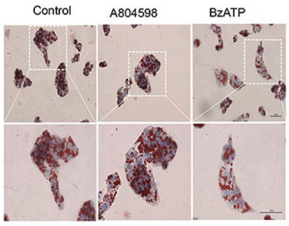

Figure 1 Activation of P2RX7 improved lipid droplet accumulation

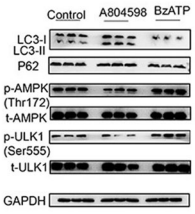

Figure 2 P2RX7 promotes the process of lipophagy

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Disclosure: **Z. Dong:** None.

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Ectopic lipid deposition upregulates SGLT2 and GLUT1 in renal tubules

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Background and aims: Glucose reabsorption in renal tubules is key to glucose homeostasis. Upregulation of renal glucose absorption may be responsible for elevated blood glucose levels in diabetes. There is now wide recognition that visceral lipid deposition as a result of dyslipidemia represents an important driver of metabolic disorders including diabetes. It remains unclear as to whether renal exposure to excessive lipids increases glucose reabsorption. The present study investigated the effect of fat on renal glucose reabsorption in both high-fat-diet-induced obese mice and human renal tubular epithelial cells.

Materials and methods: Six-week old C57BL/6 mice were fed with either high-fat diet (HFD) or normal diet (CON) over 12 weeks, followed by IPGTT and IPITT. Blood glucose, lipids and creatinine, as well as renal lipid content and 24-hour urine glucose excretion, were evaluated. Glucose transporters in the proximal tubules, including SGLT1, SGLT2, GLUT1 and GLUT2, were measured by real-time quantitative PCR, western blot and immunofluorescences. In addition, HK-2 cells (the human renal tubular epithelial cell line) were treated with 200uM palmitic acid (PA) over 24 h for evaluation of intracellular lipid droplet deposition, glucose uptake, and the expression of glucose transporters.

Results: Relative to the CON mice, mice fed with HFD achieved additional >20% weight gain, without developing diabetes during the study period. However, body weight, and renal SGLT2 and GLUT1 protein levels increased by 57.8%, 72.8%, and 17.2%. respectively ($P < 0.0001$). Treatment with PA also enhanced intracellular lipid deposition and glucose uptake in the HK2 cells, in association with upregulated SGLT2 and GLUT1 expression.

Conclusion: Our observations suggest that renal lipid deposition in obesity may upregulate the expression of SGLT2 and GLUT1 in renal tubulars, leading to increased renal glucose reabsorption and the risk of dysregulation of glycaemia.

Disclosure: **T. Li:** None.

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Regulatory loop of IR signalling is critical for adipocyte dynamics and mitochondrial homeostasis

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Background and aims: Type-2 diabetes with metabolic syndrome displays adipose-tissue insulin resistance. Insulin and IGF1 signaling initiate Tyr phosphorylation of IR/IGF1R and adaptor IRSs, leading to Ser/Thr phosphorylation of PI3K/AKT and MAPK/ERK pathways essential for the maintenance of brown and white adipose tissues (BAT & WAT). We investigated the role of insulin and IGF-1 signaling in the adult adipose tissues by an inducible adipocyte-specific IR and IGF1R gene knockout mice (Ai-DKO). Ai-DKO, causing acute metabolic disease with hyperglycemia, hyperlipidemia, and fatty liver, recovered adipose tissues by regenerating adipocytes. We aimed to uncover the mechanism of insulin resistance in adipose tissues.

Materials and methods: To identify new regulatory components of IR/IGF1R signaling, we performed RNA-seq analysis in iBAT from Control and Ai-DKO in the acute hyperglycemia phase. We identified a reduction of protein phosphatase protector, α4, which can regulate Ser/Thr phosphorylation of down-stream insulin signaling. We created inducible adipocyte-specific α4 KO mice (Ai-α4 KO) with tamoxifen-inducible Cre-ERT2 transgene and investigated the role of α4 in the IR-mediated signaling for the maintenance of BAT.

Results: shRNA-mediated α4 knockdown (α4KD) in BAT affected not only insulin-stimulated down-stream Ser/Thr phosphorylation of AKT (S473) but also affected up-stream Tyr phosphorylation of IRβ (Y1162/1163) and IRS1 (Y612), suggesting a unique regulatory role of α4 in Tyr phosphorylation. To investigate its molecular mechanism, we performed a Proteomics analysis of α4-binding molecule and identified Y-box protein 1 (YBX1), which functions as a transcription factor for Tyr phosphatase PTP1B. Knockout of α4 increased PTP1B expression in preadipocytes. The deletion of α4 in Ai-α4 KO mice impaired the fat tissue function causing cold intolerance and a significant decrease in oxygen consumption rates (VO2) with an increase of C18: 0-ceramide in adipose tissues. RNA-seq showed a marked reduction of genes associated with mitochondrial fatty acid oxidation and the increases in inflammatory cytokine pathways in Ai-α4 KO WAT and BAT, implicating the cause of fat tissue loss was due to adipocyte apoptosis. To investigate the role of α4 during aging and gaining fat mass, we created α4 knockout mice in fat tissues from birth by crossing mice with floxed α4 allele with mice carrying the adiponectin Cre transgene (Aα4KO). The apparent phenotype of Aα4KO mouse does not show significant abnormality in body weight up to 8 weeks after birth but instead gained by 12% at 16 weeks. Aα4KO mice exhibited less visceral and subcutaneous fat tissues on CT imaging, measured only 1% body fat percentage. Due to a severe loss of white and brown adipose tissues, Aα4KO mice showed severe metabolic syndrome with glucose intolerance, insulin resistance, pancreatic islet hyperplasia, and progressive NAFLD.

Conclusion: The Ser/Thr phosphatase protector α4 is essential for the maintenance of adipose tissues. α4, interacting with Ser/Thr phosphatases, suppresses YBX1 activation, leading to down-regulate PTP1B, eventually setting the IR to initiate Tyr phophorylation. The regulatory feedback loop of insulin signaling is essential for maintaining adipose tissues' insulin sensitivity, preventing the systemic metabolic disease.

Supported by: JSPS, MSD foundation, Takeda foundation

Disclosure: **M. Sakaguchi:** None.

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Neonatal overfeeding permanently programs hypertrophic adiposity: potential implications of adipose tissue turnover

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Background and aims: Childhood obesity is a public health burden worldwide. Most children with obesity will remain obese until adulthood, which in turn increases the risk of developing co-morbidities later in life, including Type 2 Diabetes (T2D) and cardiovascular disease. The mechanisms behind the persistence of obesity and its metabolic co-morbidities are poorly characterized. Our aim was to explore the mechanisms by which increased adiposity is extended from childhood to adulthood, and its relationship with metabolic disturbances.

Materials and methods: We developed a mouse model of neonatal obesity (*i.e.* childhood obesity) by litter size reduction. At birth, litters were adjusted to 8 pups per control-dam (C) and 4 pups per small litter-dam (SL). We characterized inguinal (iWAT) and epididymal (eWAT) white adipose tissue biology in young (2-week old) and adult mice (6-month old).

Results: Litter size reduction led to rapid growth rate and, by the time of weaning, SL mice were already heavier than the controls. Early growth was accompanied by rapid fat mass expansion, and 2-week old SL mice had greater iWAT and eWAT than the Controls. At weaning, all mice were maintained on a standard chow diet. Strikingly, SL mice remained heavier and maintained higher fat mass than controls until adulthood, despite showing similar food intake/ energy expenditure than the controls. Obesity in SL mice was characterized by hypertrophic adiposity: SL iWAT and eWAT adipocytes were hypertrophic as early as 2-wks, and remained larger than C until 6-mo. In young mice, hypertrophy could be largely attributed to increased lipogenesis, as assessed by increased expression (qPCR) of *Fasn*, *Scd1*, *Plin1, Glut4*, *Chrebp*. Despite minor changes in adipogenic (Pparg) or pre-adipocyte markers (*Pref1*), the adipocyte progenitor cells of SL iWAT showed normal proliferation and differentiation in vitro. In the adults, the expression of lipogenic and adipogenic genes was largely normalized. Likewise, in vitro pre-adipocyte differentiation was similar between SL and C mice (iWAT and eWAT).

Conclusion: Together, these data led us to propose that early hypertrophic adipose tissue accrual is primarily mediated by enhanced lipogenesis. Long-term adiposity (in the context of childhood obesity) cannot be attributed to changes in energy expenditure and/or defects in adipocyte proliferation/differentiation. Therefore, here we propose that stable adipose hypertrpophy could be mediated by low adipocyte turnover. We are currently testing this hypothesis in vivo (deuterium labelling). Together, we speculate that low turn-over might explain why treating obesity in young children is so challenging.

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Insulin-stimulated brain glucose uptake correlates negatively with peripheral insulin sensitivity already in the early phase of metabolic dysregulation

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Background and aims: Previous studies have shown that obesity leads to enhanced brain glucose uptake (BGU) and impaired peripheral insulin sensitivity in conditions of hyperinsulinemic-euglycemic clamp. However, most investigations have studied middle-aged obese individuals, and thus it is not known whether altered brain glucose metabolism is an early feature of insulin resistance. In the present study we evaluated whether alterations in BGU and peripheral insulin sensitivity during insulin clamp are present already in overweight subjects with risk factors for metabolic diseases in early adulthood.

Materials and methods: We studied 19 low risk and 19 high risk young (20-35 yrs) healthy male subjects. Subjects in the low-risk group had a normal BMI, had physical activity ≥ 4 h/week, and had no parental overweight or type 2 diabetes (T2D). Subjects in the high-risk group had BMI 25-30 kg/m^2, exercised < 4 h/week and had parental overweight/obesity or T2D. BGU was measured during hyperinsulinemic-euglycemic clamp using [^{18}F]FDG positron emission tomography (PET). BGU was quantified with fractional uptake rate. Endogenous glucose production (EGP) was calculated from [^{18}F]FDG disappearance rate and whole-body insulin sensitivity (M-value) was calculated. Statistical analysis was performed with statistical parametric mapping (SPM) and additionally with SPSS.

Results: The high-risk subjects had worse metabolic profile compared to the low-risk subjects comprising for BMI (27.1 ± 1.9 vs, 21.9 ± 2.0 kg/m^2), fat percentage (29.1 ± 7.6 vs. 16.4 ± 5.5 %) and visceral (VAT) (3.7 ± 1.2 vs. 1.4 ± 0.7 kg) and abdominal subcutaneous adipose tissue (SAT) (6.8 ± 2.4 vs. 2.7 ± 1.0 kg) mass (all $P < 0.0001$). M-value was lower in the high-risk compared with low-risk subjects (38.7 ± 13.7 vs. 58.0 ± 14.7 μmol/kg/min, $P = 0.0001$). Insulin-suppressed EGP did not differ between the groups (0.2 ± 6.5 vs. -2.5 ± 7.0 μmol/kg/min, *ns*). The high-risk subjects had lower rates of GU in femoral skeletal muscle (34.0 ± 11.5 vs. 52.5 ± 15.1 μmol/ kg/min, $P = 0.0001$), liver (20.9 ± 6.9 vs. 24.9 ± 6.1 μmol/kg/min, $P = 0.06$), abdominal SAT (12.3 ± 5.3 vs. 22.7 ± 7.6 μmol/ kg/min, $P < 0.0001$), VAT (19.4 ± 6.5 vs. 32.0 ± 7.46 μmol/ kg/min, $P < 0.0001$), femoral SAT (12.0 ± 5.5 vs. 19.5 ± 6.7 μmol/ kg/min, $P = 0.0005$) and brown adipose tissue (23.7 ± 9.4 vs. 31.4 ± 9.5 μmol/ kg/min, $P = 0.005$) compared to the low-risk subjects. Global BGU was higher in the high-risk as compared to the low-risk group (16.0 ± 2.2 vs. 15.5 ± 2.2, $P = 0.5$), and this result was significant in two brain regions of interest ($P = 0.05$). Only in the high-risk group, BGU correlated inversely with the M-value (r = -0.65, $P = 0.003$ in high risk group) and positively with EGP (r = 0.71, $P = 0.001$). BGU correlated negatively with skeletal muscle GU (r = -0.47, P = 0.04) and brown adipose tissue GU (r = -0.44, P = 0.07) in the high-risk group, in the low-risk group no association was found.

Conclusion: Our study adds to the current knowledge by demonstrating that BGU during insulin clamp is inversely associated with peripheral insulin sensitivity already in young, overweight subjects, suggesting that alteration of brain glucose metabolism occurs early in the pathophysiology of insulin resistance.

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Divergent effects of estradiol and its receptors on human adipocyte glucose uptake: impact of menopausal status

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Background and aims: Estrogen plays an important role in regulating metabolic health in women. The decline in circulating estrogen levels in postmenopausal women predisposes them to metabolic side effects, including intra-abdominal fat accumulation, insulin resistance and type 2 diabetes. However, cellular mechanisms are not well known. We studied the expression of estrogen receptors in adipose tissue from pre- and postmenopausal women and investigated the effects of 17-β estradiol (E2) on the glucose uptake capacity of isolated mature adipocytes. Furthermore, we explored the effects of estrogen receptor knockout on adipocyte differentiation and glucose uptake.

Materials and methods: Abdominal subcutaneous adipose tissue was obtained by needle biopsies from women (32 premenopausal, 42 postmenopausal, 19-75 years, BMI 20.9-43.7 Kg/m^2). Gene expression of estrogen receptors alpha (*ESR1*), beta (*ESR2*), and the ratio of *ESR1:ESR2* were measured in adipose tissue and correlated to anthropometric and clinical characteristics of subjects. Adipose tissue was incubated with physiological levels of E2 (0.1 nM) for 24 hours to study its effect on glucose uptake of adipocytes and estrogen receptor levels. Polymorphisms in *ESR1* and *ESR2* were also investigated using Open Targets Genetics to determine SNPs that may be involved in diabetes-related metabolic perturbations. In addition, *ESR1* and *ESR2* were knocked out in SGBS preadipocytes using CRISPR/Cas9 and adipocyte glucose uptake (per protein) and adipogenesis (fat cell differentiation) were measured.

Results: In postmenopausal women, the expression of *ESR2* in adipose tissue was higher than *ESR1* (53%; p>0.05), whereas in premenopausal women, the expression of ESRs did not differ. In late (>6 years after menopause), but not in early (<6 years) postmenopausal or premenopausal women, E2 incubation reduced basal and insulin-stimulated glucose uptake (p>0.05), suggesting that time since menopause is relevant for estradiol effects on adipocyte glucose uptake. In late postmenopausal women, E2 incubation increased the expression of the *ESR2* gene but not *ESR1* (p>0.05). In all women (pre- and postmenopausal), the expression of *ESR1* correlated negatively with BMI, body fat %, waist circumference, and waist-hip ratio. The expression of the *ESR2* gene showed a significant positive association with age and HDL and a negative association with BMI and waist circumference. *ESR1* polymorphisms were associated with weight and body fat distribution, whereas polymorphisms in *ESR2* were associated with body fat percentage. In addition, preliminary data suggests that *ERS1* knockout in a human preadipocyte cell strain (SGBS) resulted in impairment of adipogenesis as well as basal and insulin-stimulated glucose uptake, while *ESR2* knockout resulted in an increase in glucose uptake and an increasing adipogenesis trend.

Conclusion: The inhibitory effects of estradiol on adipocyte glucose utilization seem to be dependent on the time since menopause as it was reduced in late, but not early, postmenopausal women, possibly due to increased *ESR2*. Moreover, preliminary data from estrogen receptor knockouts in SGBS preadipocytes further supported the enhancing effect of *ESR1* and inhibitory effect of *ESR2*, respectively, on glucose metabolism of adipocytes.

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SO 24 Glucose-lowering drugs and the liver

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The glucose-lowering effect of the bile acid sequestrant sevelamer in patients with type 2 diabetes is not mediated by glucagon-like peptide 1

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Background and aims: Bile acid sequestrants are cholesterol-lowering drugs, which also improve glycaemic control in patients with type 2 diabetes (T2D). The mechanism behind the glucose-lowering effect has been proposed to be mediated by increased glucagon-like peptide 1 (GLP-1) secretion. Using the GLP-1 receptor antagonist exendin(9-39)NH_2, we investigated the GLP-1-mediated contribution to the glucose-lowering effect of the bile acid sequestrant sevelamer in patients with T2D.

Materials and methods: In a randomised, double-blind, placebo-controlled, crossover study, patients (9 men / 6 women) with T2D (median [interquartile range] age 68 [63-71] years, BMI 30.1 [26.6-33.0] kg/m^2, HbA1c 48 [45-61] mmol/mol (6.5 [6.3-7.7]%)) on metformin monotherapy underwent two 17-day treatment periods with either sevelamer or placebo in a randomised order. On day 15 and day 17 of each treatment period, participants were submitted to two experimental study days including four-hour liquid meal tests with concomitant infusion of exendin(9-39)NH_2 or saline.

Results: Compared to placebo, sevelamer lowered both fasting plasma glucose concentrations ((mean ± SEM) 8.7 ± 0.3 vs. 10.2 ± 0.5 mmol/l, p <0.001) and postprandial plasma glucose excursions (baseline-subtracted AUC (bsAUC): 457 ± 40 vs. 620 ± 64 mmol/l × min, p <0.001). In both treatment periods, exendin(9-39)NH_2 increased the glucose bsAUC compared to saline (p <0.001) with no absolute or relative difference between the two treatment periods (ΔbsAUC: 365 ± 34 mmol/l × min [170 ± 28%] (sevelamer) vs. 428 ± 58 mmol/l × min [165 ± 40%] (placebo treatment), p = 0.17).

Conclusion: Using the GLP-1 receptor antagonist exendin(9-39)NH_2, we were not able to detect a GLP-1-mediated glucose-lowering effect of sevelamer in patients with T2D. Thus, the mechanism behind the glucose-lowering effect of sevelamer remains unresolved.

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Metformin lowers portal pressure in patients with cirrhosis and portal hypertension: a randomised clinical trial

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Background and aims: Portal hypertension (PH) is the main determinant of clinical decompensation in patients with liver cirrhosis. In pre-

clinical data metformin lowers portal pressure, but there are no clinical data for this beneficial effect. We aimed to investigate the acute effects of metformin on hepatic venous pressure gradient (HVPG) and liver perfusion in patients with cirrhosis.

Materials and methods: In a randomized, double-blinded, placebo-controlled parallel study design we investigated 32 β-blocker naive patients with cirrhosis before and 90 minutes after an oral single-dose of 1000 mg metformin (n=16) or placebo (n=16). Liver vein catherization was performed to evaluate HVGP and indocyanine green (ICG) infusion for investigation of hepatic blood flow.

Results: The mean relative change in HVPG was -16% (95%CI: -28 to -4%) in the metformin group compared with 4% (95%CI:-6 to 14%) in the placebo group (*time x group*, p = 0.008). In patients with baseline HVPG ≥ 12 mmHg clinically significant improvements in HVPG (HVPG < 12 mmHg or a > 20% reduction in HVPG) was observed in 46% (6/13) of metformin-treated and in 8% (1/13) of placebo-treated patients (p = 0.07). There were no changes or differences in systemic blood pressure, heart rate, hepatic plasma- and blood flow, hepatic ICG clearance, hepatic O_2 uptake, or inflammation markers between groups.

Conclusion: A single oral metformin dose acutely reduces HVPG in patients with cirrhosis and PH without affecting systemic or liver hemodynamics, or inflammatory biomarkers. This offers a promising perspective of a safe and inexpensive treatment option that should be investigated in larger scale clinical studies with long-term outcomes in patients with cirrhosis and PH.

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Sodium-glucose linked transporter 2 inhibitors (SGLT2s) and alanine aminotransferase levels (ALT) in the Associated of British Clinical Diabetologists (ABCD) audits

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Background and aims: The ABCD SGLT2 audit programmes launched in 2014 with Dapagliflozin (D) and has since expanded to include Empagliflozin (E) and Canagliflozin (C). The results so far have provided valuable insight into the real-world use of these drugs. Previous analyses have demonstrated reductions in ALT associated with SGLT2 use which may have implications in fatty liver disease. Our aim is to compare changes in ALT levels following commencement across the class.

Materials and methods: Data submitted to the ABCD SGLT2 audits were included providing a baseline and follow-up ALT were available. Changes in ALT were assessed within subgroups stratified by baseline ALT: group 1 ALT≤30U/L; group 2 (slight elevation) 31-60U/L; group 3 (significant elevation) >60U/L . Association of ALT change with weight loss and HbA1c were assessed by regression analysis. Changes in ALT were assessed by by Wilcoxon Sign Rank and differences between drugs/groups by Kruskal-Wallis with Bonferroni corrections in Stata 16.

Results: 21,338 datasets met inclusion criteria (E=11,234; D=7,841; C=2,263), baseline mean±SD age 60±10.4 years (E=60±10.4; D=59.9 ±10.3; C=60.1±10.6), weight 97.5±22.1kg (E=96.6±22; D=98.7±22.1; C=97.9±21.9) and HbA1c 75.8±25.3mmol/mol (E=76.1±23.5, D=75.3 ±29.3; C=76±17.7). Median diabetes duration (IQR) was 8.2 years (4.3-12.4) and ALT levels were 28U/L (20-41). Baseline characteristics were therefore similar across all three drugs. Median follow-up of 1.5 years. ALT levels changed by a median -3U/L (95% CI -3, -3; P<0.0001) from baseline in those included. Other than group 1 canagliflozin and dapagliflozin, significant changes in ALT were noted in all groups, across all drugs, greatest in group 3 with changes of -29U/L (95% CI -28, -30). In group 3, empagliflozin use was associated with ALT changes of -30U/L (95% CI -29, -32) as compared to -26U/L (95% CI -22.7, -31) with canagliflozin and -28U/L (95% CI -26, -30) with dapagliflozin. Group 2 ALT changes were intermediary with reductions in ALT across all three drugs of -9U/L (95% CI -8, -9). All the above results are significant to P<0.001. In all analyses empagliflozin was associated with significantly larger reductions in ALT than dapagliflozin or canagliflozin (P<0.05). Weight loss was significantly associated with larger reductions in ALT level at follow-up (P<0.0001) as was change in HbA1c (P<0.001). This finding was the same across all three drugs, although weight and HbA1c reductions were greatest with empagliflozin.

Conclusion: SGLT2 use is associated with significant reductions in ALT at follow-up; these are greatest with raised ALT at baseline and correlated with change in weight and HbA1c. The effect of empagliflozin was superior and may be due to better HbA1c and weight response to treatment. These factors seem unlikely to fully explain the changes in ALT observed across the three drugs. More data is needed to assess the benefits of SGLT2s in non-alcoholic fatty liver disease.

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Long-term effects of ertugliflozin (ERTU) on liver enzymes and indices in patients with type 2 diabetes: analyses from VERTIS CV

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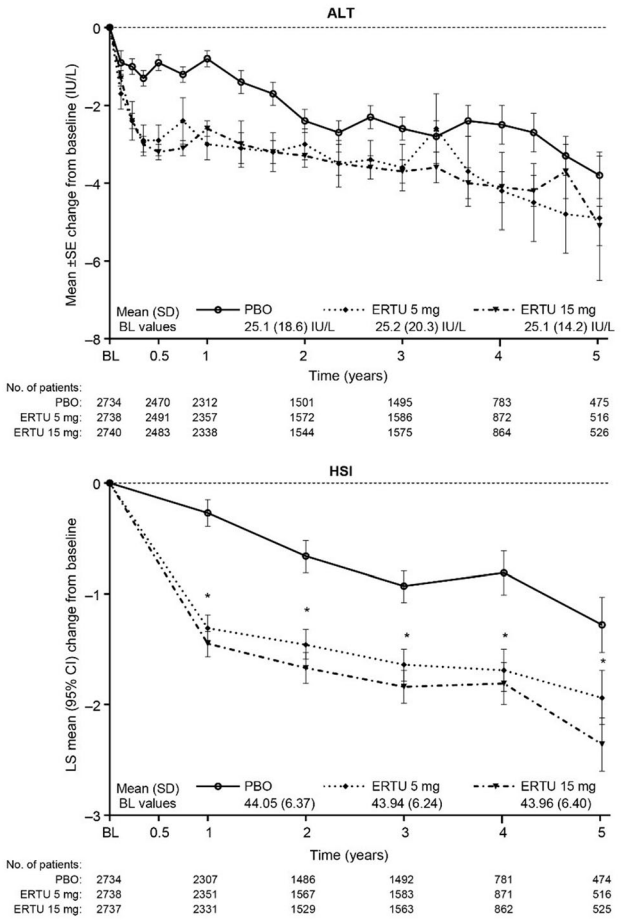
Background and aims: Non-alcoholic fatty liver disease (NAFLD) is a metabolic liver disorder associated with increased liver aminotransferases, obesity, insulin resistance and dyslipidaemia. VERTIS CV was a CV outcome trial of the SGLT2 inhibitor ERTU vs placebo (PBO) added to standard of care in patients (pts) with T2D and atherosclerotic CVD (ASCVD). ERTU reduced HbA_{1c}, BP and body weight vs PBO. Given these improvements in NAFLD risk factors with ERTU, we hypothesised that additional hepatic benefits could be evaluated by changes in liver enzymes (pre-specified) and non-invasive NAFLD scores (post-hoc).

Materials and methods: Pts were randomised to ERTU 5 mg, 15 mg or PBO. Risk of hepatic steatosis was assessed using the hepatic steatosis index (HSI) with advanced fibrosis assessed using the fibrosis-4 (FIB-4) score, and the NAFLD fibrosis score (NFS). Changes from baseline (BL) in AST, ALT, HSI, FIB-4 score and NFS were assessed.

Results: BL characteristics were balanced across treatment groups; mean (SD) BMI was 32.0 (5.4) kg/m^2 with mean AST and ALT levels within the normal range. At BL, 91% of pts had an HSI ≥36, indicative of hepatic steatosis. At BL, 2% of pts aged 36-64 and 5% ≥65 years had a FIB-4 score >2.67 while 23% of pts had a NFS >0.675, both indicative of advanced fibrosis. Small but consistent decreases in AST and ALT (**Figure**) were observed with ERTU vs PBO and were sustained for up to 5 years. There was a small but sustained decrease in HSI with PBO and ERTU (**Figure**). The mean reduction in HSI from BL was greater with ERTU vs PBO (P<0.001) at all timepoints up to 5 years. Mean HSI scores remained above the steatosis threshold with ERTU and PBO at all timepoints; the proportion of pts with HSI ≥36 decreased to 81%, 83% and 86% with ERTU 5 mg, 15 mg and PBO, respectively, by Year 5. There was no consistent change in FIB-4 score or NFS with ERTU or PBO although small sporadic changes in both scores were observed.

Conclusion: In VERTIS CV, a population with T2D, ASCVD and high-risk of NAFLD, ERTU, vs PBO, was accompanied by modest reductions in liver enzymes and indices of hepatic steatosis, but not fibrosis. It is unknown if these changes are due to ERTU benefit on HbA_{1c}, BMI or other factors.

Figure. Changes in ALT and HSI Over Time in Patients with T2D and Atherosclerotic CVD



For ALT, absolute change from BL is presented. For HSI, LS mean change from BL is presented; at each timepoint LS mean change was evaluated using a repeated measures analysis of variance model, containing baseline score, treatment, timepoint, HbA_{1c} at BL, eGFR at BL, treatment-by-timepoint interaction. Time is treated as a categorical variable. HSI was calculated as: 8 x ALT/AST + BMI + 2 (if females) + 2 (for T2D).
*P<0.001 for both ERTU 5 mg and ERTU 15 mg vs PBO.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BL, baseline; CVD, cardiovascular disease; ERTU, ertugliflozin; HSI, hepatic steatosis index; LS, least squares; PBO, placebo; T2D, type 2 diabetes.

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Effect of tirzepatide versus insulin degludec on liver fat content and abdominal adipose tissue in patients with type 2 diabetes (SURPASS-3 MRI)

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Background and aims: Tirzepatide (TZP) is a novel dual glucose-dependent insulinotropic polypeptide (GIP)/glucagon-like peptide-1 (GLP-1) receptor agonist in development for the treatment of type 2 diabetes (T2D). The effect of TZP vs insulin degludec (IDeg) on liver fat content (LFC) and volume of abdominal visceral and subcutaneous adipose tissue (VAT and SAT) was assessed in a subpopulation of patients in the SURPASS-3 study with higher likelihood of elevated LFC.

Materials and methods: This substudy of the open-label, Phase 3 trial included insulin-naive patients with T2D inadequately controlled on stable doses of metformin with/without sodium-glucose co-transporter-2 inhibitors (SGLT-2i) and Fatty Liver Index (FLI) >60 at baseline (BL). Patients had an MRI scan performed prior to randomisation (1:1:1:1) to once-weekly TZP (5, 10, 15 mg) or once-daily IDeg. LFC (expressed as percentage) and abdominal VAT and SAT volumes were assessed with MRI techniques. The primary objective was to compare the change from BL in LFC at Week 52 using pooled TZP data from 10 and 15 mg dosing arms vs IDeg. Secondary objectives included comparison of the individual TZP doses vs IDeg at Week 52 for LFC, abdominal VAT and SAT volumes; proportion of patients achieving LFC ≤10% and ≥30% relative decrease from BL in LFC.

Results: A total of 296 patients from TZP (5 mg, N=71; 10 mg, N=79; 15 mg, N=72) and IDeg (N=74) arms had evaluable MRI data during the study (mean BL age, 56.2 years; T2D duration, 8.3 years; HbA_{1c}, 67 mmol/mol (8.2%); body weight [BW], 94.4 kg; BMI, 33.5 kg/m^2; 30% on SGLT-2i). All individual TZP doses reduced LFC from BL to a greater extent than IDeg at Week 52 (Table). The absolute reduction from BL in LFC at Week 52 was significantly greater for the pooled TZP 10/15 mg arms vs IDeg arm (least squares mean treatment difference [95% CI]: -4.71% [-6.72, -2.70], p<0.001). The proportions of patients achieving LFC ≤10% and ≥30% relative decrease from BL in LFC at Week 52 were significantly greater in each TZP arm vs IDeg arm (Table). All doses of TZP reduced abdominal VAT and SAT volumes at Week 52 while IDeg increased both (Table). There were significant correlations between changes in LFC and HbA_{1c} (ρ=0.31, p≤0.01), BW (ρ=0.31, p≤0.01), SAT (ρ=0.30, p≤0.01) and VAT (ρ=0.24, p<0.05) volumes, alanine aminotransferase (ρ=0.40, p≤0.001), and aspartate aminotransferase (ρ=0.25, p<0.05) in the pooled TZP 10/15 mg arms. Baseline and endpoint values at Week 52 for all these parameters are shown in the Table (individual arms).

Conclusion: TZP demonstrated a clinically meaningful reduction in LFC and abdominal VAT and SAT volumes compared to IDeg in this subpopulation of patients with T2D and elevated LFC in the SURPASS-3 study.

Primary and Secondary Endpoints[a], Week 52	TZP 5 mg, N=71	TZP 10 mg, N=79	TZP 15 mg, N=72	IDeg, N=74
BL/endpoint LFC (%)[b]	14.88 (1.109)/10.11 (0.795)*	14.78 (1.039)/8.16 (0.792)**	16.65 (1.092)/9.59 (0.768)**	16.58 (1.053)/13.18 (0.791)
Relative change from BL in LFC (%)[b]	-29.78 (5.607)*	-47.11 (5.562)**	-39.59 (5.417)**	-11.17 (5.579)
% Patients with LFC ≤10% at BL/endpoint[c]	32.6 (5.613)/60.4 (7.979)*	38.0 (5.643)/77.9 (6.409)**	19.9 (4.873)/73.9 (6.694)**	29.5 (5.372)/34.8 (7.865)
% Patients with ≥30% relative decrease in LFC[c]	66.9 (7.394)*	81.4 (5.623)**	78.8 (5.970)**	32.1 (7.190)
BL/endpoint abdominal VAT volume (l)[d]	6.87 (0.240)/5.42 (0.187)**	6.21 (0.232)/5.00 (0.178)**	6.81 (0.238)/4.88 (0.181)**	6.34 (0.230)/6.90 (0.182)
BL/endpoint abdominal SAT volume (l)[d]	10.99 (0.506)/9.07 (0.247)**	10.21 (0.491)/8.22 (0.236)**	10.34 (0.502)/8.42 (0.234)**	10.04 (0.485)/11.10 (0.240)
BL/endpoint body weight (kg)	98.0 (1.93)/86.9 (0.85)**	93.4 (1.85)/84.9 (0.83)**	95.6 (1.92)/83.5 (0.85)**	91.3 (1.92)/97.2 (0.86)
BL/endpoint HbA_{1c} (mmol/mol)	69.8 (1.19)/44.7 (1.34)**	68.4 (1.14)/43.3 (1.32)**	65.8 (1.18)/41.2 (1.33)**	65.8 (1.18)/53.4 (1.36)
BL/endpoint HbA_{1c} (%)	8.57 (0.109)/6.24 (0.122)**	8.41 (0.104)/6.12 (0.121)**	8.15 (0.108)/5.92 (0.122)**	8.15 (0.108)/7.04 (0.124)
BL/endpoint ALT (U/l)[e,f]	28.0 (1.71)/21.8 (1.12)	25.8 (1.54)/19.1 (0.97)*	26.1 (1.59)/17.8 (0.89)**	24.7 (1.53)/22.8 (1.20)
BL/endpoint AST (U/l)[e,f]	20.7 (0.97)/18.3 (0.76)	19.6 (0.90)/17.8 (0.74)*	20.6 (0.96)/16.5 (0.67)**	20.0 (0.95)/20.4 (0.87)

Data are estimates (SE), unless otherwise noted. *p<0.05 and **p<0.001 are both vs IDeg.

TZP doses were achieved through stepwise 2.5-mg dose escalation every 4 weeks. IDeg starting dose was 10 U/day and it was titrated to a FSG <5 mmol/l following a treat-to-target algorithm. Mean IDeg dose at Week 52 was 58.8 U/day.

[a]mITT-Efficacy Analysis Set, unless otherwise noted: on treatment data prior to initiating rescue therapy from mITT population excluding patients with baseline and postbaseline data not obtained or not valid. N values vary across primary and secondary endpoints at Week 52.

[b]Missing values at Week 52 were imputed with LOCF using mITT efficacy analysis set (if early termination or unscheduled visit with MRI scan available).

[c]mITT-Safety Analysis Set: all available data from mITT population including safety follow-up regardless of adherence to study drug or use of rescue therapy including patients with non-missing baseline and at least one non-missing post-baseline record.

[d]Statistical significance based on percent change from BL vs IDeg.

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BL = baseline; FSG = fasting serum glucose; HbA_{1c} = haemoglobin A_{1c}; IDeg = insulin degludec; l = litres; LFC = liver fat content; LOCF = last observation carried forward; mITT = modified intent-to-treat (all randomised patients who took at least one dose of study drug); N = number of patients in specified dataset; SAT = subcutaneous adipose tissue; SE = standard error; TZP = Tirzepatide; VAT = visceral adipose tissue.

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Disclosure: **A. Gastaldelli:** Employment/Consultancy; Consultant for Inventiva. Honorarium; Novo Nordisk. Other; Boheringer, Eli Lilly and Company, and Gilead.

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Hepatic impairment has no impact on the clinical pharmacokinetics of tirzepatide

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Background and aims: Tirzepatide is a dual agonist of glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 receptor agonist in development as a potential weekly treatment for type 2 diabetes, weight management and nonalcoholic steatohepatitis. The impact of hepatic impairment on the pharmacokinetics (PK) of tirzepatide was assessed in participants with varying degrees of hepatic impairment (with and without type 2 diabetes) compared to healthy control participants.

Materials and methods: Participants in this parallel, single-dose, open-label study were categorized by hepatic impairment defined by baseline Child-Pugh (CP) score A (mild impairment; N=6), B (moderate impairment; N=6) or C (severe impairment; N=7) or normal hepatic function (N=13). All participants received a single subcutaneous dose of tirzepatide 5 mg. Blood samples were collected to determine tirzepatide plasma concentrations to estimate PK parameters. The primary PK parameters of area under the drug concentration-time curve from zero to infinity (AUC[0-∞]) and maximum observed drug concentration (C_{max}) were evaluated using an ANCOVA model. The geometric least squares means and mean ratios for each group, between each hepatic impairment level versus the control group, and the corresponding 90% confidence intervals (CIs) were estimated. The analysis of time of maximum observed drug concentration (t_{max}) was based on a nonparametric method. The relationships between the PK parameters and CP classification parameters (serum albumin concentration, total bilirubin concentration, and international normalized ratio) were also assessed. Adverse events were monitored to assess safety and tolerability.

Results: Tirzepatide exposure, based on AUC(0-∞) and C_{max}, was similar across the control and hepatic impairment groups. Statistical analysis showed no difference in the geometric least squares mean C_{max} or AUC(0-∞) between participants in the control group and the hepatic impairment groups, with the 90% CI for the ratios of geometric least squares means spanning unity. There was no change in median t_{max} of tirzepatide across all groups. There was no significant relationship between the exposure of tirzepatide and CP score ($p>0.1$ for C_{max}, AUC[0-∞], and apparent total body clearance. Similarly, there was no clinically relevant relationship between the exposure of tirzepatide and serum albumin concentration, total bilirubin concentration, or international normalized ratio. The geometric least squares mean $t_{1/2}$ values were also similar across the control and hepatic impairment groups. No notable differences in safety profiles were observed between subjects with hepatic impairment and healthy control subjects.

Conclusion: Tirzepatide PK were similar in participants with varying degrees of hepatic impairment compared to healthy subjects. Thus, participants with hepatic impairment treated with tirzepatide may not require dose adjustments.

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Supported by: Eli Lilly and Company

Disclosure: **C. Loghin:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

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Blueberry juice counteracts metabolic dysregulation in a rat model of prediabetes by targeting hepatic mitochondrial bioenergetics and metabolic pathways

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Background and aims: This study aimed to assess the effects of blueberry juice (BJ) on metabolic profile, gut microbiota composition, gut barrier integrity and metabolic endotoxemia, as well as on hepatic metabolism and mitochondrial-related parameters, in a prediabetic rat model.

Materials and methods: A prediabetic rat model [Male Wistar rats, 8 weeks old] was established by ingestion of a high-sucrose (HSu, 35%) diet for 9 weeks (W9), supplemented with a high-fat diet (HF, 60%) for further 14 weeks (HSuHF, W23), vs control with standard diet. Half of the animals (n=10/group) daily received BJ (25g/Kg BW, orally) between W9 and W23 (HSuHF+BJ). Along with metabolic characterization, a ^{1}H NMR-based metabolomic approach was performed to elucidated BJ effects on serum and hepatic metabolic surrogates. Gut microbiota composition, colonic ultra-structural morphology, intestinal permeability, and systemic inflammation were analyzed. Morphological and functional markers of liver damage were assessed by ultrasonography, immunohistochemical staining techniques and mitochondrial bioenergetics assays; RT-qPCR was performed to analyze hepatic expression profile of gene involved in chief metabolic pathways. Values are means ± S.E.M (ANOVA followed by post-hoc tests).

Results: BJ treatment prevented diet-evoked metabolic dysregulation. Notably, HSuHF+BJ rats ameliorated ($p<0.05$) glucose tolerance, insulin sensitivity and hypertriglyceridemia, together with attenuation of liver impairment, as seen by reduced steatosis and by improved mitochondrial function, without affecting gut microbiota composition and related barrier properties. Apart from restoring hepatic antioxidant metabolites, BJ positively affected the hepatic mRNA expression of key targets involved in fatty acid oxidation, insulin signaling, inflammatory response, as well as

mitochondrial respiratory chain-related genes, which were all downregulated ($p<0.05$) in HSuHF animals' livers.

Conclusion: BJ can be a useful nutraceutical for preventing prediabetes progression induced by hypercaloric diet, due to beneficial effects against hepatic steatosis and mitochondria dysfunction, independent of gut microbiota modulation at this early stage of disease.

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SO 25 Interaction of digestive system and glucose metabolism

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Effects of SGLT2-inhibition on postprandial insulin exposure in patients with postbariatric hypoglycaemia: a modelling analysis

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Background and aims: Insulin secretion and kinetics is key to understand postbariatric hypoglycemia (PBH), an increasingly recognized late metabolic complication of bariatric surgery, particularly gastric bypass (GB). Recently, the SGLT2-inhibitor empagliflozin was shown to reduce hypoglycaemia burden in patients with PBH. Herein, we aim to quantify the effect of SGLT2-inhibition on insulin secretion, hepatic insulin extraction and insulin kinetics by means of the Oral C-peptide Minimal Model (OCMM) coupled with a model of insulin extraction and kinetics (IMM).

Materials and methods: In a randomized two-period double-blind cross-over design, twelve post-GB adults with PBH (9F/3M; age=44±10y; BMI=27.4±3.9kg/m^2) received a single dose of empagliflozin (10mg) or placebo before a standardized liquid mixed-meal with frequent measurement of plasma glucose, C-peptide (CP) and insulin (I) over 3h. The OCMM and the IMM were used to describe CP and I data and estimate C-peptide secretion and hepatic insulin extraction. C-peptide and post-hepatic insulin kinetics, assumed to be identical on both visits, were also estimated but using a recently proposed Bayesian approach.

Results: Results are reported in Table 1. Reduced insulin exposure observed with empagliflozin was quantified by the modelling analysis by means of a significant reduction in basal beta-cell responsiveness (ϕ_b, p=0.0019) together with a significant increase in total hepatic insulin extraction (HE_{tot}, p=0.019) after empagliflozin compared with placebo. On the contrary, total beta-cell responsiveness (ϕ_{tot}) and basal hepatic insulin extraction (HE_b) resulted similar between visits.

Conclusion: The present modelling analysis suggests that the hypoglycaemia-attenuating effect of SGLT2-inhibition, apart from well-established non-insulin dependent mechanisms, is additionally mediated by an increase in HE_{tot}. These insights underscore the central role of the liver in the regulation of peripheral insulin exposure.

Estimated parameters	Unit of Measurement	PBO	SGLT-2	PBO vs. SGLT-2 (p-value)
ϕ_b	[10^{-9} min^{-1}]	8.12 ± 2.64	6.76 ± 2.03	**0.0019**
ϕ_{tot}	[10^{-9} min^{-1}]	25.7 ± 7.0	23.8 ± 8.9	0.23
C-peptide MCR	[min^{-1}]	0.083 ± 0.014		
HE_b	[%]	87 ± 0.03	85 ± 8	0.28
HE_{tot}	[%]	56 ± 15	65 ± 10	**0.019**
Post-hepatic Insulin CL	[L/min]	0.93 ± 0.35		

Table 1. Estimated OCMM and IMM parameters. OCMM: basal and total beta-cell responsiveness (ϕ_b and ϕ_{tot}, respectively) and fractional C-peptide metabolic clearance rate (MCR). IMM: basal and total hepatic insulin extraction (HE_b and HE_{tot}, respectively) and post-hepatic insulin clearance (CL). Results are presented as mean±SE. Difference between PBO and SGLT-2 visits was assessed by paired T-test (p-value < 0.05 was considered statistically significant).

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SGLT2 contributes to glucoregulatory improvements following vertical sleeve gastrectomy in mice

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Background and aims: Bariatric surgery has been demonstrated to improve insulin sensitivity and glucose clearance, but also increases glucagon secretion and cardiovascular health. Both effects have also been observed following treatment with sodium glucose co-transporter 2 (SGLT2) inhibitors. We previously demonstrated that bariatric surgery in lean and obese animals causes a significant reduction of SGLT2 gene and protein expression in the kidney cortex, pointing towards a physiologically-relevant gut-kidney axis. Therefore, we investigated the role of SGLT2 in mediating the glucoregulatory benefits of bariatric surgery.

Materials and methods: SGLT2 knockout mice were generated on a C57/BL6 background using CRISPR/Cas9 in embryonic fibroblasts to delete Exon 1. Deletion was confirmed by Sanger sequencing. Vertical Sleeve Gastrectomy (VSG) or sham surgery was performed in high-fat-fed male $SGLT2^{+/+}$ (wild type) and $SGLT2^{-/-}$ (knockout) mice. $SGLT2^{-/+}$ (het) mice were also used as a control. Glucose Tolerance tests with or without treatment with the SGLT2 inhibitor dapagliflozin were performed twelve weeks post operatively.

Results: $SGLT2^{-/-}$ sham mice demonstrated significantly improved glucose and insulin tolerance when compared to $SGLT2^{+/-}$ sham mice ($p<0.05$), as well as significantly higher fasted insulin levels ($p<0.05$). $SGLT2^{-/-}$ VSG mice had improved, but not significantly higher, glucose and insulin tolerance and secretion when compared to $SGLT2^{-/-}$ sham mice and $SGLT2^{+/+}$ VSG mice. Following dapagliflozin treatment, both $SGLT2^{-/-}$ VSG and $SGLT2^{-/-}$ sham mice had significantly improved glucose tolerance when compared to $SGLT2^{-/-}$ VSG and $SGLT2^{-/-}$ sham mice following vehicle treatment ($p<0.05$).

Conclusion: In the absence of SGLT2, the significant differences in the glucose and insulin tolerance phenotype following VSG are reduced, indicating that SGLT2 contributes to glucoregulatory improvements following bariatric surgery in mice. Moreover, dapagliflozin causes further metabolic improvement in $SGLT2^{-/-}$ mice, pointing at a potential beneficial off-target mechanism.

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Disclosure: **E. Akalestou:** None.

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Robust metabolic benefits of a novel orally administered polymeric duodenal exclusion therapy in animal models of type 2 diabetes: an alternative treatment for type 2 diabetes

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Background and aims: Bariatric surgery, by preventing nutrient exposure to proximal small intestine (duodenum and proximal jejunum) is the most successful long-term therapy for T2DM and obesity, reducing macro-, micro-vascular and mortality. However, surgical interventions are expensive, invasive and carry significant risk. Therefore, there is a need for a non-invasive oral therapy that mimics the effects of bariatric surgery. Thus, Glyscend Inc. hypothesized that the metabolic improvements of bariatric surgery could be replicated by an orally ingestible polymer that creates a lining, and has developed oral, non-absorbed polymers (GLY-100 and -200) to act locally in the gut, by creating a temporary (4-6 hrs) barrier within the lumen of the proximal small intestine. The polymer acts as a temporary barrier to the stimulation of the epithelium and indirectly modulates neurohormonal signaling via physical interactions with the intestinal wall.

Materials and methods: In a 8-week study of Goto-Kakizaki (GK) T2 diabetic rats, post-prandial glycemia (PPG) incremental area under curve (iAUC) during an oGTT and mixed meal tolerance test (mMTT) was evaluated after once daily oral gavage of GLY100 (n=7) or control (n=7). After chemical modification of GLY-100 suggested an enhancement of metabolic effects, GLY-200 was selected as the clinical candidate. Metabolic effects of once daily oral gavage of GLY-200 (n=14), versus control (n=14) were evaluated in male Zucker Diabetic Fatty (ZDF) rats. Measurements included weekly weight and fasting plasma glucose and insulin and during an oGTT, at weeks 4 and 8 of treatment.

Results: Treatment of GK rats with GLY-100 produced a profound reduction in PPG iAUC after oGTT and mMTT, commencing at day one of therapy and culminating at a ↓ of 60 to 70% at day 39 compared to controls ($p<0.0005$). Similar robust reduction in iAUC PPG was observed during mMTT (↓ of 55%; $p<0.005$). Fasting plasma glucose (FPG) was significantly lower in the GLY-100 group ($P < 0.05$) with improvement in HOMA-IR ($P < 0.005$). Over the study period, actively treated GK rats lost 6% body weight ($p<0.005$), without alteration in food intake. Similarly, GLY-200 significantly reduced peak and iAUC PPG during oGTT at week 4 ($p<0.001$) and week 8 ($p<0.001$) of treatment and simultaneously decreased early peak insulin levels. GLY 200 did not have a significant effect on food intake in the ZDF model. Safety evaluations, including histology, did not reveal significant signals.

Conclusion: Our studies confirm a key role for temporary but repeatable proximal duodenal exclusion provided by novel orally active polymers in improving PPG excursions and in parallel insulin secretion. In clinical studies, greater improvements in long-term parameters of glycaemia and weight loss should be evident by chronic administration of GLY 200 on multiple occasions daily. GLY-200 offers the potential for a novel non-invasive approach for glycemic and weight control in T2D patients that mimics the effects of gastric bypass without the associated adverse effects.

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Disclosure: **A. Nimgaonkar:** None.

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Orally administered lactate elevates glucagon like peptide-1 and slows gastric emptying in young men

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Background and aims: Fermented dairy products, often rich in lactate, are associated with improved metabolic health, including a reduced risk of type 2 diabetes mellitus. The underlying mechanisms are unknown. The aim of this study was to explore whether orally administered lactate affects appetite-regulating hormones, gastric emptying and appetite sensations.

Materials and methods: Ten healthy male volunteers were investigated on two separate study days: 1) following oral administration of D/L-Na-lactate and 2) following oral administration of isotonic iso-voluminous NaCl and intravenous iso-lactaemic lactate infusions. We obtained blood samples, evaluated appetite (questionnaire) and did an ad libitum meal test. Gastric emptying was evaluated using an acetaminophen test.

Results: Plasma concentrations of growth differential factor 15 (GDF15, primary outcome) increased during both oral and iv lactate administration with no difference between interventions (p=0.15). Oral lactate administration increased plasma concentrations of glucagon like peptide-1 (p=0.04), insulin (p<0.001), c-peptide (p<0.001) and glucagon (p<0.001) and decreased plasma concentrations of acetylated ghrelin (p=0.02) and free fatty acids (p=0.004) compared with iv administration. Furthermore, oral lactate administration slowed gastric emptying (p<0.001), increased the feeling of being "full" (p=0.008), induced satiety (p=0.06) and decreased the "anticipated future food intake" (p=0.007) compared with iv administration.

Conclusion: Orally administered lactate had a direct and significant impact on the upper gastrointestinal tract, affecting gut hormone secretion, gut motility and appetite sensations. These data suggests that lactate (e.g., lactate-rich dairy products) could play a role in the treatment of metabolic disease.

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Three weeks of time restricted eating improves fasting glucose in type 2 diabetes patients but does neither increase nocturnal fat oxidation nor insulin sensitivity

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Background and aims: Time restricted eating (TRE) is a novel strategy to improve metabolic health by limiting the daily eating time window and consequently prolonging the overnight fast. This prolonged fast increases energy storage utilization and might improve insulin sensitivity due to an increased need to replenish nutrient storages. However, to date no studies assessed the effect of TRE on nocturnal substrate oxidation and metabolic health in type 2 diabetes (T2D) patients. Here we examined if 3 weeks of TRE could improve nocturnal fat oxidation and metabolic health of overweight/obese adults with T2D.

Materials and methods: In a randomized controlled cross-over trial, 14 T2D patients (age: 68 ± 5 years, BMI: 30.5 ± 4.2 kg/m^2, fasting glucose: 7.9 ± 1.3 mmol/l, 7 females) were instructed to eat either within 10 hrs during daytime (TRE) or to spread food intake over at least 14 hrs per day (CON), for a duration of 21 days, without altering habitual energy intake and medication use. Outcome parameters included nocturnal energy expenditure and -substrate oxidation assessed by whole-room calorimetry, insulin sensitivity measured by a 2-step hyperinsulinemic-euglycemic clamp, and fasting blood metabolites.

Results: Although weight loss was not encouraged, patients lost more weight after 3 weeks TRE compared to the weight-stable CON (-1.0 ± 1.1 kg vs -0.3 ± 1.0 kg, p < 0.05). On day 20, fasting glucose levels were lower after TRE compared to CON (7.6 ± 1.3 mmol/l vs 8.6 ± 1.5 mmol/l, p < 0.05) with insulin levels being comparable (14.5 ± 8.4 mU/l vs 15.6 ± 10.4 mU/l, p = 0.27). Sleeping metabolic rate tended to be lower in TRE compared to CON (4.66 ± 0.51 kJ/min vs 4.77 ± 0.66 kJ/min, p = 0.06) without concomitant changes in substrate utilization (respiratory exchange ratio: 0.84 ± 0.03 vs 0.84 ± 0.04, p = 0.38). Peripheral insulin sensitivity did not differ between TRE and CON (rate of disappearance: 20.0 ± 5.6 μmol/kg/min vs. 19.2 ± 5.9 μmol/kg/min, respectively; p = 0.59). Also insulin-induced suppression of endogenous glucose production was similar between the two interventions (p = 0.55). During the clamp, free fatty acid levels were significantly lower with TRE during low- and high-insulin infusion (p-values respectively p = 0.01 and p = 0.04), but insulin-induced suppression of free fatty acids was similar between TRE and CON (p = 0.33). Data was analysed using the Wilcoxon matched-pairs signed rank test.

Conclusion: Three weeks of TRE resulted in a lower body weight and improved fasting glucose levels in type 2 diabetes patients. These effects were not accompanied by changes in peripheral insulin sensitivity and/or changes in nocturnal substrate oxidation. These data suggest that TRE may hold promise for long-term glucose homeostasis in type 2 diabetes patients but further research is needed to unravel underlying mechanisms.

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Remission of type 2 diabetes following a short-term intervention with insulin glargine and metformin/sitagliptin: results of the REMIT-sita randomised controlled trial

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Background and aims: Recent studies suggest that non-surgical approaches can be successful in inducing remission of type 2 diabetes, however, evidence is limited. We herein report diabetes remission following a short-term intervention with insulin glargine, metformin/sitagliptin and lifestyle approaches.

Materials and methods: We randomised 102 patients with type 2 diabetes to (i) a 12-week intensive treatment with insulin glargine and metformin/sitagliptin combined with lifestyle therapy or (ii) control group. Participants with HbA_{1C}<56 mmol/mol (7.3%) at 12 weeks were asked to stop diabetes medications and were followed for 1 year. Fasting plasma glucose (FPG) was obtained at 16 weeks, OGTT was conducted at 24 weeks, and HbA_{1C} was measured at 24, 36, 48 and 64 weeks. Diabetes relapse criteria included HbA_{1C} ≥48 mmol/mol (6.5%), glucose >10 mmol/l on ≥50% of glucometer readings, re-initiation of diabetes medications ± abnormal FPG or 2-hour plasma glucose on OGTT. Time-to-relapse analysis was conducted to compare the treatment groups with (primary analysis) and without (supplementary analysis) FPG/OGTT relapse criteria.

Results: With the FPG/OGTT criteria included, hazard ratio of relapse was 0.72 (95% CI 0.47; 1.10) in the intervention group compared to the control group (primary analysis). Removing the 2-hour plasma glucose from the relapse criteria did not affect the results (HR 0.75, 95% CI 0.49; 1.15). Without the FPG/OGTT criteria (supplementary analysis), hazard ratio of relapse was 0.60 (95% CI 0.39; 0.95).

Conclusion: A short-term intervention with insulin glargine and metformin/sitagliptin may induce sustained diabetes remission. Using the 2-hour plasma glucose from OGTT in the relapse criteria did not provide additional information regarding relapse in this study.

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Incidence and prevalence of gastrointestinal tolerability in once weekly dulaglutide (3 and 4.5 mg): a posthoc analysis

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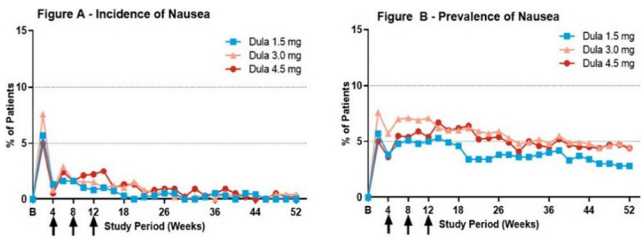
Background and aims: Glucagon-like peptide-1 (GLP-1) receptor agonists are associated with gastrointestinal adverse events (GI AEs). This post-hoc analysis of AWARD-11 in patients with inadequately controlled type 2 diabetes on metformin evaluated GI AEs with once weekly dulaglutide (DU) 1.5 mg (N=612), 3 mg (N=616) and 4.5 mg (N=614) through 52 weeks.

Materials and methods: Patients were started on once weekly DU 0.75 mg for 4 weeks followed by stepwise dose escalation every 4 weeks to their final randomized dose (Fig A & B). Safety analyses included all patients with at least 1 dose of DU.

Results: Overall nausea (15.9%), diarrhoea (10.4%), and vomiting (8.5%) were most frequent AEs with <2% of patients discontinuing treatment due to 1 of these events. Treatment discontinuation from nausea was 1.3% DU 1.5 mg, 3 mg and 1.5% 4.5 mg; from diarrhoea was 0.2% DU 1.5 mg, 1.0% 3 mg, 4.5 mg; from vomiting was 0% DU 1.5 mg, 0.8% 3 mg and 1.3% 4.5 mg . Nausea incidence for all groups peaked after initial DU 0.75 mg dose and fell to <2% by week 4 (Fig A). Most GI AEs were mild to moderate in severity and lasted <7 days. Nausea prevalence (new or ongoing events; events with no end date assumed as ongoing) peaked around treatment initiation and eventually fell to <5% (Fig B).

Conclusion: Nausea frequency was highest across all groups early after initiation and decreased during each subsequent study interval, even after escalating to 3 mg or 4.5 mg. This should allow for more patients to achieve treatment goals with acceptable GI tolerability.



All patients initiated treatment with DU 0.75 mg once weekly for 4 weeks. Thereafter, the dose of dulaglutide was increased at weeks 4, 8, and 12 to 1.5 mg, 3 mg, or 4.5 mg, respectively, until the randomized dose was reached

Clinical Trial Registration Number: NCT03495102

Supported by: Eli Lilly and Company

Disclosure: **J. Van:** Grants; Eli Lilly, Pfizer, Sanofi, Eliem, Aptinyx, Lexicon, Novo Nordisk.

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Remission of type 2 diabetes following intensive treatment with insulin glargine/lixisenatide, metformin and lifestyle approaches: results of the REMIT-iGlarLixi trial

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Background and aims: Remission of type 2 diabetes in response to non-surgical approaches has not been well studied. We investigated induction of diabetes remission following a short-term treatment with insulin glargine/lixisenatide, metformin and lifestyle changes.

Materials and methods: We randomised 160 individuals with type 2 diabetes to (i) a 12-week intensive treatment with the fixed-ratio insulin glargine/lixisenatide, metformin and lifestyle approaches or (ii) standard diabetes management. Diabetes medications were stopped in patients with HbA_{1C}<56 mmol/mol (7.3%) at 12 weeks, and participants were followed for 1 year. Diabetes relapse was defined as either the use of diabetes medications, ≥50% of capillary glucose levels ≥10 mmol/l over 1 week, or HbA_{1C}≥48 mmol/mol (6.5%) at 24, 36, 48 or 64 weeks, and analysed as time-to-event. Treatment groups were compared using a Cox Proportional Hazards model with adjustment for duration of diabetes and baseline HbA_{1C} (primary analysis). Key secondary outcomes included the proportion of participants in remission at 24, 36, 48 and 64 weeks.

Results: The hazard of diabetes relapse was significantly reduced in the intervention group compared to the control group (HR 0.57, 95% CI 0.40-0.81; p=0.002). The proportion of participants with diabetes remission was significantly higher in the intervention group compared to the control group at 24 weeks (38% vs 20%; relative risk RR 1.92 (1.14-3.24)) and at 36 weeks (32% vs 17%; RR 1.83 (1.03-3.26)), but not at 48 and 64 weeks (RR 1.88 (1.00-3.53) and 2.05 (0.98-4.29), respectively).

Conclusion: A short-term intensive treatment with insulin glargine/lixisenatide, metformin and lifestyle changes can induce sustained remission of type 2 diabetes.

Clinical Trial Registration Number: NCT03130426

Supported by: Sanofi

Disclosure: **N. McInnes:** Grants; Sanofi. Non-financial support; Sanofi.

SO 26 Cardiorenal consequences of SGLT2 inhibition

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The effect of two different sodium-glucose co-transporter 2 (SGLT2) inhibitors on decreasing serum uric acid level in patients with type 2 diabetes

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Background and aims: Sodium/glucose co-transporter-2 (SGLT2) inhibitors, the latest class of glucose lowering medication, have been proven to reduce cardiovascular risk, and prevent progression of renal impairment. Animal experiments, and retrospective data analyses of human cardiovascular safety studies have also demonstrated increased urat excretion, and decreased serum uric acid (SUA) level in cases treated with SGLT2 inhibitors. Since hyperuricaemia, a condition frequently present in type 2 diabetes patients, is also associated with higher cardiovascular risk, the lowering of SUA level may add to the beneficial cardiometabolic effect of SGLT2 inhibitors. The aim of our study was to compare the effect of two SGLT2 inhibitor molecules (dapagliflozin and empagliflozin) on SUA level and the change in eGFR over one year in patients with type 2 diabetes.

Materials and methods: A prospective observational study was conducted, whereby a total of 290 patients with type 2 diabetes were included, 171 with added SGLT2 inhibitor treatment (dapa- or empagliflozin) and 120, age, gender, and diabetes duration matched diabetic control. Exclusion criteria were type 1 diabetes, insulin treatment, presence of severe chronic kidney disease, or any diseases associated with secondary hyperuricaemia. Demographic, clinical and laboratory data (including HbA1c, SUA, creatinine) were recorded at baseline and after 12 months. Dapagliflozin, Empagliflozin and control groups were compared using independent samples t test, paired samples t test were used to compare baseline and 12 months data.

Results: HbA1c decreased in both intervention groups compared to control (ΔHbA1c in dapagliflozin vs. empagliflozin vs control group was -0.62±0.72 vs -0.65±1.08, vs. + 0.07±0.8, p =0.000). The control group had no change in serum uric acid levels or eGFR over 12 months (5.61±1.51 vs. 5.6±1.56 mg/dl, p=.86; 78.04±19. mL/min/1.73 m² vs.7.61±18.89 p=0,78), whereas SUA significantly declined in both SGLT 2 inhibitor groups compared to control (ΔSUA in dapagliflozin group vs. control was -0.87±1.35 vs. 0.03±1.61, p=0.001, in empagliflozin group was -1.02 ±1.72 vs. 0.03±1.61, p=0.02). There was no significant difference between the two SGLT 2 inhibitor treated groups. After one year no eGFR decline was to be observed in any of the groups.

Conclusion: Both groups of SGLT2 inhibitors were efficient in decreasing SUA over one year of intervention, however there were no differences in the effect of the two SGLT2 inhibitors. There was no considerable eGFR decline observed in any of the groups.

Disclosure: **M.I.M. Szabo:** None.

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Glycaemic efficacy and safety of ertugliflozin in patients with type 2 diabetes and stage 3 chronic kidney disease: an analysis from VERTIS CV

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Background and aims: VERTIS CV was a cardiovascular (CV) outcome trial that evaluated the CV safety of ertugliflozin (ERTU) in patients with type 2 diabetes and atherosclerotic CV disease (ASCVD). This analysis assessed the glycaemic efficacy and safety of ERTU in VERTIS CV patients with stage 3 chronic kidney disease (CKD).

Materials and methods: Among 8246 patients in VERTIS CV, 1776 patients with stage 3 CKD at baseline were identified based on an eGFR of 30-<60 ml min^{-1} 1.73m^{-2} (1319 patients with stage 3A CKD [eGFR 45-<60 ml min^{-1} 1.73m^{-2}]; 457 patients with stage 3B CKD [eGFR 30-<45 ml min^{-1} 1.73m^{-2}]), calculated via the Modification of Diet in Renal Disease study equation. Efficacy endpoints were changes from baseline in HbA_{1c}, body weight and systolic blood pressure (SBP) to Week 18, when doses of background antihyperglycaemic medications were to be held constant. Safety assessments included adverse event (AE) reports.

Results: Baseline characteristics did not differ across randomised groups in patients with stage 3 CKD. At Week 18, significant HbA_{1c} reductions from baseline for ERTU 5 mg and 15 mg vs placebo were observed in patients with stage 3 CKD and in the stage 3A CKD subgroup (**Table**). Significant placebo-subtracted reductions in body weight (5 mg: −1.4 kg; 15 mg: −1.5 kg) and SBP (5 mg: −2.9 mmHg; 15 mg: −3.2 mmHg) occurred in the stage 3 CKD patients receiving ERTU. The incidence of overall AEs, symptomatic hypoglycaemia, hypovolaemia, and kidney-related AEs did not differ between ERTU and placebo across stage 3 CKD subgroups.

Conclusion: In patients with type 2 diabetes, ASCVD and stage 3 CKD, ERTU improved glycaemic control, body weight and SBP, and was generally well tolerated.

Table: HbA_{1c} Reductions at Week 18 in Patients with Stage 3 CKD, Stage 3A CKD and Stage 3B CKD

BL CKD stage and mean (SD) eGFR (ml min^{-1} 1.73m^{-2})[1]	Treatment groups (N)	BL HbA_{1c} (mmol/mol)	LS mean change from BL at Week 18 (95% CI)[2]	Difference vs placebo (95% CI)	P-value[3]
Stage 3 CKD 49.3 (7.5)	Placebo (597)	66.5	−2.8 (−3.6, −2.0)		
	ERTU 5 mg (615)	66.3	−5.7 (−6.5, −4.9)	−2.9 (−4.0, −1.8)	<0.001
	ERTU 15 mg (557)	66.5	−5.8 (−6.6, −5.0)	−3.0 (−4.2, −1.9)	<0.001
Stage 3A CKD 53.0 (4.3)	Placebo (439)	66.4	−3.0 (−3.9, −2.0)		
	ERTU 5 mg (463)	65.9	−5.9 (−6.8, −4.9)	−2.9 (−4.2, −1.6)	<0.001
	ERTU 15 mg (411)	66.4	−6.3 (−7.3, −5.4)	−3.4 (−4.7, −2.1)	<0.001
Stage 3B CKD 38.8 (4.0)	Placebo (158)	66.5	−2.3 (−3.8, −0.8)		
	ERTU 5 mg (152)	67.4	−5.3 (−6.9, −3.8)	−3.0 (−5.2, −0.9)	0.006
	ERTU 15 mg (146)	66.9	−4.4 (−5.9, −2.8)	−2.1 (−4.2, 0.1)	0.064

[1]Calculated using the Modification of Diet in Renal Disease study equation. [2]Calculated using a constrained longitudinal data analysis model. [3]Nominal P-values unadjusted for multiplicity. BL, baseline; stage 3 CKD, eGFR 30 to <60 ml min^{-1} 1.73m^{-2}; stage 3A CKD, eGFR 45 to <60 ml min^{-1} 1.73m^{-2}; stage 3B CKD, eGFR 30 to <45 ml min^{-1} 1.73m^{-2}.

Clinical Trial Registration Number: NCT01986881

Supported by: Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA, and Pfizer Inc., New York, NY, USA.

Disclosure: **S. Dagogo-Jack:** Employment/Consultancy; AstraZeneca, Boehringer Ingelheim, Janssen, Merck & Co., Inc., Sanofi. Non-financial support; S.D.-J. has led clinical trials for AstraZeneca, Boehringer Ingelheim and Novo Nordisk, Inc. Stock/Shareholding; Jana Care, Inc., Aerami Therapeutics.

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Cardiorenal outcomes with ertugliflozin by baseline glucose-lowering agent: an analysis from VERTIS CV

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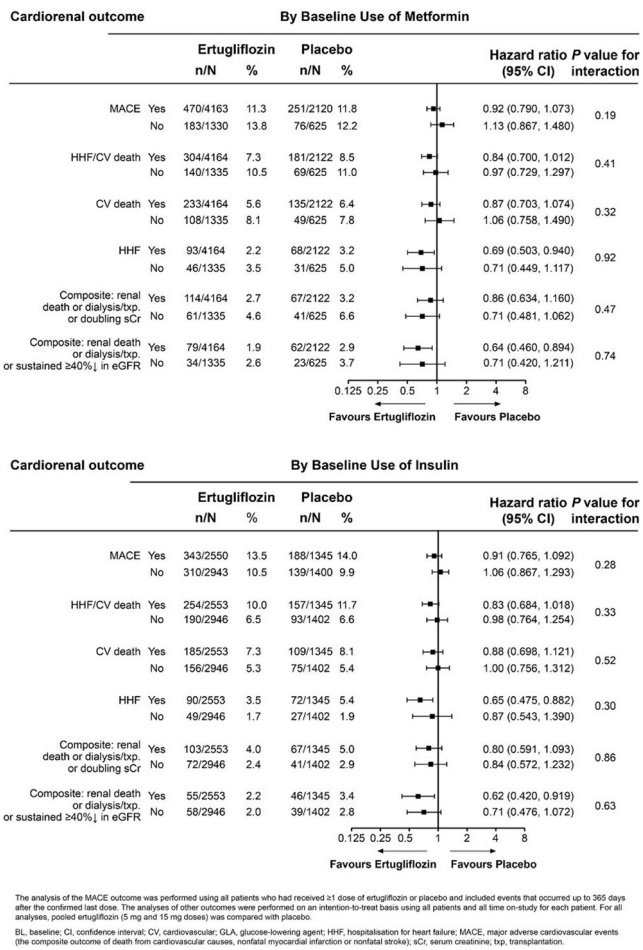
Background and aims: VERTIS CV was the cardiovascular (CV) outcome trial for the SGLT2 inhibitor ertugliflozin, conducted in patients with type 2 diabetes (T2D) and atherosclerotic CV disease. In the overall population, ertugliflozin was noninferior to placebo for major adverse CV events. Although superiority for the composite endpoint of CV death or hospitalisation for heart failure (HHF) and the renal composite (that included doubling of serum creatinine from baseline) were not met, the prespecified secondary objective of HHF showed a 30% risk reduction and a prespecified exploratory renal composite (sustained ≥40% decrease in eGFR from baseline, dialysis/transplantation, or renal death) showed improved kidney outcomes.

Materials and methods: This analysis assessed cardiorenal endpoints of VERTIS CV by Cox proportional hazard assessment according to use of baseline glucose-lowering agent (GLA).

Results: Among 8246 patients in VERTIS CV, at baseline 6286 (76%) used metformin, 3900 (47%) insulin, 3390 (41%) sulphonylureas (SU) and 911 (11%) dipeptidyl peptidase-4 inhibitors (DPP4i), alone or in combination therapy (67% used >1 GLA at baseline). For each of the GLAs, metformin, insulin, SU and DPP4i, no significant differences were observed for cardiorenal outcomes by baseline use (yes, no) of the GLA (Figure; all interaction *P* values >0.05). Cardiorenal outcomes were generally similar across the baseline GLA subgroups.

Conclusion: In VERTIS CV, the effects of ertugliflozin on cardiorenal outcomes in patients with T2D were generally consistent regardless of baseline GLA.

FIGURE. Cardiorenal outcomes with ertugliflozin vs placebo by BL GLA use



The analysis of the MACE outcome was performed using all patients who had received ≥1 dose of ertugliflozin or placebo and included events that occurred up to 365 days after the confirmed last dose. The analyses of other outcomes were performed on an intention-to-treat basis using all patients and all time on-study for each patient. For all analyses, pooled ertugliflozin (5 mg and 15 mg doses) was compared with placebo.

BL, baseline; CI, confidence interval; CV, cardiovascular; GLA, glucose-lowering agent; HHF, hospitalisation for heart failure; MACE, major adverse cardiovascular events (the composite outcome of death from cardiovascular causes, nonfatal myocardial infarction or nonfatal stroke); sCr, serum creatinine; txp, transplantation.

Clinical Trial Registration Number: NCT01986881

Supported by: Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA, and Pfizer Inc., New York, NY, USA.

Disclosure: **B. Charbonnel:** Employment/Consultancy; Merck Sharpe & Dohme, Lilly, Novo Nordisk, Sanofi. Honorarium; AstraZeneca, Boehringer Ingelheim, Lilly, Merck Sharpe & Dohme, Mundipharma, Novo Nordisk, Sanofi, and Takeda. Lecture/other fees; AstraZeneca, Merck Sharpe & Dohme, Novo Nordisk, Sanofi, and Takeda.

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Effects of canagliflozin on hospitalisation for heart failure by baseline eGFR: pooled analysis from the CANVAS Program and CREDENCE

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Background and aims: People with type 2 diabetes (T2D) are at high risk for cardiovascular (CV) events, including hospitalization for heart failure (HHF), especially as kidney function declines. We examined the effects of canagliflozin (CANA) on risk of HHF by baseline estimated glomerular filtration rate (eGFR) in patients with T2D and high CV risk and/or chronic kidney disease from the CANVAS Program and CREDENCE.

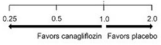
Materials and methods: Patient-level data from the CANVAS Program (N = 10,142) and CREDENCE (N = 4401) were pooled. Cox proportional hazards model for the time to first/second HHF events and proportional means model based on cumulative mean function for recurrent HHF events were used to assess effects overall as well as within baseline eGFR strata (<45, 45-60, and >60 mL/min/1.73 m^2). HHF events were independently and blindly adjudicated.

Results: In 14,540 participants with baseline eGFR values over a median follow-up of 130 weeks, 672 HHF events occurred; 357 (53%) were single events. CANA decreased the time to first HHF event (HR 0.58, 95% CI 0.48, 0.70; Figure), consistently regardless of baseline eGFR (interaction $P = 0.84$), and time to second HHF event (HR 0.67, 95% CI 0.46, 0.96; too few events to stratify by eGFR). CANA also decreased total HHF events overall (mean event ratio 0.63, 95% CI 0.54, 0.73) and across eGFR subgroups (interaction $P = 0.51$).

Conclusion: In individuals with T2D in the CANVAS Program and CREDENCE, CANA decreased the risk of first and recurrent HHF events, with consistent benefits independent of baseline eGFR.

Figure. Effects of canagliflozin versus placebo on HHF events overall and by baseline eGFR.

	Number of participants with an event (n/N)		Events per 1000 patient-years		HR (95% CI)	Interaction *P* value[a]
	Canagliflozin	Placebo	Canagliflozin	Placebo		
Risk of first HHF event						
Overall	212/7995	261/6545	7.6	13.5	0.58 (0.48, 0.70)	0.84
eGFR <45 mL/min/1.73 m^2	52/976	82/943	19.4	35.0	0.56 (0.39, 0.79)	
eGFR 45-60 mL/min/1.73 m^2	57/1559	73/1413	11.7	18.5	0.65 (0.46, 0.92)	
eGFR >60 mL/min/1.73 m^2	103/5460	106/4189	5.0	8.1	0.63 (0.48, 0.83)	
Risk of second HHF event[†]						
Overall	56/7995	60/6545	2.0	3.1	0.67 (0.46, 0.96)	–
Risk of total HHF events						
Overall	304/7995	368/6545	10.8	18.9	0.63 (0.54, 0.73)[‡]	0.51
eGFR <45 mL/min/1.73 m^2	89/976	111/943	33.0	47.1	0.72 (0.54, 0.95)[‡]	
eGFR 45-60 mL/min/1.73 m^2	82/1559	122/1413	16.8	30.8	0.54 (0.41, 0.71)[‡]	
eGFR >60 mL/min/1.73 m^2	133/5460	135/4189	6.5	10.3	0.64 (0.51, 0.82)[‡]	



HR, hazard ratio; CI, confidence interval.
[a]The interaction *P* values are based on the proportional means model including the terms of treatment, baseline eGFR, and their interaction.
[†]There were not enough events to adequately evaluate second HHF events by eGFR.
[‡]Data are presented as mean event ratio (95% CI) and were analyzed using a proportional means model using mean cumulative function.

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Effects of canagliflozin on major adverse cardiovascular events by baseline albuminuria: integrated analyses from the CANVAS Program and CREDENCE trial

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Background and aims: People with type 2 diabetes mellitus (T2DM) have a greater risk of cardiovascular (CV) disease and major adverse CV events (MACE) that is more common as renal function declines. The sodium glucose co-transporter 2 (SGLT2) inhibitor canagliflozin reduced the risk of MACE (CV death, nonfatal myocardial infarction [MI], and nonfatal stroke) in patients with T2DM and high CV risk or nephropathy in the CANVAS Program and CREDENCE trials, respectively.

Materials and methods: This post hoc analysis included integrated, pooled data from the CANVAS Program and the CREDENCE trial. The effects of canagliflozin compared with placebo on MACE were assessed in subgroups defined by baseline urinary albumin:creatinine ratio (UACR; <30, 30-300, and >300 mg/g). Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using stratified (by study) Cox regression models, with subgroup by treatment interaction terms added to test for heterogeneity. Interaction *P* values were calculated by including the terms of treatment group, baseline UACR, and their interaction in the model.

Results: A total of 14,543 participants from the CANVAS Program (N = 10,142) and CREDENCE (N = 4,401) were included, with mean estimated glomerular filtration rate of 70.3 mL/min/1.73 m^2 and median (interquartile range) UACR of 501.0 (8.4-523.6) mg/g. Among participants with baseline UACR measurements, 7038 (48.8%), 2762 (19.1%), and 4634 (32.1%) participants had baseline UACR <30, 30-300, and >300 mg/g, respectively. Rates of MACE and its components increased as UACR increased (Figure). Canagliflozin reduced the risk of MACE compared with placebo in the overall population (HR, 0.83; 95% CI, 0.75, 0.92), with consistent effects observed across UACR subgroups (interaction *P* value = 0.42). Canagliflozin also reduced the risk of the individual components of CV death (HR, 0.84; 95% CI, 0.72, 0.97), nonfatal MI (HR, 0.83; 95% CI, 0.70, 0.99), and nonfatal stroke (HR, 0.84; 95% CI, 0.69, 1.03), independent of baseline UACR (interaction *P* values = 0.40, 0.88, and 0.69, respectively). Canagliflozin was generally well tolerated in the CANVAS Program and the CREDENCE trial, with consistent results on safety outcomes across UACR subgroups.

Conclusion: Event rates of MACE and its components increased with higher UACR. Canagliflozin reduced the risk of MACE and its components in participants with T2DM and high CV risk or CKD in the CANVAS Program and CREDENCE trial, with consistent benefits observed regardless of baseline UACR.

Figure. Effects of canagliflozin on MACE and its components overall and by baseline UACR.

	Number of participants with an event (n/N)		Participants with an event per 1000 patient-years		HR (95% CI)	Interaction *P* value
	Canagliflozin	Placebo	Canagliflozin	Placebo		
MACE						
Overall	796/7942	692/6492	29.3	36.6	0.83 (0.75, 0.92)	0.42
UACR <30 mg/g	344/4028	253/3010	22.1	26.4	0.83 (0.71, 0.98)	
UACR 30-300 mg/g	182/1573	122/1189	33.4	34.4	0.95 (0.76, 1.20)	
UACR >300 mg/g	270/2341	317/2293	43.9	54.8	0.79 (0.67, 0.93)	
CV death						
Overall	376/7942	324/6492	13.1	16.2	0.84 (0.72, 0.97)	0.40
UACR <30 mg/g	141/4028	92/3010	8.6	9.0	0.91 (0.70, 1.18)	
UACR 30-300 mg/g	89/1573	56/1189	15.2	14.9	0.97 (0.69, 1.35)	
UACR >300 mg/g	146/2341	176/2293	22.8	29.1	0.76 (0.61, 0.95)	
Nonfatal MI						
Overall	284/7942	245/6492	10.3	12.8	0.83 (0.70, 0.99)	0.88
UACR <30 mg/g	140/4028	104/3010	8.9	10.7	0.84 (0.65, 1.09)	
UACR 30-300 mg/g	65/1573	47/1189	11.7	13.2	0.87 (0.59, 1.27)	
UACR >300 mg/g	79/2341	94/2293	12.6	16.0	0.80 (0.59, 1.08)	
Nonfatal stroke						
Overall	207/7942	181/6492	7.5	9.4	0.84 (0.69, 1.03)	0.69
UACR <30 mg/g	89/4028	74/3010	5.6	7.6	0.75 (0.55, 1.03)	
UACR 30-300 mg/g	45/1573	31/1189	8.0	8.6	0.96 (0.61, 1.53)	
UACR >300 mg/g	73/2341	76/2293	11.7	12.9	0.90 (0.65, 1.24)	

Clinical Trial Registration Number: NCT01032629, NCT01989754, NCT02065791

Supported by: Janssen Pharmaceutica NV

Disclosure: **D.C. Wheeler:** Lecture/other fees; Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, GlaxoSmithKline, Mitsubishi, Mundipharma, MSD, Napp, Ono Pharma, Tricida, and Vifor Fresenius. Other; Janssen, fees and travel funding for his role as a member of the CREDENCE Steering Committee.

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The effect of SGLT-2 inhibitors on mortality and heart failure in randomised trials versus observational studies

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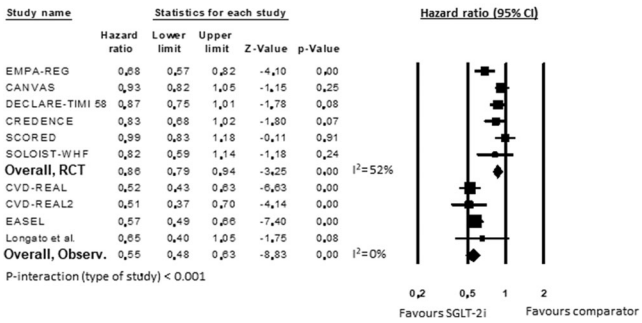
Background and aims: Randomized clinical trials (RCTs) allocating type 2 diabetes patients to treatment with sodium-glucose transport protein 2 (SGLT-2) inhibitors or placebo have found significant effects on the risk of heart failure and modest effects on mortality. In the wake of the first trials a number of observational studies have been conducted, some of these reporting a mortality reduction of 50% compared to active comparators. In this review we systematically assess and compare the results on all-cause mortality, cardiovascular mortality and heart failure hospitalization observed in randomized clinical trials with the results obtained in observational studies.

Materials and methods: We performed a systematic bibliographical search including cardiovascular outcome trials and observational studies assessing the effect of SGLT-2 inhibitors on mortality and heart failure.

Results: Seven randomized clinical trials and 23 observational studies were included in the current review. The observed heterogeneity between study results for all-cause mortality ($p<0.001$) and cardiovascular mortality ($p < 0.001$) was partly explained by study type, whereas this was not the case for heart failure ($p = 0.18$).

Conclusion: Methodological considerations such as the omission of important confounders, immortal-time bias and residual confounding such as unmeasured social economic inequality may be the cause of the inflated results observed in observational studies and that calls for caution when observational studies are used to guide treatment of patients with type 2 diabetes.

Figure 2. The effect of SGLT-2 inhibitors for all-cause mortality in trials and observational studies



Disclosure: **J. Krogh:** None.

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Sodium Glucose Co-Transporter 2 Inhibitors do not increase the risk of hyperkaleamia in type 2 diabetes: a systematic review and meta-analysis

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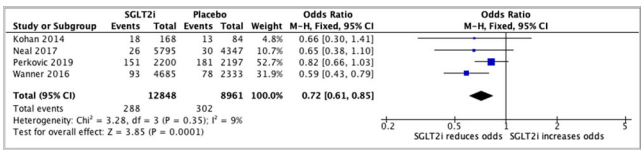
Background and aims: Sodium Glucose Co-Transporter 2 inhibitors (SGLT2i) are increasingly used in type 2 diabetes (T2DM) for cardiorenal protection as well as glycaemic control. Early studies suggested a dose-dependent risk of hyperkalaemia with SGLT2i, possibly to decreased activity of the Na/K ATPase on the basolateral membrane of the proximal convoluted tubule. We performed a systematic review and meta-analysis to assess the effects of SGLT2i on serum potassium in patients with T2DM.

Materials and methods: MEDLINE and Pubmed databases were searched using MeSH terms relating to potassium and hyperkalaemia for publications of SGLT2i in T2DM. We included English-language publications of randomised controlled trials, reporting potassium or hyperkalaemia as primary or secondary outcomes, to 31st December 2020. Study quality was assessed using the Jadad method and funnel plots were used to assess for potential bias. Cochran's Q test and the I^2 statistic were used to assess statistical heterogeneity and sensitivity analyses were performed to assess each individual study's influence on overall I2. Meta-analyses were performed using Cochrane RevMan software. Two outcomes were considered; i) The odds ratio (OR) of hyperkalaemic events (from MeDRA coding) between SGLT2i and placebo using a fixed effects model. ii) The mean difference (MD) in change from baseline serum potassium between SGLT2i and placebo using a fixed effects model.

Results: From 1724 identified publications, 12 studies were considered for qualitative analysis comprising 24,246 study participants: 14,645 randomised to SGLT2i and 9,601 randomised to placebo. Mean age was 58.6 years, in ethnically diverse populations. Study duration ranged from 8 days to 3.1 years. Ten out of 12 studies had a Jadad score >3 therefore considered to be of high quality. Of these, nine were included in the meta-analysis; three assessed hyperkalaemia, five assessed change in potassium from baseline, and one study assessed both outcome measures. The pooled OR for hyperkalaemic events for the SGLT2i group vs placebo was 0.72 [95% CI= 0.61, 0.85, P<0.001] with an I^2 of 9% (Figure). The pooled MD in serum potassium concentration from baseline in the SGLT2i group vs placebo was -0.04 mmol/L [95% CI = -0.08, -0.00 mmol/L; P=0.04] with an I^2 of 89%.

Conclusion: The use of SGLT2i in people with T2DM led to decreased odds of developing hyperkalaemia, compared to those on placebo. A statistically significant, but not clinically meaningful difference, in mean change in potassium was observed with SGLT2i. Prevention of hyperkalaemia likely reflects improved renal outcomes from SGLT2i.



Disclosure: **C. Charlwood:** None.

SO 27 Glucose-lowering agents: Real World Evidence

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Healthcare resource utilisation after empagliflozin initiation in Europe: real-world evidence from the EMPRISE study

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Background and aims: The EMPA-REG-OUTCOME® trial showed that empagliflozin (EMPA) reduced the risk of cardiovascular (CV) death (hazard ratio [HR] 0.62; 95%CI 0.49-0.77), hospitalization for heart failure (HF) (0.65; 0.50-0.85), all-cause hospitalizations (0.83; 0.76-0.91) and mortality (0.68; 0.57-0.82) in adults with type 2 diabetes (T2D) and established CV disease (CVD). How EMPA impacts healthcare resource utilization (HCRU) in routine practice is less clear, as there are no European data in this respect. This analysis compared HCRU between T2D patients initiating EMPA or a dipeptidyl peptidase-4 inhibitor (DPP-4i) in Sweden (SE) and Finland (FI), as part of the multi-country EMPRISE study.

Materials and methods: Non-interventional cohort study using data from Nordic national registries. T2D patients ≥18 years newly initiated on EMPA or a DPP-4i from May-2014 to Dec-2018 were matched 1:1 using propensity scores based on >180 covariates (sociodemographic, laboratory, clinical and drug use at any time; HCRU and costs in the last year). Balance was assessed by standardized differences (threshold 0.1). Outcomes included outpatient visits, emergency room (ER) visits, all-cause hospital admissions, first hospitalization and hospital length of stay (LOS). Follow-up continued until death, end of study, drug withdrawal or switch. Analyses were conducted in the main cohorts and in subgroups with/without CVD or HF at baseline.

Results: A total of 15,785 and 5,838 matched patient pairs were identified in SE and FI, respectively. The mean follow-up times were 6.5-7.7 months in the EMPA group and 9.9-10.3 months in the DPP-4i group. All-cause hospitalizations (rate ratio [RR]=0.85; 95%CI: 0.80-0.89 in SE and RR=0.77; 0.72-0.82 in FI), risk of first hospitalization stay (HR=0.87; 0.81-0.93 in SE and HR=0.87; 0.79-0.95 in FI), and outpatient visits (RR=0.96; 0.94-0.98 in SE and RR=0.87; 0.85-0.89 in FI) were lower in EMPA initiators compared to DPP-4i initiators. In FI, EMPA was also associated with lower risk of ER care need (RR=0.83; 0.76-0.91), without significant differences in SE (Table). Hospital LOS was lower with EMPA than DPP-4i (1.5 vs 2.0 days per member per year [PMPY] in SE and 1.8 vs 2.9 days PMPY in FI). In both countries, results trended in the same direction across subgroups with/without CVD or HF at baseline. Results from other European countries are forthcoming.

Conclusion: These findings in routine clinical practice suggest that patients who initiated EMPA experienced reduced inpatient care need and lower burden of outpatient visits compared to DPP-4i initiators.

	Sweden			Finland		
Patient baseline characteristics	**EMPA (N= 15,785)**	**DPP-4i (N=15,785)**	**Std**	**EMPA (N= 5,838)**	**DPP-4i (N= 5,838)**	**Std**
Age, mean (SD)	63.49 (10.90)	63.36 (11.49)	0.01	62.60 (11.27)	62.40 (12.01)	0.02
Male, n (%)	10,202 (64.63)	10,255 (64.97)	0.01	3,410 (58.41)	3,420 (58.58)	0.00
Prior HF diagnosis, n (%)	1,212 (7.68)	1,202 (7.61)	0.00	436 (7.47)	442 (7.57)	0.00
Prior CVD, n (%)	8,523 (53.99)	8,389 (53.15)	-0.02	3,053 (52.30)	3,014 (51.63)	-0.01
All-cause hospitalizations 365 days prior to drug start, n (%)	3,063 (19.40)	3,044 (19.28)	0.00	1,388 (23.78)	1,364 (23.36)	0.01
HCRU Outcomes	**N events (PMPY)**	**N events (PMPY)**	**RR/HR* (95%CI)**	**N events (PMPY)**	**N events (PMPY)**	**RR/HR* (95%CI)**
Outpatient visits	17,590 (2.07)	27,888 (2.17)	0.96 (0.94–0.98)	12,140 (3.32)	18,472 (3.81)	0.87 (0.85–0.89)
Emergency room visits	1,000 (0.12)	1,613 (0.13)	0.94 (0.87–1.01)	709 (0.19)	1,131 (0.23)	0.83 (0.76–0.91)
All-cause hospital admissions	2,413 (0.28)	4,310 (0.33)	0.85 (0.80–0.89)	1,410 (0.39)	2,440 (0.50)	0.77 (0.72–0.82)
First hospitalization stay	1,401 (0.18)	2,230 (0.19)	0.87 (0.81–0.93)	770 (0.23)	1,106 (0.26)	0.87 (0.79–0.95)

**RR for all outcomes except for first hospitalization stay, which was analyzed through HR. CI, confidence interval; CVD, cardiovascular disease; DPP-4i, dipeptidyl peptidase-4 inhibitor; EMPA, empagliflozin; HCRU, healthcare resource utilization; HR, hazard ratio; HF, heart failure; PMPY, rate per member per year; RR, rate ratio; SD, standard deviation; Std, standardized difference.*

Clinical Trial Registration Number: EUPAS27606, NCT03817463

Supported by: This project is funded by BI

Disclosure: **L. Niskanen:** None.

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Effectiveness and safety of empagliflozin in routine care in Europe and East Asia: results from the empagliflozin comparative effectiveness and safety (EMPRISE) study

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Background and aims: The EMPRISE study evaluates the effectiveness and safety of empagliflozin (EMPA) in a broader population of patients with type 2 diabetes (T2D) in routine clinical care across countries.

Materials and methods: The study included 67,140 pairs of 1:1 propensity score (PS) matched patients ≥18 years with T2D newly initiating EMPA or any dipeptidyl peptidase-4 inhibitor (DPP-4i) use from large databases/registers in Israel, Finland, Germany, Spain, Sweden, South Korea, Taiwan and Japan using an as-treated approach; followed until first event or, switch/discontinuation of treatment, concomitant use of any SGLT-2i/DPP-4i, death or end of study period to calculate outcomes. Study inclusion periods were 2015-2018 (Finland, Sweden, Israel, Japan), 2016-2017 (South Korea, Taiwan), 2014-2019 (Germany). Mostly, PS matching used 100+ baseline covariates, and pooled HR with 95% CI were computed using random-effects meta-analysis models.

Results: Proportion of women was 33-43% across countries, and mean age varied between 56-65 years at baseline. In Japan, 27% of patients had congestive heart failure (HF) at baseline, the proportion was 2-8% for the remaining countries. EMPA compared to DPP-4i, was associated with 27-47% lower risk of hospitalization for heart failure (HHF), stroke, all-cause mortality (ACM), and two composite outcomes: HHF plus ACM; and myocardial infarction, stroke plus ACM. Safety analyses associated EMPA with 46% lower risk of acute kidney injury requiring dialysis.

Conclusion: Results from the EMPRISE study program showed that EMPA is associated with reduced risk of cardiovascular (CV) disease, including HHF, in patients with T2D in routine clinical care settings in Israel, Europe and East Asia. The EMPRISE study results complement the results from EMPA-REG OUTCOME® trial, and are in line with the 2019 update from ADA and EASD consensus report to prescribe SGLT-

2i for patients with T2D and high-risk, vascular or chronic kidney disease or heart failure to reduce HHF, major adverse CV events, and CV death.

Incidence rates and hazard ratios for outcomes	N (paired patients)	Pooled N events (incidence rate per 1,000 person-years)		Pooled HR (95% CI)
		Empagliflozin	DPP-4i	
Hospitalization for Heart Failure (HHF)	67,140	565 (13.85)	823 (17.61)	0.72 (0.62-0.83)
Myocardial infarction (MI)	67,140	202 (4.93)	265 (5.65)	0.93 (0.77-1.12)
Stroke	67,140	223 (5.45)	340 (7.24)	0.73 (0.62-0.87)
All-Cause Mortality (ACM)	66,301	377 (9.43)	849 (18.43)	0.53 (0.44-0.65)
Composite outcome including HHF and ACM	66,301	876 (22.03)	1,542 (33.73)	0.65 (0.53-0.79)
Composite outcome including MI, Stroke, and ACM	66,301	750 (18.84)	1,346 (29.41)	0.67 (0.57-0.78)
Lower-Limb Amputation	55,339	31 (0.86)	51 (1.30)	0.68 (0.42-1.08)
Bone Fracture	67,140	235 (5.32)	287 (5.72)	0.94 (0.79-1.12)
Diabetic ketoacidosis	56,298	45 (1.32)	27 (0.67)	1.82 (1.12-2.95)
Acute Kidney Injury that Required Dialysis	41,011	83 (2.98)	153 (5.11)	0.54 (0.41-0.70)

Supported by: This study was funded by Boehringer Ingelheim

Disclosure: **A. Karasik:** Grants; Grant received to support this work from Boehringer Ingelheim. Honorarium; From Boehringer Ingelheim. Lecture/other fees; From Boehringer Ingelheim.

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Real-world use of once-weekly semaglutide in diverse patient populations with type 2 diabetes: pooled analysis of four SURE studies

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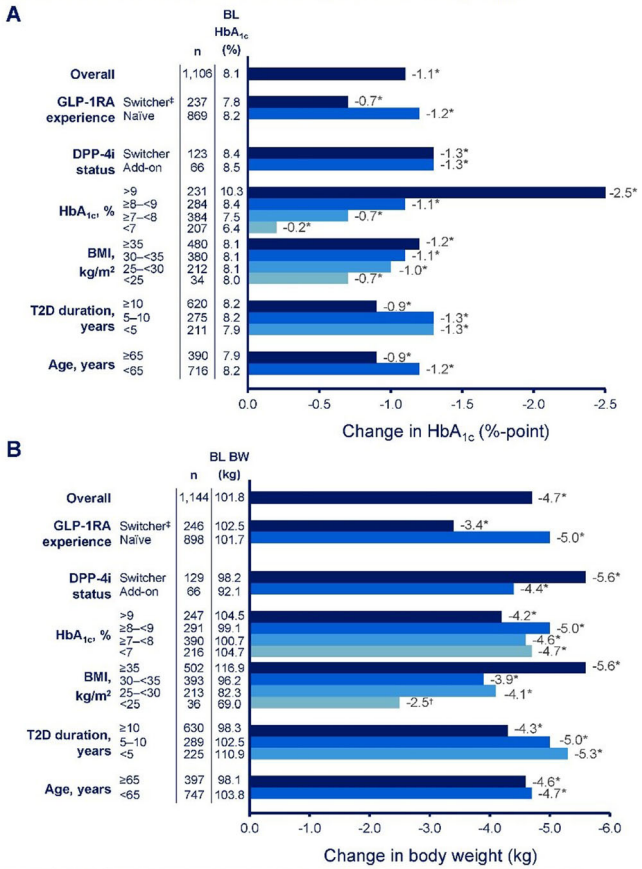
Background and aims: Once-weekly semaglutide, a glucagon-like peptide-1 receptor agonist (GLP-1RA) approved for the treatment of type 2 diabetes (T2D), consistently demonstrated superior HbA_{1c} and body weight reductions vs placebo and active comparators in the SUSTAIN clinical trials. Observational studies reflect real-world use of a medication in diverse patient populations and provide evidence on outcomes in routine clinical practice. Results from the first four individual SURE studies (Canada, Denmark/Sweden, Switzerland and UK) investigating real-world use of semaglutide consistently showed significant HbA_{1c} and body weight reductions. This pooled *post hoc* analysis of these four SURE studies evaluated use of semaglutide in the overall population and according to various baseline (BL) characteristics.

Materials and methods: Data from populations of patients enrolled in the studies (age ≥18 years; T2D with ≥1 documented HbA_{1c} value ≤12 weeks before semaglutide initiation) were pooled for this analysis. Semaglutide and other anti-hyperglycaemic drugs were prescribed at the physician's discretion. Change from BL to end of study (EOS; ~30 weeks) in HbA_{1c} and body weight are reported in the overall population, and the following BL subgroups: GLP-1RA experience (switcher/naïve [defined as not receiving a GLP-1RA ≤12 weeks prior to semaglutide initiation]); dipeptidyl peptidase-4 inhibitor (DPP-4i) status; HbA_{1c} (<7% / ≥7-<8% / ≥8-<9% / ≥9%); BMI (<25 / 25-<30 / 30-<35 / ≥35 kg/m^2 [country-specific cutoffs <28 / ≥28 kg/m^2 also reported]); age (<65 / ≥65 years) and diabetes duration (<5 / 5-10 / ≥10 years).

Results: Overall, 1,212 patients were included in the full analysis set, with BL characteristics reflective of real-world practice. Significant HbA_{1c} ($p<0.0001$) and body weight ($p<0.01$) reductions were observed with semaglutide in the overall population and all subgroups (Figure), including by BL BMI cutoffs <28 and ≥28 kg/m^2. The greatest HbA_{1c} reduction was observed with BL HbA_{1c} >9%, and the smallest with BL HbA_{1c} <7%; the greatest body weight reductions were observed with BL BMI ≥35 kg/m^2 or in DPP-4i switchers, and the smallest with BL BMI <25 kg/m$_2$. The discontinuation rate in the overall population was 9.5%, and no new safety signals were identified.

Conclusion: In a pooled analysis of real-world data from Canada, Denmark/Sweden, Switzerland and the UK, patients with T2D initiating semaglutide experienced significant reductions in HbA_{1c} and body weight, in the overall population and in subgroups based on various BL characteristics, including switching from another GLP-1RA.

Figure: Change from baseline to EOS in HbA_{1c} (A) and body weight (B) in the overall population and by baseline subgroups



*p<0.0001. †p=0.0092. ‡No stop date was recorded for baseline GLP-1RA within the first 4 weeks following visit 1 in six patients in the 'GLP-1RA Switcher' group. P-values are reported for no average change in response from baseline to week 30. Data are from in-study period which represents the time period during which patients are considered to be in the study, regardless of semaglutide treatment status. Response was analysed using baseline, T2D duration, age, BMI, time, time-squared, pre-initiation use of GLP-1RA (except in 'GLP-1RA experience' subgroups), pre-initiation use of DPP4i (except in 'DPP-4i status' subgroups), pre-initiation use of insulin, number of OADs used pre-initiation (0–1/2+) and sex with random intercept and random time coefficient (slope). BL, baseline; BW, body weight; DPP-4i, dipeptidyl peptidase-4 inhibitor; GLP-1RA, glucagon-like peptide-1 receptor agonist; OAD, oral anti-hyperglycaemic drug; T2D, type 2 diabetes.

Clinical Trial Registration Number: NCT03648281, NCT03631186, NCT03876015, NCT03457012

Supported by: Trial sponsored by Novo Nordisk

Disclosure: **J. Yale:** Grants; Grants received from Novo Nordisk for SURE trials.

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Patients reaching treatment targets with once-weekly semaglutide in real-world practice: pooled analysis of four SURE studies

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Institutet, Stockholm, Sweden, [5]Joanne F. Liutkus Medicine Professional Corporation, Cambridge, Canada, [6]Novo Nordisk Service Centre India Private Ltd., Bangalore, India, [7]St George's Medical Practice, Darlington, UK.

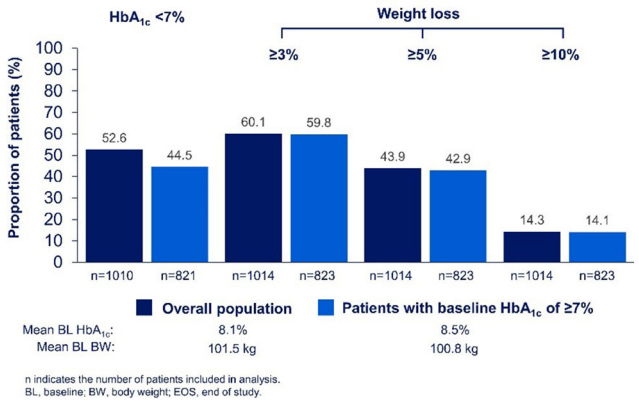
Background and aims: Once-weekly semaglutide, a glucagon-like peptide-1 receptor agonist approved for the treatment of type 2 diabetes (T2D), consistently demonstrated superior, clinically relevant reductions in HbA_{1c} and body weight compared with placebo and active comparators in the SUSTAIN clinical trials. Real-world evidence studies are important to understand the use of a drug in routine clinical practice and diverse patient populations. The results from the first four individual SURE real-world studies (Canada, Denmark/Sweden, Switzerland, and UK) consistently showed significant HbA_{1c} and body weight reductions from baseline following initiation of semaglutide. Achieving glycaemic and weight-loss treatment targets is important in T2D management to avoid long-term complications. This pooled *post hoc* analysis of the first four SURE studies evaluated patients achieving HbA_{1c} and weight-loss targets.

Materials and methods: Patients (age ≥18 years) with T2D with ≥1 documented HbA_{1c} value ≤12 weeks before semaglutide initiation were enrolled, and for this analysis the patient populations were pooled. Semaglutide and other anti-hyperglycaemic drugs were prescribed at the physician's discretion. The proportion of patients achieving HbA_{1c} <7%, weight loss from baseline ≥3%, ≥5% and ≥10%, and a composite endpoint of HbA_{1c} reduction ≥1% and weight loss ≥3% at end of study (EOS; ~30 weeks) are reported in the overall pooled population and in the subgroup of patients with a baseline HbA_{1c} level ≥7%.

Results: The pooled population included 1,212 patients in the full analysis set, 981 of whom had a baseline HbA_{1c} ≥7%; baseline characteristics were reflective of real-world practice. At EOS, 531 (52.6%) patients in the overall population and 365 (44.5%) with a baseline HbA_{1c} ≥7% achieved HbA_{1c} <7% (Figure). In the overall population, 609 (60.1%), 445 (43.9%) and 145 (14.3%) patients achieved a weight loss of ≥3%, ≥5% and ≥10%, respectively; similar proportions of patients with a baseline HbA_{1c} ≥7% achieved the same weight-loss targets (Figure). In the overall population, 297 (29.4%) patients achieved the composite endpoint of an HbA_{1c} reduction ≥1% and weight loss ≥3%, and 283 (34.6%) of patients with a baseline HbA_{1c} ≥7% achieved the same composite endpoint.

Conclusion: In a pooled analysis of real-world data from SURE Canada, Denmark/Sweden, Switzerland, and UK, over half of patients with T2D initiating semaglutide achieved HbA_{1c} <7% and weight loss ≥3%, by EOS. The proportion of patients achieving glycaemic and weight-loss targets was similar in the overall population and in the subset of patients who had a baseline HbA_{1c} ≥7%.

Figure: Proportion of patients achieving treatment targets at end of study (full analysis set)



Clinical Trial Registration Number: NCT03648281, NCT03631186, NCT03876015, NCT03457012

Supported by: SURE trials sponsored by Novo Nordisk

Disclosure: **G. Rudofsky:** Other; Gottfried Rudofsky was head of a participating study centre.

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Trends in initiation of GLP-1 RA in patients with type 2 diabetes during 2014 - 2019: a US database study

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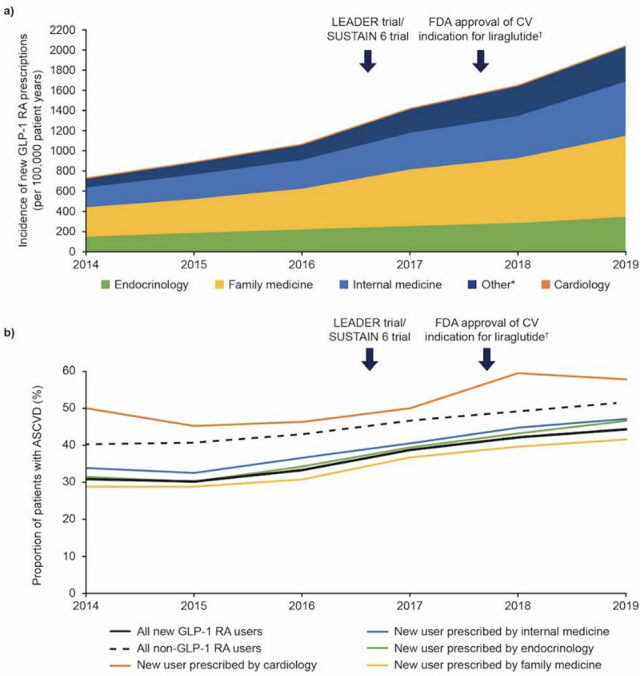
Background and aims: Evidence of cardiovascular (CV) benefit of certain glucagon-like peptide 1 receptor agonists (GLP-1 RAs) from CV outcomes trials (CVOTs) resulted in updates to international guidelines and label indications in patients with type 2 diabetes (T2D) at high CV risk. To evaluate the impact of new evidence, we investigated the incidence of new GLP-1 RA use and trends in prescriber and patient characteristics over a 5-year period encompassing key CVOT publications and an updated label indication.

Materials and methods: Data from consecutive cross-sectional cohorts of patients with T2D and no GLP-1 RA prescription for ≥1 year prior to the start date, corresponding to each year between 2014 and 2019, were extracted retrospectively from US Optum's de-identified Clinformatics® Data Mart Database (2007-2019). For each year, incidence of new GLP-1 RA use, overall and according to prescriber speciality and the proportion of patients with atherosclerotic CV disease (ASCVD) among the new users were evaluated. Speciality was categorised using prescriber information within the database, while ASCVD was identified with ICD-9 CM or 10 CM codes reported prior to GLP-1 RA prescription or year-start date.

Results: During the study period, 2,966,970 eligible patients with T2D were identified, 108,541 of whom were new users of GLP-1 RAs. Overall incidence of new GLP-1 RA use increased each year, with a 3-fold increase in prescriptions from 727 to 2045 per 100,000 patient years between 2014 and 2019 (Figure a). Among new GLP-1 RA users, mean age was 60.1 years, increasing from 56.8 to 61.7 years. Mean HbA_{1c} was 8.6% and was stable over time. Further analysis of new GLP-1 RA prescriptions identified family medicine as the greatest contributor to the absolute increase in incidence between 2014 and 2019, comprising nearly 40% of total prescriptions each year, whereas cardiology contributed the least, at <1% each year. The overall proportion of patients with ASCVD among those newly initiated on a GLP-1 RA and those initiated by a cardiology specialist increased after 2015, from 30.8-44.3% and 45.2-57.9% in 2019, respectively (Figure b). However, this increase in the proportion of patients with ASCVD was also observed among the total population of patients with T2D without new GLP-1 RA use (Figure b).

Conclusion: New use of GLP-1 RAs in patients with T2D increased in absolute terms between 2014 and 2019. Interestingly, the proportion of patients with ASCVD among those newly initiating the class has not changed relative to non-GLP-1 RA users over time, highlighting a gap in adoption of CVOT data in clinical practice by all specialities and the potential opportunity to further reduce CV risk in these patients.

Figure: (a) Incidence of new GLP-1 RA prescriptions in patients with type 2 diabetes (T2D) during 2014–2019 according to speciality. **(b)** Prevalence of ASCVD among patients with T2D and a new GLP-1 RA prescription during 2014–2019, overall and according to the prescriber speciality, and among those who were not using a GLP-1 RA during 2014–2019.



Blue arrows demarcate publication of landmark cardiovascular outcomes trials and updates to GLP-1 RA label indication. *Other: All other specialities that prescribed GLP-1 RA, as reported in the database. †Within patients with T2D. ASCVD was identified with International Classification of Diseases, ninth (430-438; 410-414) and tenth (I60-I69; G45; I20-I25; Z95; I70; I73.9) Clinical Modification codes. ASCVD, atherosclerotic cardiovascular disease; CV, cardiovascular; FDA, US Food and Drug Administration; GLP-1 RA, glucagon-like peptide 1 receptor agonist; LEADER, Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results; SUSTAIN 6, Trial to Evaluate Cardiovascular and Other Long-term Outcomes with Semaglutide in Subjects with Type 2 Diabetes.

Supported by: Novo Nordisk A/S

Disclosure: **J. Broe Honoré:** Employment/Consultancy; Novo Nordisk.

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Greater adherence and persistence with dulaglutide compared to injectable semaglutide at 6- and 12- months follow-up in U.S. real-world data

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Background and aims: The objective of this retrospective, observational study was to compare 6- and 12-month adherence and persistence among dulaglutide (DU) vs matched injectable semaglutide (SEMA) initiators using real-world administrative claims data from the IBM MarketScan Databases.

Materials and methods: Adult patients with type 2 diabetes newly initiating DU or SEMA between January 2018 - January 2020 (index date=earliest fill date), without evidence of prior glucagon-like peptide-1 receptor agonist (GLP-1 RA) use, pregnancy, gestational diabetes, or bariatric surgery in the 6-month baseline period, and with continuous enrolment in the 6-month baseline and 6- or 12-month follow-up periods were included. Patients are considered persistent if patient is on continuous therapy for index drug from the index date through the end of follow-up (6 or 12-month), allowing for a maximum fixed gap between fills (45 or 60 days). DU initiators were propensity-score matched 1:1 to SEMA initiators for each 6- and 12-month follow-up (26,284 and 13,837 pairs, respectively). Baseline characteristics were balanced, with mean age 53 years and 50% females.

Results: More DU patients were adherent than SEMA patients (6 months: 63% vs 48%, 12 months: 54% vs 43%, p values < 0.001). More DU patients were persistent (45-day permissible gap) on therapy compared to SEMA patients (6 months: 72% [18,897] vs 62% [16,351], 12 months: 56% [7.684] vs 45% [6,265], p values < 0.001) and had more mean persistent days (6 months: 145 vs 132, 12 months: 254 vs 221, p-values <0.001). Among patients who escalated and remained on a higher dose of index drug, most were adherent at 6 months (DU 83% vs SEMA 75%) and 12 months (DU 76% vs SEMA 72%).

Conclusion: At both 6 and 12 months, DU initiators had significantly higher adherence and greater persistence on their therapy than SEMA initiators.

Table: Adherence (PDC) at 6- and 12-months follow-up among matched dulaglutide and injectable semaglutide initiators

		6-month Follow-Up		12-month Follow-Up	
		DU (N=26,284)	SEMA (N=26,284)	DU (N=13,837)	SEMA (N=13,837)
PDC†, mean (SD)		0.77 (0.28)	0.70 (0.27)*	0.70 (0.32)	0.64 (0.31)*
PDC ≥80%, n (%)	Overall	16,655 (63.4)	12,554 (47.8)*	7,534 (54.4)	5,986 (43.3)*
Index Dose	Low	13,882 (63.7)	10,405 (47.7)	6,305 (55.4)	4,966 (43.6)
	High	2,773 (62.0)	2,149 (48.0)	1,229 (50.0)	1,020 (41.5)
Dosing Pattern	Starts on low dose, never uses high dose	6,553 (55.6)	7,566 (42.0)	2,925 (42.1)	2,840 (33.6)
	Starts on high dose, never uses low dose	2,637 (61.5)	1,908 (46.7)	1,151 (49.2)	868 (40.1)
	Starts on low dose, changes to high dose and stays on high dose	6,014 (82.7)	2,832 (74.6)	3,063 (76.4)	1,860 (71.8)
	All other dose patterns	451 (80.7)	448 (68.5)	395 (73.8)	398 (68.5)

*p<0.001 vs. dulaglutide; p-values for categorical variables were obtained using Chi-square tests; p-values for continuous variables were obtained using t-tests
† Proportion of Days Covered (PDC) is calculated as the number of days with drug on-hand divided by the number of days in the specified time-period (6- or 12-month follow-up period in this study)

Supported by: Eli Lilly and Company

Disclosure: **R. Mody:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

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Mobile health application as a real-world data resource: self-recorded weight reduction with once-weekly semaglutide

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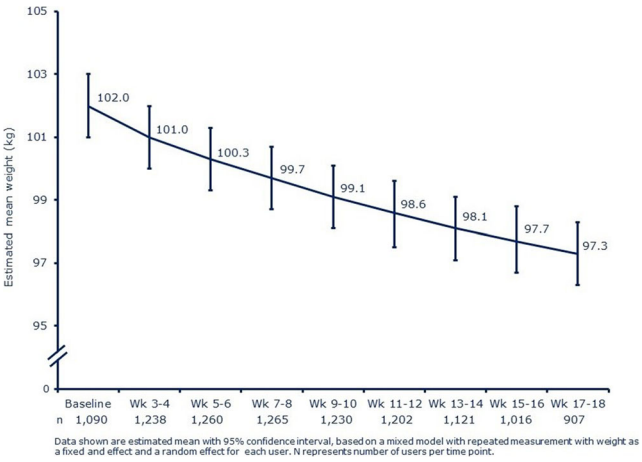
Background and aims: Supporting treatment initiation and adherence enables successful management of type 2 diabetes. Once-weekly (OW) semaglutide is a glucagon-like peptide-1 receptor agonist, approved for the treatment of type 2 diabetes. A mobile health application (mHealth app) that includes treatment planning, and body weight and dose-tracking features, has been developed to support patients during the initiation phase (first 8-12 weeks) of semaglutide treatment. The app is not limited to the initiation period and can be used afterwards to support treatment. Since August 2018, the app has been launched in 13 countries: Australia, Belgium, Canada, Chile, Colombia, Denmark, Finland, Luxembourg, Russia, Sweden, Switzerland, UK and USA. Users are required to register in the app by confirming they have been prescribed OW semaglutide according to local regulations. Here, we report the app users' self-recorded body weight data.

Materials and methods: Baseline body weight was defined as the first weight reported between weeks 1 and 2 after initiating treatment; subsequently reported weights were averaged over 2-week intervals. Mean weight over time (up to 18 weeks) was estimated using a mixed model for repeated measurements. Data were analysed for active users, defined as those who interact with the app (open or respond 'yes' when reminded about taking their dose) at least once every 3 weeks during the 12-week period.

Results: Of 2,820 active users, 1,506 had at least four recorded body weight entries and were included in the analysis. Of 1,506 users, 1,090 had baseline measurements (mean 102.0 kg), and 907 had week 17-18 measurements (mean 97.3 kg) and were included in the primary analysis. The mean self-recorded weight loss was 4.6% (4.7 kg; p<0.0001) from baseline to week 18. A sensitivity analysis for users reporting a baseline body weight (n=1,090) showed similar mean weight loss (4.5% [4.6 kg]). The recorded changes in

body weight are in line with those from retrospective real-world evidence (RWE) studies such as SPARE (n=937; 3.9% weight loss over 12-24 weeks) and prospective RWE studies such as SURE (Canada [n=452]: 4.3%; Switzerland [n=331]: 5.0%; Denmark/Sweden [n=214]: 5.3%; and UK [n=215]: 5.9%; all in a period of ~30 weeks).

Conclusion: We report patient self-recorded body weight data from a medication-specific mHealth app for people with type 2 diabetes who are prescribed OW semaglutide. One limitation of the study is that the data are collected from a mobile app and are patients' self-recorded body weight measurements which depend on the patients' level of engagement with the app. Nonetheless, the findings indicate that mHealth app may assist in generating RWE from a new source of data. Use of mHealth app anchors new habits that may contribute to patient adherence although more research is needed to support this.



Disclosure: **U. Bodholdt:** None.

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Efficacy and safety of alogliptin versus acarbose in Chinese type 2 diabetes individuals with cardiovascular risk or coronary heart disease: a randomised prospective study

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Background and aims: Chinese guidelines recommend aspirin to individuals with type 2 diabetes (T2D) and established coronary heart disease (CHD) or high cardiovascular (CV) risks. Previous studies revealed that these individuals did not achieve sufficient glycemic control and experienced gastrointestinal (GI) adverse events. Both alogliptin and acarbose are recommended for treatment of individuals with T2D inadequately controlled with metformin. This present study evaluated efficacy, safety, and tolerability of alogliptin versus acarbose in Chinese T2D individuals with CHD or high CV risk inadequately controlled with metformin monotherapy or treatment-naïve.

Materials and methods: This 16-week multicenter, randomized, open-label, prospective study included Chinese adults (age ≥18 years) with T2D who were either treatment-naïve or treated with metformin monotherapy. All individuals were treated with aspirin (100 mg) throughout the study. Individuals were randomized (2:1) to receive alogliptin 25 mg q.d. (n = 725) or acarbose 100 mg t.i.d. (n = 363) if they had HbA1c between ≥7.5% to ≤ 11.0%, FPG ≤13.3mmol/l and documented history of CHD or high CV risk at screening. Efficacy and safety outcomes were analyzed by analysis of covariance (ANCOVA) model, Pearson's Chi-square test and Cochran-Mantel-Haenszel test.

Results: After the 16-week treatment period, mean change in HbA1c was similar for alogliptin (least square (LS) means -1.090 [SE 0.037] %) and acarbose (-1.043 [0.0504] %), showing non-inferiority of alogliptin to acarbose (LS mean difference -0.048 [95% CI: -0.1687 to 0.0737] %], P<0.0001). Significantly lesser GI adverse events were observed with alogliptin than acarbose (8.9% vs 33.6%, P<0.0001). Number of individuals achieving HbA1c <7% were similar in both treatment groups (52.0% vs 51.7%, P=0.9058), with a trend for greater HbA1c <7% without GI effect with acarbose (48.0% vs 32.7%, P<0.0001). The event of at least 1 hypoglycemia was rare in both treatment groups (0.4% vs 1.1%, P=0.1820). Treatment-emergent adverse events were more frequent with acarbose than with alogliptin (50.7% vs 33.3%), with the most common events related to gastrointestinal, cardiac disorders (acute myocardial infarction, palpitations, unstable angina, coronary artery disease, myocardial ischemia) and infections. Patient discontinuation due to adverse drug reaction were significantly rare with alogliptin compared with acarbose (0.3% vs 1.9%; P=0.0046) (Table 1).

Conclusion: Alogliptin provides comparable glycemic control to acarbose with consistently lesser hypoglycemia, GI-related adverse events and reduced CV events in Chinese individuals with T2D.

Table 1: Analysis Of Efficacy And Safety Outcomes Of Alogliptin And Acarbose Over 16 weeks

ITT Population (Efficacy)			Alogliptin (N = 715) N (%)	Acarbose (N = 357) N (%)	LS mean difference (95% CI)/Risk difference (95% CI)	P-value
HbA1c (%)	Week 16	LS mean change from baseline to Month 4 (SE)	-1.090 (0.0357)	-1.043 (0.0504)	-0.048 (0.1687, 0.0737)	<0.0001
HbA1c<7% (%)	Week 16	N (%)	356 (52.0)	172 (51.7)	0.004 (-0.0611, 0.0694)	0.9058
HbA1c<7% without GI effect	Week 16	N (%)	328 (48.0)	109 (32.7)	0.1522 (0.0894, 0.215)	<0.0001
Safety Population			**Alogliptin (N = 723) N (%)**	**Acarbose (N = 363) N (%)**	**Risk difference (95% CI)**	**P-value**
GI tolerability (%)	Week 16	N (%)	64 (8.9)	122 (33.6)	-24.8 (-30.0, -19.5)	<0.0001
At least one hypoglycemia event (≤3.9 mmol/l)	Week 16	N (%)	3 (0.4)	4 (1.1)	-0.7 (-1.9, 0.5)	0.1820
Any TEAE	Week 16	N (%)	241 (33.3)	184 (50.7)	-	-
Gastrointestinal disorders	Week 16	N (%)	62 (8.6)	124 (34.2)	-	-
Infections and infestations	Week 16	N (%)	38 (5.3)	27 (7.4)	-	-
Cardiac disorders	Week 16	N (%)	1 (0.1)	7 (1.9)	-	-
Adherence and tolerability to treatment	Week 16	N (%)	2 (0.3)	7 (1.9)	-1.7 (-3.1, -0.2)	0.0046

CI: confidence interval; ITT: intention to treat; GI: gastrointestinal; SE: standard error; TEAE: treatment emergent adverse event

Clinical Trial Registration Number: NCT03794336
Supported by: Sanofi
Disclosure: **B. Gao:** None.

SO 28 GLP-1 receptor agonists and weight loss

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Once-weekly semaglutide 2.4 mg improved glucose metabolism and prediabetes in adults with overweight or obesity in the STEP 1 trial

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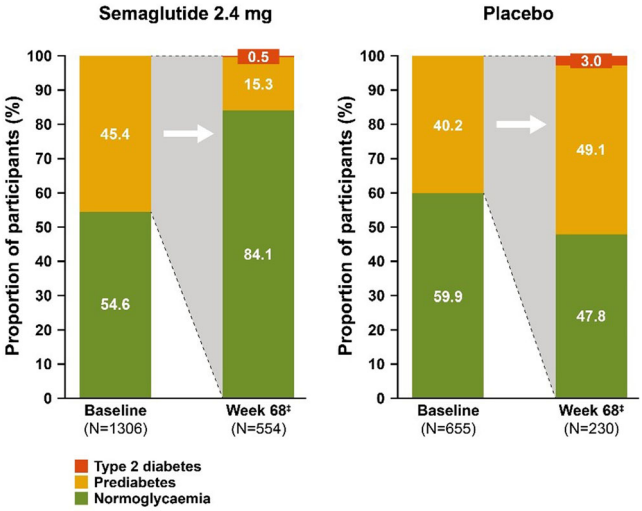
Background and aims: Semaglutide 2.4 mg demonstrated 14.9% body weight loss in adults with overweight/obesity in STEP 1. We evaluated effects on glucose metabolism in participants with prediabetes at baseline.

Materials and methods: Participants (N=1961) were randomised 2:1 to once-weekly s.c. semaglutide 2.4 mg or placebo, plus lifestyle intervention, for 68 weeks. Post-hoc analyses included change from baseline to week 68 in glycaemic status (normoglycaemia, prediabetes or type 2 diabetes; all investigator assessed), HbA_{1c}, fasting plasma glucose (FPG) and HOMA-IR. Statistical analyses were not adjusted for multiplicity.

Results: At baseline, 856 (43.7%) participants had prediabetes (593 semaglutide/263 placebo; mean HbA_{1c}: 5.9/5.9%, FPG: 98.7/97.6 mg/dL, HOMA-IR: 4.2/4.1, body weight: 106.9/106.9 kg). By week 68, 84.1% of those with prediabetes at baseline were normoglycaemic with semaglutide vs 47.8% with placebo ($p<0.0001$) (Figure); of those who reverted from prediabetes to normoglycaemia in the semaglutide group, the majority achieved weight loss ≥10%. Treatment with semaglutide vs placebo lowered HbA_{1c} (difference: -0.35 percentage points; $p<0.0001$), FPG (-8.49 mg/dL; $p<0.0001$) and HOMA-IR (relative % change: -28%; $p<0.0001$).

Conclusion: Once-weekly semaglutide 2.4 mg allowed most adults with overweight/obesity and prediabetes to revert to normoglycaemia at week 68.

Figure: Shift in glycaemic status* from baseline to week 68 in participants with prediabetes at baseline†



Data are observed data during the in-trial period (regardless of treatment discontinuation or rescue intervention).
***Glycaemic category was evaluated by the investigator based on all available relevant information (e.g. concomitant medication, medical records, and blood glucose parameters), and in accordance with the American Diabetes Association's definitions;**
†p<0.0001 (chi-squared test for independence for the proportion of participants changing glycaemic status from prediabetes at baseline to normoglycaemia at week 68); ‡data for participants with an assessment at week 68.

Clinical Trial Registration Number: NCT03548935

Supported by: Novo Nordisk A/S

Disclosure: **L. Perreault:** Employment/Consultancy; Novo Nordisk, Sanofi, Boehringer Ingelheim, Lilly, AstraZeneca, Janssen, Merck, Medscape, and UpToDate (all consultancy only). Lecture/other fees; Novo Nordisk, Sanofi, Boehringer Ingelheim, Lilly, AstraZeneca, Janssen, Merck, Medscape, and UpToDate.

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Effect of semaglutide 2.4 mg on achievement of weight loss and HbA_{1c} <7%, without hypoglycaemia, in adults with overweight/obesity and type 2 diabetes in STEP 2

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Background and aims: After 68 weeks in the STEP 2 RCT, the glucagon-like peptide-1 receptor agonist semaglutide 2.4 mg led to mean weight loss (WL) of 9.6% vs 7.0% with semaglutide 1.0 mg and 3.4% with placebo (PBO; both p<0.0001). Significant reduction in HbA_{1c} was achieved with semaglutide 2.4 mg vs PBO (1.6% vs 0.4%, respectively; p<0.0001). Effects on composite endpoints relating to WL and HbA_{1c} goals, without hypoglycaemia, were assessed. As sulphonylureas (SUs) are associated with weight gain and a higher risk of hypoglycaemia, these endpoints were also analysed based on baseline use of SUs.

Materials and methods: In STEP 2, 1210 adults with BMI ≥27 kg/m^2, type 2 diabetes and HbA_{1c} 7-10% (53-86 mmol/mol) while on 0-3 oral antihyperglycaemic drugs were randomised to once weekly s.c. semaglutide 2.4 mg (N=404), 1.0 mg (N=404) or PBO (N=403). The composite endpoints of WL ≥5%, ≥10%, ≥15% or ≥20% and HbA_{1c} <7%, without hypoglycaemia, were analysed for all patients and in a subgroup analysis based on baseline use of SUs. *Post-hoc* logistic regression analyses were performed based on the treatment policy estimand for the in-trial period (not adjusted for multiplicity). Hypoglycaemia was defined as clinically significant or severe events (ADA 2018 level 2/3).

Results: At baseline, participants had a mean age of 55 years, HbA_{1c} of 8.1%, body weight of 99.8 kg and BMI of 35.7 kg/m^2; in total, 25.1% of patients (n=304) were on SUs (n=108 in the semaglutide 2.4 mg group; n=99 in the semaglutide 1.0 mg group and n=97 in the PBO group). At week 68, patients treated with semaglutide 2.4 mg were more likely to achieve WL ≥5% (data not shown), ≥10%, ≥15% or ≥20% and HbA_{1c} <7%, without hypoglycaemia, vs semaglutide 1.0 mg and PBO; similar results were seen for those not on SUs at baseline (all p<0.05; Table). In those on SUs at baseline, semaglutide 2.4 mg vs PBO was more likely to result in WL ≥5% or ≥10% and HbA_{1c} <7%, without hypoglycaemia, but no differences were seen vs semaglutide 1.0 mg (Table). By week 68, ~1/3 patients in the semaglutide groups had stopped SU treatment vs ~1/7 patients in the PBO group.

Conclusion: More patients on semaglutide 2.4 mg achieved clinically meaningful WL (≥5-≥20%) and glycaemic target of HbA_{1c} <7%, without hypoglycaemia, vs semaglutide 1.0 mg and PBO. These results were more pronounced when patients were not on SUs at baseline.

Table. Achievement of categorical weight loss and HbA_{1c} <7% with no hypoglycaemic episodes in the STEP 2 trial

	Patients achieving endpoint at week 68, n (%)								
	WL ≥10%, HbA_{1c} <7% & no hypoglycaemia			WL ≥15%, HbA_{1c} <7% & no hypoglycaemia			WL ≥20%, HbA_{1c} <7% & no hypoglycaemia		
Treatment arm	Total	SU treatment	No SU treatment	Total	SU treatment	No SU treatment	Total	SU treatment	No SU treatment
Semaglutide 2.4 mg	155 (40.7)	21 (22.8)	134 (46.4)	89 (23.4)	12 (13.0)	77 (26.6)	45 (11.8)	9 (9.8)	36 (12.5)
Semaglutide 1.0 mg	97 (25.8)***	18 (22.8)	79 (26.6)***	46 (12.2)***	11 (13.9)	35 (11.8)***	16 (4.3)**	4 (5.1)	12 (4.0)**
Placebo	23 (6.1)***	3 (3.6)*	20 (6.9)***	9 (2.4)***	2 (2.4)	7 (2.4)***	5 (1.3)***	1 (1.2)	4 (1.4)**

Proportions are observed in-trial data for the full analysis set for patients with a body weight and HbA_{1c} assessment at week 68 (semaglutide 2.4 mg: n=381 of which 92 on SU at baseline; semaglutide 1.0 mg: n=376 of which 79 on SU at baseline; placebo: n=374 of which 83 on SU at baseline). *p<0.05, **p<0.001, ***p<0.0001 for higher odds ratio with semaglutide 2.4 mg. *Post-hoc* logistic regression analyses were based on the treatment policy estimand, with randomised treatment, stratification groups (oral antihyperglycaemic drug treatment status and HbA_{1c} category at screening) and the interaction between stratification groups as factors and baseline HbA_{1c} as covariate. Missing observations were multiply (x1000) imputed from retrieved patients of the same randomised treatment arm. SU, sulphonylurea; WL, weight loss.

Clinical Trial Registration Number: NCT03552757

Supported by: Novo Nordisk

Disclosure: **R. Goldenberg:** Employment/Consultancy; Amgen, AstraZeneca, Boehringer Ingelheim, Eli Lily, HLS, Janssen, Merck, Novo Nordisk, Sanofi (all consultancy only). Honorarium; Amgen, AstraZeneca, Boehringer Ingelheim, Eli Lily, HLS, Janssen, Merck, Novo Nordisk, Sanofi.

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Effect of once weekly dulaglutide 3.0 and 4.5 mg in patients with different baseline renal function: post hoc analysis from the AWARD-11 trial

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Background and aims: AWARD-11 study in patients with type 2 diabetes on metformin demonstrated similar safety and superior efficacy of dulaglutide (DU) 3.0 and 4.5 mg vs. DU 1.5 mg. A post hoc analysis assessed consistency in safety and efficacy of the three DU doses across patients with different baseline renal function (RF) levels.

Materials and methods: Participants randomized to DU 1.5, 3.0, and 4.5 mg were categorized into three RF levels based on baseline estimated glomerular filtration rate (eGFR; eGFR≥90, eGFR 60-90, and eGFR 30-60; eGFR unit: ml min^{-1} 1.73m^{-2}). Key safety and efficacy parameters were explored at week 52.

Results: There was no evidence of inconsistency in the effect of DU treatment across patients in any RF subgroup for incidence of gastrointestinal events, hypoglycaemia, new onset albuminuria or macroalbuminuria, or change from baseline in heart rate, blood pressure, or PR interval (interaction *p* value >0.05 for all). There was no evidence of inconsistency in the effect of DU treatment patients in any RF subgroups for change from baseline in HbA_{1c} (interaction *p* value=0.128) and bodyweight values (interaction *p* value=0.607).

Conclusion: Effect of DU treatment was associated with consistent safety and efficacy patterns for all patients regardless of their RF status at baseline.

Table: Summary of safety and efficacy parameters across dulaglutide doses in patients with different baseline renal function[a] status.

		Events, n/N (%) DU 1.5 mg (N = 612)	DU 3.0 mg (N = 616)	DU 4.5 mg (N = 614)	
Gastrointestinal events[c] at week 52	Baseline eGFR ≥90	87/408 (21.3%)	99/393 (25.2%)	111/397 (28.0%)	
	60 ≤ Baseline eGFR <90	35/171 (20.5%)	52/198 (26.3%)	50/184 (27.2%)	0.657[b]
	30 ≤ Baseline eGFR < 60	6/33 (18.2%)	3/25 (12.0%)	10/33 (30.3%)	
Hypoglycemia[d] at week 52	Baseline eGFR ≥90	6/408 (1.5%)	0/393 (0.0%)	4/397 (1.0%)	
	60 ≤ Baseline eGFR < 90	3/171 (1.8%)	2/198 (1.0%)	3/184 (1.6%)	0.188[b]
	30 ≤ Baseline eGFR < 60	0/33 (0.0%)	0/25 (0.0%)	0/33 (0.0%)	
HbA_{1c} CFB to week 52 (mmol/mol); LSM change ± SE	Baseline eGFR ≥90	-17.5 ± 0.7	-18.6 ± 0.7	-20.8 ± 0.7	
	60 ≤ Baseline eGFR < 90	-14.2 ± 0.9	-16.6 ± 0.8	-18.6 ± 0.9	0.128[e]
	30 ≤ Baseline eGFR < 60	-13.1 ± 1.9	-17.5 ± 2.2	-18.6 ± 1.86	
Bodyweight CFB to week 52 (kg); LSM change ± SE	Baseline eGFR ≥90	-3.1 ± 0.26	-3.7 ± 0.27	-4.8 ± 0.26	
	60 ≤ Baseline eGFR < 90	-4.1 ± 0.39	-5.2 ± 0.36	-5.5 ± 0.38	0.607[e]
	30 ≤ Baseline eGFR < 60	-4.3 ± 0.87	-6.0 ± 0.99	-5.7 ± 0.85	

[a] Renal function level as measured by eGFR using cystatin C equation (in mL min^{-1}1.73m^{-2})
[b] Comparison *p* value for test of homogeneity of odds ratios in treatment groups and renal subgroups is from a Breslow Day test for a Cochrane Mantel Haenszel test
[c] Gastrointestinal events at week 52; Included nausea, vomiting and diarrhoea
[d] Hypoglycaemia; defined as plasma glucose <3.0 mmol/L
[e] Interaction *p* values are from a mixed model for repeated measures to assess the change from baseline which includes an interaction between the treatment groups and the renal subgroups.
Abbreviations: CI = confidence interval; DU = dulaglutide; eGFR = estimated glomerular filtration rate; HbA_{1c} = glycated haemoglobin; LSM = least squares mean; SE = standard error

Clinical Trial Registration Number: NCT03495102
Supported by: Eli Lilly and Company
Disclosure: **L. García-Pérez:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

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Influence of baseline characteristics on weight loss with semaglutide 2.4 mg in adults with overweight/obesity and type 2 diabetes (STEP 2)

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Background and aims: Weight loss with semaglutide 2.4 mg vs placebo in the STEP 2 trial was analysed according to baseline characteristics.

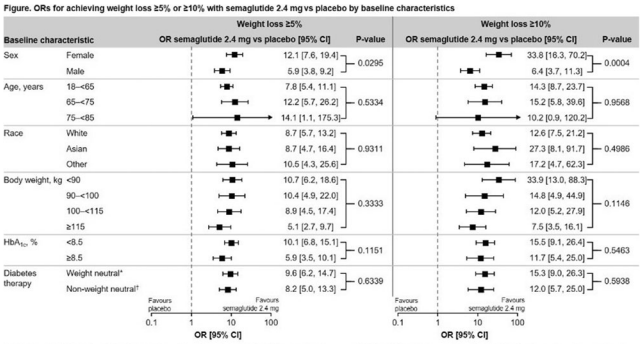
Materials and methods: STEP 2 was a 68-week RCT. Adults (N=1210) with BMI ≥27 kg/m^2 and type 2 diabetes (HbA_{1c} 7-10% [53-86 mmol/mol]) were randomised to once weekly s.c. semaglutide 2.4 mg, 1.0 mg (data not shown) or placebo. Categorical weight loss was evaluated at week 68 by baseline age, sex, race, body weight, HbA_{1c} and

diabetes therapy. The odds of achieving weight loss ≥5% or ≥10% for these parameters were compared using post-hoc logistic regression analyses (not controlled for multiplicity). Logistic regression analyses included body weight responders at week 68, based on the trial product estimand for the on-treatment period (N=320 for semaglutide 2.4 mg; N=313 for placebo), with treatment, subgroup, stratification and the interaction between subgroup and stratification as factors and baseline body weight as covariate. Missing week 68 assessments were imputed using a mixed model repeated measurement model with treatment and stratification as factors and baseline body weight as covariate, all nested within visit.

Results: At week 68, the proportions of patients with weight loss ≥5% and ≥10% were 73.2% and 49.9% for semaglutide 2.4 mg vs 27.6% and 7.1% for placebo. Patients were significantly more likely to achieve weight loss ≥5% or ≥10% with semaglutide 2.4 mg vs placebo in most subgroups (Figure). There were no significant interactions for any subgroups, except sex; the ORs for achieving weight loss ≥5% or ≥10% with semaglutide 2.4 mg vs placebo were more than double for females vs males (p<0.05 for interaction). Across all subgroups, treatment with semaglutide 2.4 mg resulted in clinically relevant weight loss at week 68.

Conclusion: With the exception of female sex, which was associated with a greater weight loss response to semaglutide 2.4 mg, weight loss was not influenced by baseline characteristics including body weight category, HbA_{1c} and use of non-weight neutral therapy.

Figure. ORs for achieving weight loss ≥5% or ≥10% with semaglutide 2.4 mg vs placebo by baseline characteristics



*Defined as diet/physical activity alone or in combination with one OAD (either metformin or an SGLT2i); †defined as treatment with 1–3 OADs, where monotherapy is not metformin or an SGLT2i. OAD, oral antihyperglycemic drug; OR, odds ratio; SGLT2i, sodium-glucose co-transporter-2 inhibitor.

Clinical Trial Registration Number: NCT03552757

Supported by: Novo Nordisk A/S

Disclosure: **C.W. le Roux:** Lecture/other fees; Novo Nordisk, GI Dynamics, Sanofi, Boehringer Ingelheim, Herbalife, Johnson &Johnson, Keyron. Stock/Shareholding; Keyron.

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Higher doses of dulaglutide induce weight loss in patients with type 2 diabetes regardless of baseline BMI: post hoc analysis of AWARD-11

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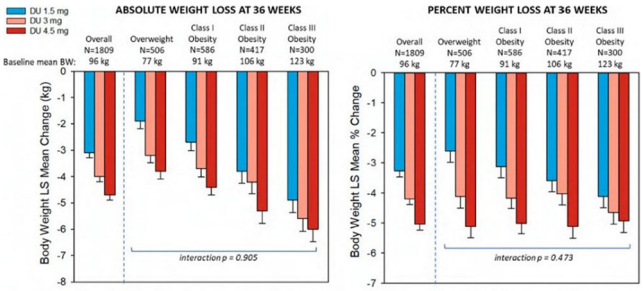
Background and aims: The AWARD-11 trial demonstrated that dulaglutide (DU) 3 mg and 4.5 mg once weekly improved glycated haemoglobin (HbA_{1C}) and bodyweight (BW), compared to DU 1.5 mg once weekly, in patients with type 2 diabetes inadequately controlled with metformin monotherapy. The aim of this post-hoc analysis was to assess the effect of DU on BW in clinically relevant baseline body mass index (BMI) categories as defined by clinical practice guidelines.

Materials and methods: Eligible patients had screening HbA_{1C} 58 - 97 mmol/mol (7.5 - 11%) and BMI ≥ 25 kg/m^2. Patients (N=1842) were randomized to DU 1.5 mg, DU 3 mg, or DU 4.5 mg. Total treatment period was 52 weeks with primary efficacy endpoint at 36 weeks. Baseline BMI (kg/m^2) was categorized as overweight (<30), obesity Class I (30 - <35), Class II (35 - <40) or Class III (≥40). Mixed model for repeated measures was used within the BMI subgroups for assessing change in BW.

Results: At 36 weeks, mean absolute reduction in BW within each DU dose group increased by baseline BMI category, whereas mean percentage weight loss was similar regardless of BMI category in DU 3 mg and 4.5 mg groups (Figure). Treatment-by-BMI subgroup interaction was not significant for either change or % change in BW (p = 0.905 and 0.473, respectively). The pattern of common adverse events was similar across BMI subgroups.

Conclusion: Treatment with DU 3 mg and 4.5 mg induces weight loss across a range of clinically relevant BMI categories in patients with type 2 diabetes.



N = total number of patients overall and in each BMI category at baseline. Analyses included patients with non-missing baseline value and at least one non-missing post-baseline weight measurement. P-value is for interaction of subgroup and treatment at Week 36 evaluated using a significance level of 0.10, unadjusted. DU, dulaglutide; LS, least squares

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Disclosure: **E. Bonora:** Employment/Consultancy; Abbott, Astrazeneca, Becton Dickinson, Boehringer Ingelheim, Bristol-Myers Squibb, Bruno Farmaceutici, Janssen, Johnson & Johnson, Eli Lilly, Merck Sharp and Dohme, Mundipharma, Novartis, Novo Nordisk, Roche, Sanofi, Servier, Takeda, Bayer, Daiichi-Sankyo.

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The use of semaglutide alongside other diabetes medications: real-world results from the Association of British Clinical Diabetologists (ABCD) audit programme

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Background and aims: The ABCD semaglutide audit launched in 2019

with the aim of establishing real-world outcomes and our results so far have demonstrated comparable HbA1c and weight reductions to randomised controlled trials. Further HbA1c and weight reductions still occurred in those who had been on glucagon like peptide 1 (GLP1) receptor agonists prior, but to a lesser extent. Our aim is to establish if HbA1c and weight changes associated with the commencement of semaglutide are enhanced or attenuated by its use alongside other classes of diabetes drugs or in multidrug combinations.

Materials and methods: Data submitted to the ABCD audit programme were included providing sufficient follow-up data were available. HbA1c and weight changes between baseline and follow-up were assessed using paired T-Tests and ANOVA with stratified analyses also performed by number of additional diabetes drugs and classes included. Analyses performed in Stata 16.

Results: 773/1,517 had sufficient follow-up data for inclusion in the analysis. Baseline characteristics are displayed in **table 1.** Reductions in HbA1c and weight were observed in combination with all other drugs and with any number of drug combinations. HbA1c reduced by 13.2mmol/mol (95% CI -11.8, -14.5; P<0.0001) across the entire population. The number of drugs in the regimen did not impact the magnitude of HbA1c change (P=0.76). Sodium glucose linked transporter 2 inhibitors (SGLT2) use was associated with attenuated reductions in HbA1c (11.4mmol/mol vs 14.1 not taking, P<0.05). Larger HbA1c reductions of 15.9mmol/mol (vs 12.4 without, P<0.05) were seen in insulin users. Other drug classes had no impact on the extent of HbA1c reduction. Weight decreased by 4.7kg across the population (95% CI 4.1, 5.3, P<0.001). No drugs appeared to enhance or attenuate weight loss, nor did the number of drugs in the regimen (P=0.06). Although reductions still occurred, previous GLP1 use was also associated with lesser degrees of HbA1c and weight reductions similar to previously demonstrated (P<0.0001).

Conclusion: Semaglutide use is associated with HbA1c and weight reductions irrespective of other diabetes therapy but may be enhanced or attenuated in combination with certain drugs. Previous GLP1 use was associated with attenuated HbA1c and weight reductions compared to those unexposed, although further significant reductions were still observed.

Table 1. Baseline characteristics of observed population

Characteristic	n=773
Age, years ± SD	59.3 ± 10.5
Male, %	50.2%
Median diabetes duration, year (IQR)	11.6 (7-15.9)
Mean Hba1C, % ± SD	9.32 ± 1.68
mmol/mol ± SD	78.4 ± 18.6
Mean BMI, kg/m2 ± SD	36.3 ± 7.2
Mean weight, kg ± SD	107.4 ± 23.2
Mean serum creatinine, umol/L ± SD	80.4 ± 26.9
Mean eGFR, mL/min/1.73m^2 ± SD	87.0 ± 26.5
Mean systolic BP, mmHg ± SD	133.6 ± 14.8
Mean diastolic BP, mmHg ± SD	78.5 ± 10.0
Previous GLP1 use, %	25.9%
Sodium glucose linked transporter 2 (SGLT2) use, %	34.5%
Insulin use, %	22.9%
Thiazolidinediones (TZD) use, %	5.1%
Dipeptidyl peptidase 4 inhibitor use (DPP4), %	8.9%
Metformin use, %	78.8%
Sulphonylurea use, %	26..9%
Semaglutide monotherapy, %	8.2%
Semaglutide + one other diabetes drug, %	30.4%
Semaglutide + two other diabetes drugs, %	40.5%
Semaglutide + three or more diabetes drugs, %	21.0%

BMI, body mass index; eGFR, estimated glomerular filtration rate; BP, blood pressure; IQR, interquartile range; SD, standard deviation

Supported by: ABCD

Disclosure: **T.S.J. Crabtree:** Lecture/other fees; Sanofi, Novo Nordisk. Non-financial support; Sanofi, Novo Nordisk.

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Body weight loss with oral semaglutide is predominantly mediated by effects other than gastrointestinal adverse events

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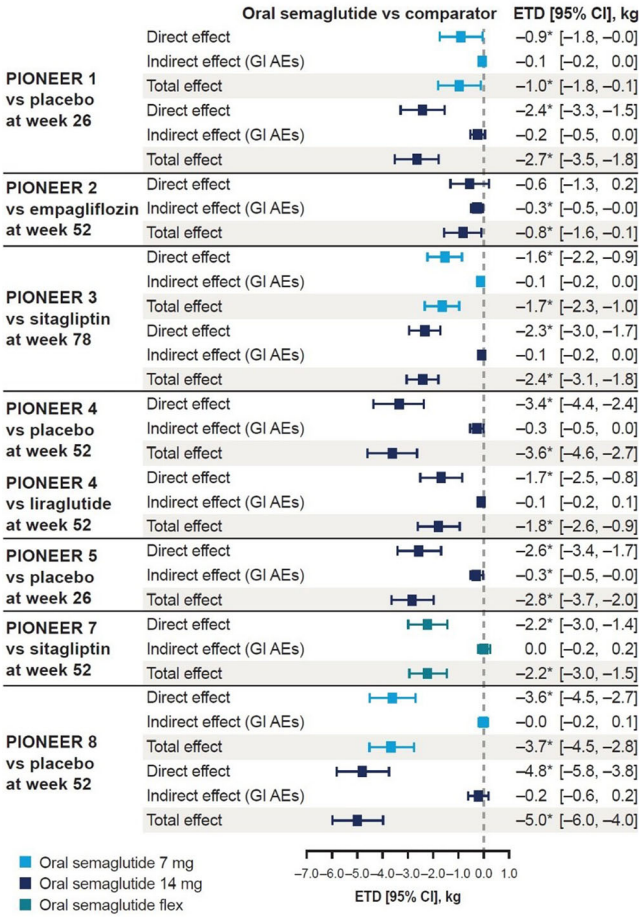
Background and aims: Glucagon-like peptide-1 receptor agonists (GLP-1RAs) reduce body weight. Evidence suggests that GLP-1RAs directly modulate appetite and reduce food intake through central mechanisms. However, GLP-1RAs are also associated with gastrointestinal (GI) adverse events (AEs), which may contribute to reduced appetite and food intake. We sought to assess whether the body weight loss observed in phase 3 clinical trials with oral semaglutide was primarily due to indirect effects (defined as GI AEs, specifically nausea, vomiting and diarrhoea) or direct effects (not explained by GI AEs).

Materials and methods: Data from PIONEER 1-5, 7 and 8 were assessed and a mediation analysis was performed to separate the overall contribution of direct and indirect effects to changes in body weight. A mediation analysis explores the effect of a third variable (occurrence of GI AEs: nausea, diarrhoea or vomiting) on the relationship between two other variables (treatment and body weight).

Results: Significantly greater (p<0.05) body weight loss was observed vs all comparators favouring oral semaglutide 7 mg (estimated treatment difference [ETD]: 1.0-3.7 kg vs placebo, 1.7 kg vs sitagliptin), oral semaglutide 14 mg (ETD: 2.7-5.0 kg vs placebo, 0.8-2.4 kg vs active comparators) and oral semaglutide flexibly dosed (ETD: 2.2 kg vs sitagliptin) (Figure). In the mediation analysis, oral semaglutide 7 mg, 14 mg and flexibly dosed provided significantly (p<0.05) more weight loss via direct effects than placebo (ETD: 0.9-4.8 kg), as well as the active comparators sitagliptin (ETD: 1.6-2.3 kg) and liraglutide (ETD: 1.7 kg). GI AEs, the indirect effects in the mediation analysis, accounted for 0.0-0.3 kg of the body weight loss across oral semaglutide doses vs all comparators (Figure).

Conclusion: GI AEs appear to have no clinically relevant impact on total body weight loss observed with oral semaglutide vs placebo and most active comparators.

Figure. Contribution of direct and indirect effects to the estimated treatment differences in body weight with oral semaglutide vs comparators at end of treatment.



Analysed by an effect model with imputation-based estimation: included the interaction between treatment and any nausea, diarrhoea or vomiting together with the baseline variables of body weight, region, and stratification factors as main effects, assuming no interaction between natural effects and baseline variables.
*p<0.05 vs comparator. Data shown are for the trial product estimand (on trial product without rescue medication) at the end of treatment. Due to rounding of data, direct and indirect effects may not always equal the total effect. AE, adverse event; ETD, estimated treatment difference; flex, flexibly dosed; GI, gastrointestinal.

Clinical Trial Registration Number: NCT02906930 [PIONEER 1], NCT02863328 [PIONEER 2], NCT02607865 [PIONEER 3], NCT02863419 [PIONEER 4], NCT02827708 [PIONEER 5], NCT02849080 [PIONEER 7], NCT03021187 [PIONEER 8]

Supported by: Novo Nordisk A/S

Disclosure: **J.J. Meier:** Employment/Consultancy; AstraZeneca, BMS, Boehringer Ingelheim, Eli Lilly, MSD, Novo Nordisk, Sanofi-Aventis (all consultancy only). Grants; Boehringer Ingelheim, MSD, Novo Nordisk, Sanofi-Aventis. Lecture/other fees; AstraZeneca, Berlin-Chemie, Boehringer Ingelheim, BMS, Eli Lilly, MSD, Novo Nordisk, Novartis, Roche, Sanofi-Aventis.

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Benefits of insulin glargine/lixisenatide fixed-ratio combination for patients inadequately controlled on premixed insulin and oral agents

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Background and aims: Achieving and maintaining glycemic control is essential for reducing the risk of diabtes-aasociated complications. Unfortunately, studies have shown that between 40-60% of patients worldwide are not achieving their glycemic goals. The premixed insulin are still routinely in management T2DM. The fixed - ratio combination insulin glargine/lixisenatide (IglarLixi) combines two different glucose-lowering agents, basal insulin and a glucagon-like peptide 1 receptor agonist, with complementary mechanisms of action, addressing both fasting plasma glucose (FPG) and postprandial glucose levels. The aim of study was to evaluate benefit on glycemic and non glycemic parameters after switched from premixed insulin to IGlarLixi.

Materials and methods: Fifty patients with T2DM who receiving premixed insulin with metformin and inadequately controlled were switched to IGlarLixi to 24 weeks. All patients continued to receive metformin. We analyzed change in HbA1c, fasting plasma glucose (FPG), self-monitored plasma glucose (SMPG) profile, body weight, proportion patient achieving HbA1c<7%.

Results: At week 26, HbA1c reduction was -1,2% . Mean HbA1c was 8.6 at baseline and 7.4% at the end of study. 20 patients (40%) achieved HbA1c <7%. Mean FPG significantly lower at the end of study.The difference in FPG change relative to baseline after IglarLixi treatment was -1.6 mmol/l. Parameters derived from seven point SMPG profile (preprandial and 2h postprandial glucose) were significantly lower at the end of study (preprandial breakfast SMPG -1.1 mmol/l, postprandial breakfast SMPG -1.5 mmol/l, preprandial lunch SMPG -1.4 mmol/l , postprandial lunch SMPG 1.1 mmol/l, preprandial dinner SMPG -1.3 mmol/l, postprandial dinner SMBG 1.2 mmol/l). IGLarLixi was associated with weight reduction -2.1 kg from baseline, lower BMI from baseline (30.1 at baseline to 29.2 kg/m^2 at the end of study).

Conclusion: In patients with T2DM inadequately controlled on premixed insulin switched to IglarLixi fixed - ratio combination was associated with better glycemic control and weight reduction.

Disclosure: **I. Risovic:** None.

SO 29 Novel glucose-lowering agents

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Dual GLP-1 and glucagon receptor agonism with cotadutide significantly increases insulin secretion in overweight and obese adults with type 2 diabetes

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Background and aims: Cotadutide is a GLP-1/glucagon receptor dual agonist in development for non-alcoholic steatohepatitis and type 2 diabetes (T2D) with diabetic kidney disease. We conducted a mechanistic phase 2a, randomized, double-blind, placebo-controlled trial in adults (BMI 28-40 kg/m^2) with T2D (HbA1c ≤8.0%) to assess the effects of cotadutide on glucose homeostasis measured by a mixed-meal tolerance test (MMTT) and frequently sampled iv glucose tolerance test (FSivGTT) performed before and during study dosing.

Materials and methods: Participants underwent a 16-day single-blind placebo run-in and were randomized 2:1 to double-blind sc cotadutide (n=19) or placebo (n=9) for 42 days. The dose was uptitrated every 4 days from 100 µg to 300 µg daily. The MMTT (cotadutide, n=12; placebo, n=7) was used to assess change in $AUC_{0\text{-}4\ h}$ for glucose and insulin (Day -1 to Day 59). A subgroup of patients underwent FSivGTT (cotadutide, n=9, placebo, n=4; change from baseline to Day 42). We calculated insulin sensitivity (S_I) and glucose effectiveness (S_G) from the minimal model ; first-phase insulin response (FPIR) as incremental AUC in the first 10 minutes after glucose challenge. Gastric emptying time (GET) was assessed before and after treatment. Data are mean (SD) for MMTT and median (IQR) for FSivGTT unless otherwise indicated.

Results: In the MMTT, there was a significant reduction in glucose $AUC_{0\text{-}4\ h}$ in the cotadutide group (35.5 [10.2] to 26.9 [3.1] mmol/L*h) with no change in the placebo group (30.5 [3.7] to 30.3 [4.8] mmol/L*h); least-squares (LS) mean % change vs placebo was - 12.3 (90% CI: - 19.7, -4.9, p=0.010). Insulin $AUC_{0\text{-}4\ h}$ during MMTT increased significantly with cotadutide (889.6 [449.1] to 985.7 [409.4] hr.pmol/L; LS mean % change from baseline, 27.3 [90% CI: 10.3, 44.4]); LS mean % change vs placebo was 29.6 (90% CI: 1.5, 57.7, p=0.085). There was no change in fasting insulin after cotadutide (LS mean change from baseline vs placebo: 11.4 [90% CI: - 25.6, 48.5] pmol/L, p=0.598). In the FSivGTT, FPIR increased with cotadutide (117.4 [- 192.5, 647.9] to 1966.9 [1302.9, 7474.3] mU/L*min) and nominally decreased with placebo from 489.4 (125.6, 1297.6) to 441.8 (64.2, 1047.9) mU/L*min (p=0.003). Changes in S_I and S_G were not significantly different between groups. GET significantly increased following cotadutide treatment (LS mean change from baseline 6.7 h (90% CI: 5.0, 8.4) and 2.6 h (90% CI: 0.4, 4.7) for cotadutide vs placebo (p=0.019).

Conclusion: Glucose-lowering effects of cotadutide in overweight/obese adults with T2D are mediated predominantly by an increase in insulin secretion and delayed GET rather than a change in responsiveness to insulin.

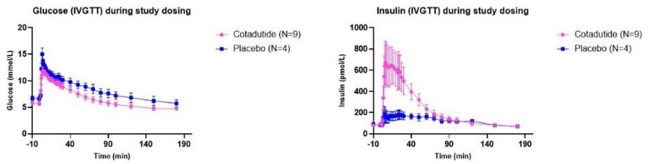

Figure. Intravenous glucose tolerance test data for glucose and insulin during study dosing

Abbreviation: IVGTT- intravenous glucose tolerance test
Data are mean (SEM)

Clinical Trial Registration Number: NCT03596177

Supported by: AstraZeneca

Disclosure: **R. Golubic:** Other; The study was funded by AstraZeneca.

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A novel, stable mutation of FGF1 separates the glucose lowering effect from its proliferative properties through interaction with the insulin receptor

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Background and aims: Since the last decade, the acidic fibroblast growth factor 1 (FGF1) has been associated with its metabolic roles in glucose homeostasis in type 2 diabetes (T2D). A single injection of FGF1 can reduce high blood glucose levels in mice model of diabetes. However, due to the proliferative properties of FGF1 and its low thermal stability, therapeutical use of it in T2D remains unavailable. Here we evaluate the antidiabetic and mitogenic effect of a novel FGF1 variant with increased thermal stability and with altered affinity to cognate receptors.

Materials and methods: The glucose uptake assay was carried out in 3T3-L1 cells differentiated into adipocytes. Cells were treated with proteins at 10-1000 ng/ml. To study diabetes improving effect, proteins were administered to *db/db* mice s.c. three times, every 48 h, at 1 or 5 mg/kg, and blood glucose concentrations were measured at different time points. The protein stability analyses were performed using Differential Scanning Calorimetry (DSC). Proliferation was studied in NIH3T3 fibroblasts by use of MTT assay. Cells were treated with increasing concentrations of proteins (from 0-1000 ng/mL) for 48 h. To study the effect on tumour growth female SCID mouse model implanted with an RT112 cell line (bladder carcinoma). Proteins were administered every other day, s.c. at 0.1, 1 or 5 mg/kg for 29 days. Binding affinity assay was performed using microscale thermophoresis (MST) with MonolithNT instrument.

Results: Herein we present a novel, stable mutation of FGF1 ($FGF1^{CP43}$), with a denaturation temperature of 63°C ± 0.5°C and decreased binding affinity to FGFR1IIIc receptor domain. In adipocytes (*in vitro*) $FGF1^{CP43}$ changed glucose uptake dosage-dependently and at 1000 ng/mL increased glucose uptake from 1 ± 0.05 to 2.3 ± 0.5. In *db/db* mice, $FGF1^{CP43}$ lowered blood glucose from 387 ± 13 to 156 ± 6 mg/dl after the third injection. The duration of the effect was dose-dependent and lasted for 48h after each injection. No hypoglycemia or other visible outcomes on proliferation and body weight were observed during 7 days of treatment. As a strong candidate for further clinical development against T2D, the proliferative properties of $FGF1^{CP43}$ were tested. Interestingly, the MTT assay showed no-mitogenic properties of $FGF1^{CP43}$ *in vitro* in NIH3T3 fibroblasts. Importantly, in SCID mice implanted with RT112 cells, after 29 days of treatment, $FGF1^{CP43}$ tumour volume was decreased from 1174 mm^3 ± 172mm^3 in a control group to 959 mm^3± 176 mm^3 in mice treated with $FGF1^{CP43}$ at 5 mg/kg. To elucidate the mechanism underlying divergent metabolic and proliferative effects of both proteins, the interaction with different FGFR isoforms and InsR was studied. Interestingly, $FGF1^{CP43}$ showed increased binding affinity to the insulin receptor (InsR) (kD1 1.79 µM ± 0.05 µM) as compared to wild type (wt) FGF1 (kD1 4.8 µM ± 0.5 µM). $FGF1^{CP43}$ induced the phosphorylation cascade of InsR signalling and showed stronger glucose uptake properties after induction of insulin resistance in adipocytes.

Conclusion: These findings lead to the strong assumption, that novel mutein $FGF1^{CP43}$ has significant potential in the clinical development in the field of T2D due to the separation of mechanistic effects of glucose lowering and proliferation.

Supported by: NCBiR

Disclosure: **M. Janiszewski:** None.

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VEGF-a/ang-2 neutralisation causes sustained prevention of subretinal macrophage infiltration in a mouse model of spontaneous choroidal neovascularisation

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Background and aims: Faricimab, currently in phase 3 trials, is the first bispecific antibody for intraocular use. It independently binds and neutralizes both angiopoietin-2 (Ang-2) and VEGF-A, key drivers of vascular instability (vascular leakage, neovascularization [NV], and inflammation). Faricimab demonstrated sustained efficacy compared with anti-VEGF monotherapy in the phase 2 clinical trial for neovascular AMD. This abstract presents new preclinical data on the anti-inflammatory effect of targeting Ang-2 and supporting the vessel stabilization potential of Ang-2 inhibition in a mouse model of spontaneous choroidal NV (sCNV) in the context of observed phase 2 clinical data.

Materials and methods: 7-week-old JR5558 mice developing bilateral spontaneous neovascular lesions were treated intraperitoneally with mouse cross-reactive tool antibodies against VEGF-A, Ang-2, or both (bispecific anti-VEGF-A/Ang-2 antibody), and IgG as controls. Subretinal macrophage infiltration, detected by Iba1 immunostaining, was evaluated ex vivo by flat-mounted retinal pigment epithelium (RPE)/choroid histology at 1, 3, and 5 weeks post treatment to assess immediate and long-term effects on the number of inflammatory cells around lesions.

Results: Treatment with the bispecific anti-VEGF-A/Ang-2 antibody significantly reduced the number of Iba1-positive macrophages around lesions on flat-mounted RPE/choroid histology by 23% and 38% ($P<0.05$) vs IgG control at 1 and 3 weeks post treatment, respectively. The effect of VEGF-A or Ang-2 inhibition alone was not significant. At 5 weeks post treatment, only anti-Ang-2- and anti-VEGF-A/Ang-2-treated mice showed significant reduction in the number of Iba1-positive macrophages by 53% and 49% ($P<0.0001$), respectively, vs IgG control. Anti-VEGF-A treatment alone did not prevent subretinal infiltration of Iba1-positive immune cells.

Conclusion: Preclinical experiments further elucidated the potential role of Ang-2 inhibition alone and in combination with anti-VEGF in reducing inflammation in the retina, and demonstrated that the prolonged anti-inflammatory effect was driven by Ang-2 neutralization. In a mouse model of sCNV, dual Ang-2/VEGF-A inhibition was superior to VEGF-A monotherapy in causing sustained prevention of subretinal macrophage infiltration around lesions on RPE/choroid, supporting the results of the phase 2 clinical trial.

Supported by: F. Hoffmann-La Roche Ltd.

Disclosure: **J. Canonica:** Employment/Consultancy; F. Hoffmann-La Roche Ltd.

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Novel GLP-1 analogue, GZR18: a preclinical evaluation in type 2 diabetes models

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Background and aims: Glucagon-like peptide 1 receptor agonists (GLP-1 RAs) are an approved treatment option for patients with type 2 diabetes (T2D) to improve glycemic control and reduce body weight, given their ability to increase insulin secretion, suppress glucagon secretion, and slow gastric emptying. This study aimed to evaluate the pharmacokinetics (PK) and pharmacology of a novel GLP-1 analog, GZR18, in GLP-1 receptor (GLP-1R)-expressing cell lines, primary mice islets, and animal models.

Materials and methods: GZR18 binding to GLP-1R was characterized *in vitro* by radio-ligand competitive binding assays in the presence of human serum albumin (HSA). GZR18 activation of GLP-1R was assessed in cell lines expressing human (h)GLP-1R in the presence of HSA. Glucose-stimulated insulin secretion (GSIS) was assessed in primary mice islets. *In vivo* efficacy compared to vehicle controls was evaluated in db/db mice in single-dose (n=3; 0.3, 1, 3, 10, 30, and 100 nmol/kg GZR18) and in 5-week repeated-dose (n=12; 30, 100, and 300 μg/kg GZR18; dulaglutide [300 μg/kg]; Q3D) studies, using subcutaneous (s.c.) injection. The PK profiles of GZR18 were studied in cynomolgus monkeys (CM; n=4; 60 μg/kg single s.c. injection).

Results: GZR18 bound hGLP-1R with a half-maximal inhibitory concentration (IC_{50}) of 3.7±2.0 nM and 5552.3±378.8 nM, and activated GLP-1R with a half-maximal concentration of 1.9±0.5 nM and 696.0 ±149.8 nM in the presence of 0.005% and 2% HSA, respectively. GZR18 (30 nM) increased GSIS in isolated mice islets 1.68-fold compared with vehicle control. A single dose of GZR18 in db/db mice lowered random blood glucose with a median effective dose of 3.0 nmol/kg after 6h. The repeated-dose study in db/db mice demonstrated ameliorated glucose intolerance 48h after treatment with 100 and 300 μg/kg GZR18 ($p<0.01$); reduced HbA1c after the 11th dose of GZR18 (17.3%, 22.1%, and 26.4% in the 30, 100, and 300 μg/kg GZR18 groups, respectively) and 300 μg/kg dulaglutide (16.7%) ($p<0.01$); and reduced serum triglyceride ($p<0.05$), compared to vehicle controls. Repeated doses of GZR18 in db/db mice lowered food and water consumption, and body weight, dose-dependently ($p<0.05$ vs. vehicle controls). A PK study of GZR18 performed in CM demonstrated that the C_{max} was 527 ±140 nmol; the average terminal half-life ($T_{1/2}$), 61.3±5.34 h; the T_{max}, 14 ±6.73 h; the elimination rate, 0.0114±0.000956 h^{-1}; and bioavailability, 73.3%.

Conclusion: Results demonstrate that GZR18 can specifically bind and activate GLP-1R, and improve glucose homeostasis in db/db mice. Moreover, $T_{1/2}$ of GZR18 in CM is 61.3 hours. These results suggest that GZR18 may have the potential to treat diabetic patients as a long-acting GLP-1R analog.

Supported by: Gan & Lee Pharmaceuticals

Disclosure: **Y. Wang:** Employment/Consultancy; Employee of Gan & Lee Pharmaceuticals.

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Oz101, an oligofructose prebiotic, may ameliorate beta cell deterioration associated with long-term sulphonylurea therapy in type 2 diabetes patients: a pilot study

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Background and aims: The sulphonylurea (SU) class of anti-diabetes drugs are inexpensive and widely used to treat type 2 diabetes (T2D), but

are associated with loss of efficacy over time in part due to progressive beta cell failure. Naturally occurring gut bacteria, important to glucose and insulin homeostasis, are under-represented in the intestinal tracts of patients with T2D. Long-term use of SUs may further exacerbate gut microbial dysbiosis as they are derivatives of sulphonamide antibiotics. We conducted a study, in a resource constrained area in India where SU is widely used, to test the hypothesis that the dietary fiber comprised of an oligofructose prebiotic (OZ101), administered as an adjunctive therapy, can improve beta cell function and glycaemic control in SU-treated T2D patients.

Materials and methods: Subjects with T2D on SU monotherapy (n=36) were randomized in a 24-week parallel, open label, proof-of-concept study to either continue receiving their usual SU-only treatment or add a thrice daily regimen of 13.5 or 27 g/d doses of OZ101 taken with meals. HOMA-B and glycaemic parameters after 12 hours fasting, glucose total area under the curve (AUC) over 240 minutes after intake of a pre-defined calorie milkshake and stool samples for gut microbiome were collected and measured at baseline and 24 weeks.

Results: No safety or tolerability issues were observed. Subjects on SU-only therapy showed decline in beta cell function based on a 36% decrease in HOMA-B from baseline ($p<0.01$), whereas subjects taking SU+13.5 g/d OZ101 showed an improvement in beta cell function based on a 23% increase in HOMA-B from baseline ($p<0.031$). This was also associated with a statistically significant reduction in primary outcomes. Compared to the SU-only treated subjects, in subjects treated with SU+ 13.5 g/d OZ101 HbA1c and AUC were reduced by 0.95% (10 mmol/mol, $P<0.047$) and 607 mmol/L∗240 min ($p<0.039$), respectively. Two- to ten-fold increase in a number of Bifidobacteria- and Lactobacillus- strains were observed in OZ101-treated subjects. Subjects treated with SU+27 g/d OZ101 showed smaller and non-significant reductions in HbA1c (0.29%, N.S.) and AUC (88 (mmol/L) ∗240 min, N.S.).

Conclusion: In this pilot trial, in a resource constrained area, adjunctive intake of OZ101 in patients on SU therapy was safe, well tolerated and associated with improved beta cell function and better glycaemic control over 24 weeks. These effects may be partly due to an increase in a number of commensal bacteria previously shown to influence glucose and insulin homeostasis. The lack of dose response may be explained by further changes in the gut microbiome dynamics at higher doses of OZ101. The limited sample size and inter-subject variability justifies future studies designed to confirm and expand on these observations and to determine the mechanistic link between the changes in the gut microbiome and the improved metabolic responses.

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Disclosure: **M.A. Noor:** Stock/Shareholding; Share holder at OzStar Therapeutics Pty Ltd.

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Beneficial antidiabetic actions of a novel V1a and V1b receptor specific AVP analogue in high fat fed mice

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Background and aims: Recent evidence highlight beneficial metabolic effects of arginine vasopressin (AVP), on metabolism, mediated by activation of V1a and V1b receptors. The well characterised role of AVP on fluid retention relates to activity at V2 receptors. In the current study, six novel N-terminally acetylated and/or amino acid substituted AVP peptides were screened for enzymatic stability, insulin secretory activity and receptor activation profile. The lead peptide, Ac3IV a V1a and V1b receptor specific AVP analogue, was progressed to sub-chronic antidiabetic efficacy testing in high fat fed (HFF) mice.

Materials and methods: : AVP analogues were incubated with murine plasma (0-4 h) to assess *in vitro* enzyme stability. Insulin secretory activity (5.6 and 16.7 mM glucose ;20 min) of the AVP analogues (10^{-12} - 10^{-6} M) was assessed in BRIN-BD11 beta-cells (n=8). Fully characterised V1a, V1b and V2 receptor antagonists, namely SR-49059, SR-149415 and tolvaptan respectively, were employed to determine receptor specificity of AVP peptide. To examine antidiabetic efficacy, benefits of the lead AVP analogue, Ac3IV, were compared directly against exendin-4 in HFF mice. These mice (n=8) and received twice daily (09:30 and 17:00 h) injections of saline vehicle, Ac3IV or exendin-4 (both at 25 nmol/kg bw) for 22 days. Energy and fluid intake, body weight, blood glucose and plasma insulin concentrations were measured at regular intervals. Intraperitoneal glucose tolerance (18 mmol/kg), and insulin sensitivity (15 U/kg) tests were conducted at the end of the study. Terminal analyses included assessment of circulating glucagon and lipids as well as body composition and examination of pancreatic hormone content and islet architecture.

Results: All N-terminally acetylated AVP analogues remained fully intact following incubation in murine plasma for 4 h. All AVP analogues stimulated significant ($P<0.001$) insulin release from BRIN-BD11 beta-cells at both 5.6 and 16.7 mM glucose. Receptor selectivity experiments confirmed that Ac3IV was a V1a/V1b receptor specific peptide, and along with stability and *in vitro* bioactivity data this peptide was selected as the lead compound for further studies. Twice daily administration of Ac3IV or exendin-4 for 22 days reduced ($P<0.05$ to $P<0.001$) energy intake as well as body weight and fat content in HFF mice. Fluid intake was similar in all groups of HFF mice, confirming lack of activity of Ac3IV at V2 receptors. Both Ac3IV and exendin-4 decreased ($P<0.01$) circulating glucose levels, enhanced ($P<0.01$) insulin sensitivity and substantially improved ($P<0.05$-$P<0.01$) glucose tolerance and related insulin secretion in response to an intraperitoneal or oral glucose challenge. Only Ac3IV decreased ($P<0.05$-$P<0.001$) circulating total- and LDL-cholesterol, triacylglycerol and glucagon concentrations while also increasing ($P<0.01$) HDL-cholesterol, with exendin-4 improving only some of these parameters. Both treatments partially reversed ($P<0.05$-$P<0.001$) elevations of islet and beta cell areas and decreased ($P<0.01$) beta-cell apoptosis and, in the case of exendin-4, also decreased ($P<0.05$) alpha-cell apoptosis.

Conclusion: Sustained activation of V1a and V1b receptor pathways by Ac3IV exerts notable antidiabetic benefits in HFF mice, which were equivalent or superior to exendin-4. As such, future investigation into the therapeutic potential of AVP for the treatment of obesity and related diabetes is fully warranted.

Supported by: Ulster University VCRS

Disclosure: **C.R. Moffett:** None.

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DA-1241 a novel GPR119 agonist: Data on safety, tolerability and pharmacokinetics (PK), from part 1 of a phase 1b multiple ascending dose (MAD) study in healthy volunteers (HV)

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Background and aims: DA-1241 is a novel small molecule selective GPR119 agonist. In preclinical studies DA-1241 enhanced insulin and GLP-1 secretion, reduced glucose excursions, lowered blood glucose and improved dyslipidemia. The primary objective of Part 1 of this study was to assess the safety and tolerability of multiple once daily oral doses of DA-1241 compared to placebo in HV. Secondary objective was to estimate the plasma pharmacokinetic (PK) characteristics.

Materials and methods: Part 1 was a double blind placebo-controlled, single-center study of DA-1241 in HV. Three sequential cohorts of HV (n=8/cohort) were blinded and randomized (3:1) to receive DA-1241: 50, 100 or 200 milligram (mg; n=6/cohort) or placebo (n=2/cohort), as single daily oral doses for 28 days. Safety data reviews and dose escalation decisions between cohorts took place after all subjects of an ongoing cohort had completed procedures through day 14.

Results: Overall 24 male subjects participated in Part 1 (age 38.4 ± 10.7, BMI 26.0 ± 2.7). All doses tested were safe and well tolerated. There were no Serious Adverse Events (SAEs) and no discontinuations due to Adverse Events (AEs). All TEAE's were mild with no obvious dose relation and resolved spontaneously. The day 28 C_{max} and $AUC_{0\text{-}tau}$ PK parameters showed dose proportional characteristics across the tested dose range, with C_{max} (50mg: 464.9 ± 284.1; 100mg: 569.3 ± 173.2; 200mg: 1653.2 ± 615.4 ng/mL) and $AUC_{0\text{-}tau}$ (50mg: 5168.4 ± 1651.2; 100mg: 7620.0 ± 1837.7; 200mg: 19167 ± 9088.8 h*ng/mL). T_{max} was reached at 2.9 ± 1.8; 2.3 ± 1.2 and 1.9 ± 0.6 hours respectively with a $t_{1/2}$ of 590 ± 370; 368 ± 355 and 537 ± 395 hours, values for 50, 100, and 200 mg doses respectively.

Conclusion: Data from this phase 1b study in HV confirmed a favorable safety, tolerability and PK profile of DA-1241, and supported a progression of the clinical development program into patients.

Clinical Trial Registration Number: NCT03646721

Disclosure: **B. Franey:** Employment/Consultancy; ProSciento.

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A novel, long-acting dual agonist for GIPR/GLP-1R, HISHS-2001, demonstrates effects on HbA_{1c} and weight loss in the db/db mouse model of type 2 diabetes

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Background and aims: HISHS-2001 is a novel long-acting, glucose-dependent insulinotropic polypeptide (*GIP*) and glucagon-like peptide-1 (GLP-1) dual receptor (R) agonist.

Materials and methods: cAMP *in vitro* assay was performed in human GIP/GLP-1 receptor-expressing cells. Type-2 diabetes db/db mouse model was used for *in vivo* experiment.

Results: In *in vitro* cAMP assays in human GIP/GLP-1 receptor-expressing cells, HISHS-2001 exerted half-maximal effects at concentrations of 2.3 nM and 4.1 nM on the receptors for GIP (GIPR) and GLP-1 (GLP-1R) respectively. After SC dosing every third day for four weeks, HISHS-2001 at 3, 10 and 21 nmol/kg demonstrated robust effects on HbA1c in *db/db* mice which were superior to semaglutide and tirzepatide (Table). The effect on body weight was more pronounced with HISHS-2001 than semaglutide and similar to tirzepatide which was associated with a reduction in food intake. Interestingly, HISHS-2001 led to a significant increase in uncoupling protein-1 (UCP-1) levels in intrascapular brown adipose tissue, in inguinal white adipose tissue and serum and liver fibroblast growth factor-21 (FGF-21) levels. A decrease in serum interleukin-6 (IL-6) level was also observed.

Conclusion: HISHS-2001 is a potent, long acting GIP/GLP-1R dual agonist, which provides improved control of glucose homeostasis in diabetic mice compared to an existing GLP-1 agonist and a GIP/GLP-1R dual agonist in development.

Table: Changes in HbA1c, Body weight, UCP-1, FGF-21 and IL-6

(n=8)	Δ%HbA1c Reduction vs. Diabetic Control Day 28	Body Weight Change (%) Baseline vs. Day 28 Mean ± SD	UCP-1 Intrascapular Brown Adipose tissue (ng/g) Day 28 Mean ± SD	UCP-1 Inguinal White adipose tissue (ng/g) Day 28 Mean ± SD	FGF-21 Serum (ng/mL) Day 28 Mean ± SD	FGF-21 Liver (ng/g) Day 28 Mean ± SD	IL-6 Serum (pg/mL) Day 28 Mean ± SD
Diabetic Control	0	3.9 ± 3.1	14.8 ± 3.9	3.6 ± 1.7	1.10 ± 0.44	7.2 ± 0.9	28.5 ± 12.4
HISHS-2001							
3nmol/kg (14.3 µg/kg)	-3.11***	-6.8 ± 2.4***	30.6 ± 3.6***	7.5 ± 0.8**	2.7 ± 0.90	16.0 ± 2.4**	13.1 ± 4.2**
10nmol/kg (47.5 µg/kg)	-3.76***##$$	-8.5 ± 4.2***#	35.3 ± 5.4***	9.9 ± 1.7***	3.02 ± 1.40*	20.9 ± 3.5***	12.3 ± 1.2**
21nmol/kg (99.8 µg/kg)	-3.21***	-8.9 ± 1.7***##	40.8 ± 3.1***##	10.8 ± 0.8***##	3.23 ± 0.72**	21.1 ± 5.1***	9.9 ± 1.4***
Semaglutide							
21nmol/kg (86.4 µg/kg)	-2.75***	-3.4 ± 2.9**	29.3 ± 5.0***	7.1 ± 1.0***	2.56 ± 0.36	16.2 ± 3.2**	9.8 ± 6.4***
Tirzepatide							
21nmol/kg (101.4 µg/kg)	-2.89***	-6.3 ± 2.7**	34.6 ± 4.7***	9.5 ± 1.3***	2.75 ± 0.88	19.0 ± 3.6***	12.0 ± 1.0**

UCP-1: Uncoupling Protein-1; FGF-21: Fibroblast Growth Factor-21; IL-6: Interleukin-6
Data were analysed using One way ANOVA followed by Bonferroni's post test, where
*=p<0.05, **=p<0.01, ***=p<0.001 vs. Diabetic Control;
#=p<0.05, ##=p<0.01, ###=p<0.001 vs. semaglutide and
$=p<0.05, $$=p<0.01, $$$=p<0.001 vs. tirzepatide

Disclosure: **R. Thennati:** None.

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Effect of probenecid and cyclosporin on the pharmacokinetics of SNAC in healthy subjects

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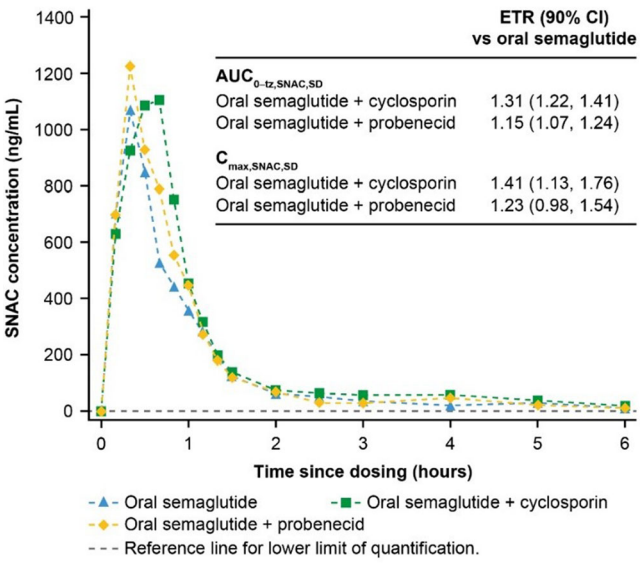
Background and aims: Oral semaglutide is a coformulation of semaglutide (a glucagon-like peptide-1 analogue) and sodium N-(8-[2-hydroxybenzoyl] amino) caprylate (SNAC; 300 mg) (an absorption enhancer). SNAC is partly metabolised by glucuronidation and is a substrate for specific organic anion transporters and other proteins.

Materials and methods: This single-centre, randomised, open-label, 3-period crossover trial assessed whether co-dosing with the perpetrator drugs probenecid (500 mg, twice daily to steady state) or cyclosporin (600 mg, single dose) affects the pharmacokinetics of SNAC and SNAC metabolites in healthy subjects (N=21). Primary endpoints were the area under the SNAC plasma concentration-time curve from time 0 to time of last quantifiable concentration ($AUC_{0\text{-}tz,SNAC,SD}$) and the maximum SNAC plasma concentration ($C_{max,SNAC,SD}$) after a single dose of oral semaglutide 3 mg.

Results: For each endpoint (N=21 analysed), the 90% CIs for the estimated treatment ratios (oral semaglutide with probenecid or cyclosporin vs oral semaglutide alone) fell within the pre-specified 'no-effect' interval of 0.50-2.00 (Figure). Estimated treatment ratios vs oral semaglutide alone for $AUC_{0\text{-}tz,SNAC,SD}$ were 1.15 (90% CI: 1.07, 1.24) for oral semaglutide plus probenecid and 1.31 (90% CI: 1.22, 1.41) for oral semaglutide plus cyclosporin. Estimated treatment ratios for $C_{max,SNAC,SD}$ were 1.23 (90% CI: 0.98, 1.54) and 1.41 (90% CI: 1.13, 1.76), respectively. At 24 hours, SNAC (Figure) and SNAC metabolites were at, or close to, the lower limit of quantification. There were 40 adverse events reported in 13 subjects (61.9%) during the trial, with no unexpected events. Most adverse events were mild, and no serious adverse events and no withdrawals were reported.

Conclusion: Once-daily oral semaglutide can be dosed with cyclosporin and probenecid with no clinically relevant effects on exposure of SNAC or SNAC metabolites, and with no unexpected safety findings.

Figure. Mean plasma concentration of SNAC from 0–6 hours and estimated treatment ratios (90% CI) for $AUC_{0-tz,SNAC,SD}$ and $C_{max,SNAC,SD}$.



$AUC_{0-tz,SNAC,SD}$, area under the SNAC plasma concentration–time curve from time 0 to time of last quantifiable concentration; $C_{max,SNAC,SD}$, maximum SNAC plasma concentration; ETR, estimated treatment ratio; SNAC, sodium N-(8-[2-hydroxybenzoyl] amino) caprylate.

Clinical Trial Registration Number: NCT03466567

Supported by: Novo Nordisk A/S

Disclosure: **T.K. Thorning:** Employment/Consultancy; Novo Nordisk A/S.

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Effects of anti-diabetes therapies on irisin secretion in type 2 diabetes patients

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Background and aims: Irisin is a hormone secreted by skeletal muscle following physical activity or excess of saturated fatty acids, able to improve metabolic homeostasis and promote energy expenditure. Serum irisin levels are reduced in type 2 diabetes (T2D), while exogenous irisin administration improves glycemic control in diabetic mice. Indeed, in a previous study, we have demonstrated that irisin promotes beta-cell function and viability. This study investigated the changes in serum irisin levels in T2D patients according to their anti-diabetes treatment.

Materials and methods: 127 T2D patients (aged 18 to 70 years) were enrolled and stratified by anti-diabetes therapy: only diet (17), metformin (met, 37), and met plus sulfonylureas (5), or pioglitazone (7), or GLP-1 receptor agonists (GLP-1RAs, 31), or DPP-4 inhibitors (DPP-4i, 15), or SGLT2 inhibitors (SGLT2i, 15). The control group included 36 sex-, and body mass index (BMI)-matched subjects without diabetes. In addition, human skeletal muscle cells were treated *in vitro* with exendin-4 (1-100 nM) for different times (8-24h). Irisin levels in patient serum or in skeletal muscle cell culture medium were measured using a specific ELISA assay.

Results: T2D patients showed lower irisin levels than the control group (21.5 [10.1-50.8] vs 29.1 [14.1-43.5] ng/mL, P<0.01). Serum irisin levels were positively associated with diabetes duration (r=0.214, P<0.05), and negatively associated with total (r=-0.196, P<0.05) and LDL cholesterol (r=-0.214, P<0.05), in patients with T2D. Patients treated with met plus GLP-1RAs or DPP-4i showed increased serum irisin levels (28.1 [12.4-50.8] and 25.8 [13.2-37.8] ng/mL, respectively) compared to patients treated with diet or met (19.8 [13.5-40.5] and 16.9 [10.7-28.8] ng/ml, respectively; P<0.05). Interestingly, human skeletal muscle cells treated *in vitro* with exendin-4 released more irisin in the culture medium compared to untreated control cells.

Conclusion: In conclusion, treatment with met plus GLP-1RAs or DPP-4i significantly increased irisin serum concentration to levels comparable to those of non-diabetic subjects. This effect could be due to a direct stimulation of skeletal muscle by GLP-1RAs, suggesting a new possible mechanism underlying their beneficial effects.

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Disclosure: **A. Natalicchio:** None.

SO 30 The advantage of dual agonists

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Effect of tirzepatide versus insulin degludec on glycaemic control captured with continuous glucose monitoring in patients with type 2 diabetes (SURPASS-3 CGM)

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Background and aims: Tirzepatide (TZP) is a novel dual GIP/GLP-1 receptor agonist in development for type 2 diabetes (T2D) treatment. The purpose of this study was to compare the percentage of time in the euglycemic range and to evaluate the glucose variability captured with continuous glucose monitoring (CGM) over 24 hours for TZP versus insulin degludec (IDeg) in insulin-naïve patients with T2D inadequately controlled on metformin with/without SGLT-2i.

Materials and methods: In this sub-study of the 52-week, open-label, parallel-arm, Phase 3, SURPASS-3 trial, a subset of 243 randomized patients who received at least 1 dose of study drug and had CGM data were included. Patients were randomized 1:1:1:1 to receive once-weekly TZP [5, 10, 15 mg] or IDeg once daily. Interstitial glucose values were collected by CGM at 5-minute intervals for approximately 7 days at baseline, 24 weeks, and 52 weeks. The primary objective was to compare TZP versus IDeg for the percentage of time CGM glucose values were within euglycemic range (3.9 to 7.8 mmol/L) during a 24-hour period at 52 weeks. Secondary objectives included comparing TZP versus IDeg for the percentage of time spent in euglycemic range at 24 weeks.

Results: In the TZP (5 mg N=64, 10 mg N=51, 15 mg N=73) and IDeg (N=55) groups, the baseline glycated haemoglobin (HbA1c) concentrations were 8.17% (65.8 mmol/mol), 7.92% (63.1 mmol/mol), 8.31% (67.3 mmol/mol), and 8.09% (64.9 mmol/mol) (p=0.158), respectively, and the fasting serum glucose concentration was 9.73, 9.40, 9.53, and 8.84 mmol/L, respectively (p=0.300). Pooled TZP 10 mg and 15 mg significantly increased the percentage of time CGM glucose values were within euglycemic range (3.9 to 7.8 mmol/L) compared to IDeg at 52 weeks (72.60 ± 2.45% vs 48.04 ± 3.74% p<0.001). All doses of TZP significantly increased the percentage of euglycemic time in range compared to IDeg at 52 weeks (Table). All doses of TZP significantly reduced the percentage of time CGM glucose values were ≤3.9 mmol/L compared to IDeg at 52 weeks (Table). All doses of TZP significantly reduced the Within-day Coefficient of Variation compared to IDeg at 52 weeks (Table).

Conclusion: Individuals with T2D treated with TZP spent significantly more time in euglycemic range in comparison to insulin degludec without increasing hypoglycemia. Additionally, TZP-treated participants had improved glycemic variability.

	TZP 5 mg	TZP 10 mg	TZP 15 mg	IDeg
Percent Euglycemic TIR 3.9-7.8 mmol/L				
Baseline	22.7 (2.97)	25.5 (3.42)	21.1 (2.77)	22.2 (3.22)
24 weeks	59.9 (3.48)	70.8 (4.07)**	72.7 (3.33)**	51.6 (3.91)
52 weeks	59.6 (3.59)*	72.4 (3.97)**	72.6 (3.50)**	48.0 (4.01)
Percent TIR 3.9-10.0 mmol/L				
Baseline	51.9 (3.88)	60.7 (4.47)	49.4 (3.62)	53.9 (4.20)
52 weeks	84.9 (2.54)*	91.0 (2.78)**	91.2 (2.49)**	75.0 (2.84)
Percentage of time >10.0 mmol/L at 52 weeks	14.9 (2.60)	8.2 (2.85)**	8.5 (2.54)**	22.5 (2.90)
Percentage of time ≤3.9 mmol/L at 52 weeks	0.6 (0.18)**	1.0 (0.25)*	0.8 (0.20)**	2.4 (0.42)
Incidence of hypoglycemia BG ≤3.9 mmol/L (midnight to 0600) at 52 weeks	15.3 (5.10)*	21.2 (6.36)	22.0 (5.96)	38.3 (8.23)
Within-day Coefficient of Variation at 52 weeks	18.6 (0.55)**	16.2 (0.60)**	16.1 (0.54)**	24.4 (0.61)

Unless otherwise stated, a constrained longitudinal data analysis model (cLDA) was used. Baseline and post-baseline CGM measures were considered as dependent variables, in conjunction with the constraint of a common baseline mean across the treatment groups. Percentage of time ≤3.9 mmol/L at 52 weeks used a constrained tobit mixed-effects model. Values are over a 24-hour period.
Data are least square mean (SE) actual values, unless otherwise stated.
TZP doses were achieved through stepwise 2.5-mg dose escalation every 4 weeks. IDeg was titrated to a FSG <5 mmol/L following a treat-to-target algorithm. Mean IDeg dose at 52 weeks was 0.6 U/kg/day.
*p<0.05 and **p<0.001 vs insulin degludec.
Subjects were included in the analysis of actual values if they had at least one non-missing value at either baseline or post-baseline.
Percent TIR was calculated as the number of observations within the specified range divided by the total number of observations in the time interval.
BG = blood glucose; IDeg = insulin degludec; N = number of patients; TIR = time in range; TZP = Tirzepatide

Clinical Trial Registration Number: NCT03882970

Supported by: Eli Lilly and Company

Disclosure: **T. Battelino:** Grants; TB's institution has received grant support & travel expenses from NIH-NIDDK, EC/IMI, Slovenian Research Agency, Abbott Diabetes Care, Medtronic, Novo Nordisk, Sanofi, Sandoz, Novartis, & Zealand. Honorarium; TB received honoraria for participation on advisory boards & as a speaker for Novo Nordisk, Sanofi, Eli Lilly & Company, Boehringer, Medtronic, Indigo, AstraZeneca, & Roche. Stock/Shareholding; TB owns stocks of DreaMed Diabetes.

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Patient-reported outcomes in patients with type 2 diabetes treated with tirzepatide or placebo as an add-on to basal insulin (SURPASS-5)

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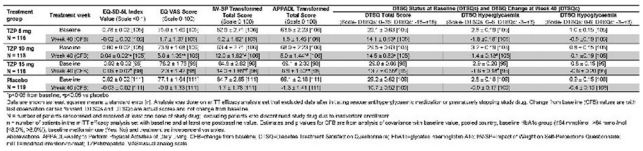
Background and aims: Tirzepatide (TZP), a novel dual glucose-dependent insulinotropic polypeptide/glucagon-like peptide-1 (GIP/GLP-1) receptor agonist in development for treatment of type 2 diabetes (T2D), has shown clinically meaningful glycemic control improvements and bodyweight (BW) loss in patients with T2D inadequately controlled with insulin glargine with or without metformin in SURPASS-5 study. Additionally, we evaluated the effect of TZP treatment vs placebo (PBO) when added to titrated insulin glargine in patient-reported outcomes (PROs) measuring health status, self-perceptions impacted by BW, treatment satisfaction, and ability to perform activities of daily living.

Materials and methods: Patients were randomised (1:1:1:1) to once weekly TZP 5, 10, 15 mg, or PBO. PRO measures assessed at baseline and week 40 were: EQ-5D-5L, Impact of Weight on Self-Perceptions Questionnaire (IW-SP), Diabetes Treatment Satisfaction Questionnaire (DTSQ), and Ability to Perform Physical Activities of Daily Living (APPADL). Higher PRO scores indicate better outcomes except for DTSQ Hyperglycaemia and Hypoglycaemia scores where lower scores indicate better outcomes.

Results: TZP 10 mg and 15 mg groups significantly improved EQ-5D-5L, IW-SP, and APPADL scores at week 40 vs PBO (p<0.05), except there was no statistically significant difference between the TZP 15 mg dose group and PBO in EQ VAS score (Table). All 3 TZP doses had a significantly higher total DTSQ change scores vs PBO (p<0.05), indicating greater improvement in satisfaction as compared with PBO. There was significant difference for each of the 3 TZP doses vs PBO (p<0.05) in self-reported DTSQ perceived hyperglycaemia change score, but none in the self-reported DTSQ perceived hypoglycaemia change score.

Conclusion: Improvements in EQ-5D-5L, IW-SP, and APPADL scores indicate that patients' overall assessment of their health, BW-related self-perception, and physical well-being improved with TZP 10 and 15 mg compared with PBO. Patients reported improved treatment satisfaction and lower perceived frequency of hyperglycaemia with all 3 TZP doses relative to PBO. The addition of TZP to treatment did not change patient self-reported perception of hypoglycaemia compared to placebo. Thus, there was a positive overall health-related quality of life trend with the addition of TZP to existing insulin glargine treatment, including those related to activities of daily living.



Clinical Trial Registration Number: NCT04039503

Supported by: Eli Lilly and Company

Disclosure: **M. Yu:** Employment/Consultancy; Employee of Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

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Efficacy and safety of tirzepatide, a dual GIP/GLP-1 receptor agonist, compared to insulin degludec in patients with type 2 diabetes (SURPASS-3)

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Background and aims: Tirzepatide (TZP) is a novel dual glucose-dependent insulinotropic polypeptide (GIP)/glucagon-like peptide-1 (GLP-1) receptor agonist under development for the treatment of type 2 diabetes (T2D). The efficacy and safety of TZP vs titrated insulin degludec (IDeg) were assessed in insulin-naive patients with T2D inadequately controlled on metformin with/without sodium-glucose co-transporter-2 inhibitors (SGLT-2i).

Materials and methods: In this open-label, 52-week, Phase 3 study, 1444 patients with T2D were randomised (1:1:1:1) to once-weekly TZP (5, 10, 15 mg) or once-daily IDeg (mean baseline [BL] age, 57.4 years; T2D duration, 8.4 years; HbA_{1c}, 65.78 mmol/mol (8.17%); BMI, 33.5 kg/m^2; 32% on SGLT-2i). The primary efficacy endpoint was mean change in HbA_{1c} from BL to Week 52. Secondary efficacy endpoints included mean change in fasting serum glucose (FSG) and body weight (BW) and proportion of subjects achieving HbA_{1c} and BW goals. Safety data included all data through safety follow-up from the modified intent-to-treat population.

Results: All TZP doses were superior to IDeg in mean change from BL in HbA_{1c} at Week 52. Least squares mean treatment difference values vs IDeg (95% CI) were -6.4 (-7.9, -4.9) mmol/mol (-0.59% [-0.73, -0.45]) for TZP 5 mg, -9.4 (-10.9, -7.9) mmol/mol (-0.86% [-1.00, -0.72]) for TZP 10 mg, and -11.3 (-12.8, -9.8) mmol/mol (-1.04% [-1.17, -0.90]) for TZP 15 mg ($p<0.001$ all doses). All TZP doses were also superior to IDeg in the proportion of subjects achieving HbA_{1c} <53 mmol/mol (7.0%) at Week 52 (Table). Among patients taking TZP, 25.8–48.4% achieved HbA_{1c} <39 mmol/mol (5.7%) vs 5.4% with IDeg. FSG was significantly reduced ($p<0.001$) from BL to Week 52 in all treatment arms and to a similar extent with TZP 10 and 15 mg vs IDeg (Table). All TZP doses decreased BW from BL to Week 52 while IDeg increased BW. Significantly larger proportion of patients ($p<0.001$) achieved BW loss goals in all TZP arms vs IDeg (Table). The most common adverse events in TZP-treated patients were mild to moderate gastrointestinal events. Higher incidence of nausea (11.5–23.7%), diarrhoea (15.4–16.7%), decreased appetite (6.1–12.0%), and vomiting (5.9–10.0%) was reported in patients treated with TZP vs IDeg (1.7%, 3.9%, 0.6%, and 1.1%, respectively). Hypoglycaemia incidence (<3.0 mmol/l or severe) was lower in all TZP arms (1.11–2.23%) vs IDeg (7.26%). One patient in the TZP 15 mg group had one episode of severe hypoglycaemia while receiving 2.5 mg at Day 28.

Conclusion: In patients with T2D, TZP demonstrated clinically meaningful reductions in HbA_{1c} and BW that were significantly greater vs titrated IDeg at Week 52. TZP was associated with lower incidence of hypoglycaemia.

Primary and Secondary Endpoints[a], Week 52	TZP 5 mg, N=358	TZP 10 mg, N=360	TZP 15 mg, N=359	IDeg, N=360
% of Subjects Completing the Treatment, n (%)	316 (88.0)	294 (81.4)	300 (83.6)	320 (87.7)
Baseline HbA_{1c}, mmol/mol	65.8 (0.53)	66.0 (0.53)	66.3 (0.53)	65.4 (0.53)
Baseline HbA_{1c}, %	8.17 (0.049)	8.19 (0.049)	8.21 (0.049)	8.13 (0.049)
Change from Baseline in HbA_{1c}[b], mmol/mol	-21.1 (0.54)**	-24.0 (0.55)**	-26.0 (0.55)**	-14.6 (0.54)
Change from Baseline in HbA_{1c}[b], %	-1.93 (0.050)**	-2.20 (0.051)**	-2.37 (0.050)**	-1.34 (0.049)
% of Subjects Achieving HbA_{1c} <53 mmol/mol (7.0%)[b]/<39 mmol/mol (5.7%)	82.4**/25.8**	89.7**/38.6**	92.6**/48.4**	61.3/5.4
Baseline FSG, mmol/l	9.54 (0.135)	9.48 (0.135)	9.35 (0.135)	9.24 (0.136)
Change from Baseline in FSG, mmol/l	-2.68 (0.101)*	-3.04 (0.103)	-3.29 (0.103)	-3.09 (0.100)
Baseline BW, kg	94.5 (1.07)	94.3 (1.08)	94.9 (1.07)	94.2 (1.08)
Change from Baseline in BW[b], kg	-7.5 (0.37)**	-10.7 (0.37)**	-12.9 (0.37)**	2.3 (0.37)
% of Subjects Achieving BW Loss ≥5%/≥10%/≥15%	66.0**/37.4**/12.5**	83.7**/55.7**/28.3**	87.8**/69.4**/42.5**	8.3/2.9/0.0

Data presented for key efficacy variables are model estimates (estimate [SE] at baseline and change from baseline at Week 52, unless otherwise noted). All changes from baseline at Week 52 are significant ($p<0.001$) vs baseline values. *$p<0.01$ and **$p<0.001$ vs IDeg. TZP doses were achieved through stepwise 2.5-mg dose escalation every 4 weeks. IDeg starting dose was 10 U/day and was titrated to a FSG <5.0 mmol/l following a treat-to-target algorithm. Mean IDeg dose at Week 52 was 48.8 U/day. Note: for HbA_{1c} and BW loss goals, missing values at endpoint were predicted from MMRM analysis using mITT efficacy analysis set. The percentages are based on average number of subjects achieving target in imputed data for missing values.
[a]mITT-Efficacy Analysis Set: on treatment data prior to initiating rescue therapy from mITT population excluding patients who discontinued study drug due to inadvertent enrolment.
[b]Tested for superiority, controlled for Type 1 error.
BW=body weight; FSG=fasting serum glucose; HbA_{1c}=haemoglobin A_{1c}; IDeg=insulin degludec; mITT=modified intent-to-treat (all randomised participants who took at least 1 dose of study drug); MMRM=mixed model repeated measures; N=number of patients in specified dataset; n=number of subjects with events meeting specified criteria; SE=standard error; TZP=tirzepatide.

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Supported by: Eli Lilly and Company

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Weekly dual GIP/GLP-1 receptor agonist tirzepatide monotherapy improved markers of islet cell function and insulin sensitivity in people with type 2 diabetes (SURPASS-1)

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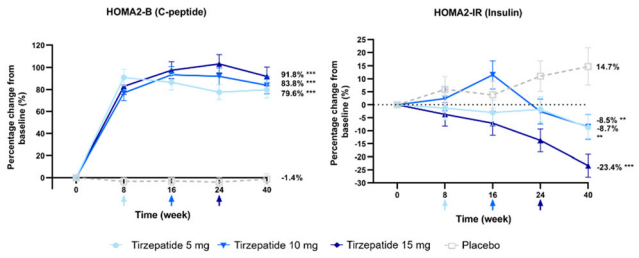
Background and aims: Tirzepatide, a novel dual GIP and GLP-1 receptor agonist, achieved significantly greater HbA1c and body weight reductions versus placebo in a 40-week, randomised, double-blind Phase 3 monotherapy trial of people with early type 2 diabetes. This analysis aimed to assess the changes in markers of islet cell function and insulin sensitivity with tirzepatide in the absence of concurrent use of oral antihyperglycemic medication (OAM).

Materials and methods: A total of 478 patients were randomised (mean baseline HbA1c 63 mmol/mol [7.94%], age 54.1 years; diabetes duration 4.7 years; no prior OAM use [treatment-naïve] 54%, body mass index 31.9 kg/m^2) and 475 were included in analyses. Once weekly tirzepatide doses were gradually escalated to 5, 10, or 15 mg. Fasting markers related to pancreatic beta and alpha cell function and insulin sensitivity were assessed at weeks 0, 8, 16, 24 and 40 by mixed model repeated measures in the randomised and treated population prior to rescue therapy initiation for hyperglycaemia or study drug discontinuation.

Results: Markers of beta and alpha cell function were improved with tirzepatide 5, 10, and 15 mg doses at 40 weeks. HOMA2-B indices, calculated with C-peptide, significantly increased by 80-92% with tirzepatide compared to a reduction of 1.4% with placebo. In addition, fasting glucagon levels, adjusted for fasting serum glucose, significantly decreased by 37-44% with tirzepatide compared to an increase of 5% with placebo. Significant increases in HOMA2-B and reductions in fasting glucagon levels observed with tirzepatide were achieved by week 8 and maintained by all doses of tirzepatide over the duration of the study. The early improvement in beta and alpha cell functions were accompanied by

similarly early improvement in fasting glucose levels, which decreased by 1.98 to 2.26 mol/L with tirzepatide at week 8 and were maintained over time compared to an increase by 0.67 mol/L with placebo. Tirzepatide improved insulin sensitivity as reflected by significant reductions of HOMA2-IR indices, calculated with insulin, of 9-23% with tirzepatide as compared to an increase of 15% with placebo. Fasting insulin levels were significantly reduced by 5% with 5 mg and by 12% with 15 mg tirzepatide compared to an increase of 15% with placebo. In the tirzepatide 15 mg group, progressive improvements in insulin sensitivity were observed over time throughout the entire study period as demonstrated by reductions in HOMA2-IR indices.

Conclusion: Monotherapy with the dual GIP and GLP-1 receptor agonist, tirzepatide significantly improved markers of pancreatic beta and alpha cell function and insulin sensitivity in people with early type 2 diabetes.



*Data are percentage change from baseline (Estimated Mean +/-SE) for HOMA2-IR and HOMA2-B from 0 to 40 weeks. Mixed model repeated measures analysis on log-transformed data then converted back to original scale. Arrows indicate maintenance dose reached and continued for 4 weeks (TZP 5mg,10mg, 15mg) **p<0.01 vs placebo, ***p<0.001 vs placebo.*

Clinical Trial Registration Number: NCT03954834

Supported by: Eli Lilly and Company

Disclosure: **C.J. Lee:** Employment/Consultancy; Employee of Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

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Efficacy and safety of once weekly tirzepatide, a dual GIP/GLP-1 receptor agonist versus placebo as monotherapy in people with type 2 diabetes (SURPASS-1)

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Background and aims: Tirzepatide (TZP) is a novel dual GIP/GLP-1 receptor agonist (RA) in development for type 2 diabetes (T2D). The efficacy and safety of TZP vs placebo (PBO) were assessed in people with T2D inadequately controlled with diet and exercise alone.

Materials and methods: In this double-blind, PBO-controlled, 40-wk Phase 3 study, people with T2D (N=478; mean baseline [BL] HbA1c 63 mmol/mol (7.94%); age 54.1 y; T2D duration 4.7 y; body mass index 31.9 kg/m^2) were randomised (1:1:1:1) to TZP (5, 10, 15 mg) or PBO. Primary efficacy measure was mean change in HbA_{1c} from BL at 40 wk. The secondary measures were mean change in fasting serum glucose (FSG) and body weight (BW) and the proportion achieving HbA1c targets and BW loss goals. HbA_{1c}, HbA_{1c} targets <7% and <5.7%, FSG and body weight were tested for superiority. Safety data included all data through safety follow-up from the mITT population (safety analysis set).

Results: At 40 weeks, all TZP doses were superior to PBO in mean HbA_{1c} change from baseline (Table). LSM treatment differences vs PBO (95% CI) were -20.8 mmol/mol (-23.9, -17.8) (-1.91% [-2.18, -1.63]) for TZP 5 mg, -21.1 mmol/mol (-24.1, -18.0) (-1.93% [-2.21, -1.65]) for TZP 10 mg, and -23.1 mmol/mol (-26.2, -20.0) (-2.11% [-2.39, -1.83]) for TZP 15 mg (p<0.001, all TZP doses). TZP 5, 10 and 15 mg were also superior to PBO in achieving HbA_{1c} targets <7% and <5.7%. Fasting serum glucose significantly decreased from baseline with TZP 5, 10 and 15 mg vs PBO (-3.13 mmol/L [-57 mg/dL], -3.26 mmol/L [-59 mg/dL], and -3.45 mmol/L [-62 mg/dL]; p<0.001, all TZP doses). Significant BW loss was achieved with TZP 5, 10 and 15 mg vs PBO (-6.3 kg [-7.8, -4.7], -7.1 kg [-8.6, -5.5], -8.8 kg [-10.3, -7.2]; p<0.001, all TZP doses). A greater proportion of patients achieved BW loss ≥5%, ≥10% and ≥15% with TZP vs PBO (p≤0.011, all TZP doses). TZP was well tolerated and the most common adverse events were gastrointestinal and mild to moderate in severity. Nausea, diarrhoea, decreased appetite, constipation and vomiting was reported in 12-18%, 12-14%, 4-8%, 5-7% and 3-6% of patients treated with TZP doses vs 6%, 8%, 1%, 1% and 2% with PBO, respectively. There was no severe or clinically significant (blood glucose [BG] <54 mg/dL) hypoglycaemia with TZP.

Conclusion: TZP as a first-in class dual GIP/GLP-1 RA for T2D monotherapy demonstrated robust clinically meaningful reductions in HbA_{1c} and BW without increased risk of severe or clinically significant (BG <54 mg/dL) hypoglycaemia and safety profile similar to GLP-1 RAs. Among those taking TZP 15 mg, 52% achieved normoglycaemia (HbA_{1c} <5.7%) and 27% achieved ≥15% BW loss.

Primary and Secondary Endpoints[1], Week 40	TZP 5 mg, (N=121)	TZP 10 mg, (N=121)	TZP 15 mg, (N=120)	PBO, (N=113)
Baseline HbA_{1c}, mmol/mol	63.6 ± 0.86	62.6 ± 0.87	62.6 ± 0.88	64.8 ± 0.89
Change from baseline in HbA_{1c}[2], mmol/mol	-20.4 ± 1.03	-20.7 ± 1.05	-22.7 ± 1.07	0.4 ± 1.15
Baseline HbA_{1c}, %	7.97 ± 0.08	7.88 ± 0.08	7.88 ± 0.08	8.08 ± 0.08
Change from baseline in HbA_{1c}[2], %	-1.87 ± 0.094	-1.89 ± 0.096	-2.07 ± 0.098	0.04 ± 0.105
% of patients achieving HbA_{1c} <5.7 mmol/mol (7.0%)[3] / ≤48 mmol/mol (6.5%) / <39 mmol/mol (5.7%)[3]	37**[3] / 83**[3] / 34**	92**[3] / 81**[3] / 31**	88**[3] / 86**[3] / 32**	20[3] / 10[3] / 1
Baseline fasting serum glucose, mmol/L	8.53 ± 0.203	8.47 ± 0.205	8.58 ± 0.207	8.61 ± 0.211
Change from baseline in fasting serum glucose[2], mmol/L	-2.42 ± 0.189	-2.55 ± 0.192	-2.73 ± 0.201	0.71 ± 0.222
Baseline body weight, kg	87.0 ± 1.79	85.7 ± 1.81	85.9 ± 1.83	84.4 ± 1.86
Change from baseline in body weight[2], kg	-7.0 ± 0.52	-7.8 ± 0.53	-9.1 ± 0.54	-0.7 ± 0.57
% of patients achieving body weight loss ≥5% / ≥10% / ≥15%	67** / 31** / 13*	78** / 60** / 37*	77** / 67** / 52*	34 / 1 / 0

*Data are LSM ± SE at baseline from ANCOVA and change from baseline at 40 weeks from MMRM analysis, unless otherwise noted. Primary and other secondary endpoint data are from the efficacy analysis set (mITT on treatment without rescue therapy and excluding patients who discontinued study drug due to inadvertent enrollment). Note: proportion of patients achieving HbA_{1c} and weight loss goals was obtained by dividing the number of patients reaching respective goals at Week 40 by the number of patients with baseline value and at least one non-missing postbaseline value. Missing value at Week 40 was predicted from MMRM analysis. *p<0.05 and **p<0.001 vs placebo.*

[1]mITT-Efficacy Analysis Set: on treatment data prior to initiating rescue therapy from mITT population excluding patients who discontinued study drug due to inadvertent enrolment (efficacy-estimand).

[2]Tested for superiority, controlled for Type 1 error.

ANCOVA=analysis of covariance; HbA_{1c}=glycosylated haemoglobin; LSM=least squares mean; mITT=modified intent-to-treat (all randomised patients who took at least 1 dose of study drug); MMRM=mixed model repeated measures; N=number of patients in specified dataset; n=number of subjects with events meeting specified criteria; SE=standard error.

Clinical Trial Registration Number: NCT03954834

Supported by: Eli Lilly and Company

Disclosure: **J. Rosenstock:** Grants; Applied Therapeutics, Merck, Pfizer, Sanofi, Novo Nordisk, Eli Lilly and Company, GlaxoSmithKline, Genentech, Hanmi, Oramed, Janssen, Lexicon, Boehringer Ingelheim, Intarcia. Honorarium; Applied Therapeutics, Eli Lilly and Company, Sanofi, Novo Nordisk, Hanmi, Oramed, Boehringer Ingelheim, Intarcia. Other; Applied Therapeutics, Merck, Pfizer, Sanofi, Novo Nordisk, Eli Lilly and Company, GlaxoSmithKline, Genentech, Hanmi, Oramed, Janssen, Lexicon, Boehringer Ingelheim, Intarcia.

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Patient-reported outcomes in patients with type 2 diabetes treated with tirzepatide or placebo (SURPASS-1)

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Background and aims: Tirzepatide (TZP), a dual GIP/GLP-1 receptor agonist (RA) for type 2 diabetes (T2D), has shown clinically meaningful glycaemic control improvements and bodyweight (BW) loss in patients with T2D inadequately controlled with diet and exercise alone. We evaluated the effect of TZP treatment vs placebo (PBO) in patient-reported outcomes (PROs) measuring health status, self-perceptions impacted by BW, and ability to perform activities of daily living.

Materials and methods: Patients were randomized (1:1:1:1) to once weekly TZP 5, 10, 15 mg, and PBO. PRO measures assessed at baseline and week 40 were: EQ-5D-5L, Impact of Weight on Self-Perceptions Questionnaire (IW-SP), and Ability to Perform Physical Activities of Daily Living (APPADL). Higher PRO scores indicate better outcomes.

Results: Scores for all PRO measures improved significantly from baseline at 40 week for all TZP doses (p<0.05) and only IW-SP for PBO. TZP 10 mg and 15 mg groups significantly improved in EQ VAS and IW-SP scores at week 40 vs PBO (p<0.05). There were no statistically significant differences between the TZP doses and placebo in EQ-5D-5L index and APPADL scores.

Conclusion: Improvements in EQ VAS and IW-SP scores indicate that patients' overall assessment of their health and BW-related self-perception improved with TZP compared with PBO. These PROs may help clinicians understand patient perspectives regarding their quality of life after starting tirzepatide treatment.

Table: Patient-Reported Outcome Measures for SURPASS-1 Study

Treatment group	Treatment week	EQ-5D-5L Index Value (Scale <0-1)	EQ VAS Score (Scale 0-100)	IW-SP Transformed Total Score (Scale 0-100)	APPADL Transformed Total Score (Scale 0-100)
TZP 5 mg	Baseline	0.84 ± 0.017 [108]	80.4 ± 1.29 [108]	65.7 ± 2.57 [108]	70.7 ± 1.95 [108]
N = 121	CFB at week 40	0.03 ± 0.013* [108]	4.2 ± 1.01**# [108]	10.5 ± 1.77** [108]	4.5 ± 1.31** [108]
TZP 10 mg	Baseline	0.88 ± 0.017 [104]	82.8 ± 1.32 [104]	67.6 ± 2.62 [104]	79.4 ± 1.99 [104]
N = 121	CFB at week 40	0.03 ± 0.013* [104]	5.3 ± 1.03**# [104]	14.5 ± 1.81**# [104]	4.8 ± 1.32** [104]
TZP 15 mg	Baseline	0.88 ± 0.018 [93]	83.8 ± 1.39 [93]	68.2 ± 2.76 [94]	79.7 ± 2.09 [94]
N = 120	CFB at week 40	0.04 ± 0.014* [93]	6.4 ± 1.09**# [93]	13.8 ± 1.90**# [94]	5.9 ± 1.39** [94]
Placebo	Baseline	0.87 ± 0.021 [70]	83.4 ± 1.61 [70]	67.5 ± 3.17 [71]	77.1 ± 2.41 [71]
N = 113	CFB at week 40	0.00 ± 0.016 [70]	0.2 ± 1.25 [70]	5.9 ± 2.19* [71]	1.8 ± 1.60 [71]

*p<0.05 from baseline; **p<0.001 from baseline; #p<0.05 vs placebo.
Data are shown as least squared means ± standard error [n]. Change from baseline (CFB) values are with last observation carried forward.
N = number of patients randomized and received at least one dose of study drug; excluding patients who discontinued study drug due to inadvertent enrolment and data after initiating rescue antihyperglycaemic medication or prematurely stopping study drug (mITT efficacy analysis set).
n = number of patients in the mITT efficacy analysis set with baseline and at least one postbaseline value.
7%, 3.3%, 1.7%, and 25.2% of patients in the TZP 5, 10, 15 mg and placebo arm, respectively, initiated rescue therapy postbaseline.
Estimates and p-values for CFB are from analysis of covariance with baseline value, country, baseline HbA1c Group (≤8.5% [69 mmol/mol], >8.5% [69 mmol/mol]) and prior use of oral antihyperglycaemic medication (Yes, No) as independent variables
Abbreviations: APPADL=Ability to Perform Physical Activities of Daily Living; CFB=change from baseline; HbA1c=glycated haemoglobin A1c; IW-SP=Impact of Weight on Self-Perceptions Questionnaire; TZP=Tirzepatide; mITT=modified intention-to-treat; VAS=visual analog scale.

Clinical Trial Registration Number: NCT03954834

Supported by: Eli Lilly and Company

Disclosure: **K. Boye:** Employment/Consultancy; Employee of Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

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Efficacy, safety and tolerability of cotadutide as an add-on therapy in overweight subjects with type 2 diabetes treated with dapagliflozin and metformin

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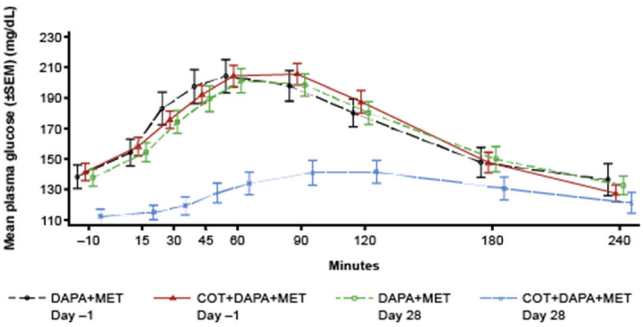
Background and aims: Cotadutide (COT) is a glucagon-like peptide 1 (GLP-1)/glucagon receptor dual agonist in development for the treatment of non-alcoholic steatohepatitis and type 2 diabetes (T2D) with diabetic kidney disease. We evaluated the efficacy and safety of COT in overweight T2D subjects treated with dual therapy dapagliflozin (DAPA) and metformin (MET).

Materials and methods: In this exploratory, double-blind, phase 2a study (NCT03444584), 49 adult obese subjects with T2D (BMI 25-40 kg/m^2; HbA_{1c} 7.0-10%) treated with DAPA 10 mg and MET (>1 g) were randomized to once daily s.c. COT+DAPA+MET at a titrated target dose of COT 100 μg, 200 μg, 300 μg, or DAPA+MET only, for 28 days; up-titrated weekly from 100 μg to 300 μg. Primary endpoint was percent change in glucose $AUC_{0\text{-}4h}$ by standardised mixed meal tolerance test (MMTT) from baseline (BL) to treatment end. Safety, continuous glucose monitoring (CGM) and PK profile (COT and DAPA) (secondary endpoints), and fasting β-hydroxybutyrate (β-OHB) and weight change (exploratory endpoints) were assessed.

Results: 47 of 49 subjects completed therapy. A significantly greater reduction in MMTT plasma glucose $AUC_{0\text{-}4h}$ was seen from BL to Day 28 for COT+DAPA+MET versus DAPA+MET (least squares [LS] mean difference: -22.17%; p<0.0001) (**Figure**). Also, on Day 28, a significantly greater reduction from BL in 24-h CGM mean glucose was seen for COT+DAPA+MET compared with DAPA+MET (LS mean difference: -34.06 mg/dL; p=0.0001). Significant body weight reductions from BL to Day 29 were seen for COT+DAPA+MET versus DAPA+MET (LS mean difference: -2.13 kg; p=0.0002) and percent body weight (LS mean difference: -2.26%; p=0.0002). Treatment-related AEs occurred more often with COT+DAPA+MET than with DAPA+MET (40.0% vs 16.7%); most events were mild to moderate in severity. Nausea, emesis and constipation were the most common AEs with COT. No clinically relevant trends in laboratory safety, ECG and BP were seen. C_{max}, but not AUC, of DAPA was decreased by co-administration with COT. Fasting plasma β-OHB levels were similar by treatment; mean maximal fasting ketone concentrations with COT+DAPA+MET ranged from 0.31 to 0.39 mmol/L compared with 0.29 to 0.35 mmol/L for DAPA+MET (Day -1 to 28).

Conclusion: Treatment with COT+DAPA+MET over 28 days provided a marked reduction in fasting and post-prandial glucose, a significant improvement over DAPA+MET in 24-h glycaemic control as measured by CGM, and an acceptable tolerability and safety profile. A marginal impact on DAPA oral absorption was seen, likely related to delay in gastric emptying driven by GLP-1/glucagon pharmacology.

Mean Plasma Glucose Values during MMTT from Day -1 and Day 28



Clinical Trial Registration Number: NCT03444584

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Disclosure: **A. Flor:** Employment/Consultancy; Employee of AstraZeneca.

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Semaglutide 2.4 mg improves patient-reported outcome measures of physical functioning in adults with overweight or obesity and type 2 diabetes in the STEP 2 trial

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Background and aims: An analysis of the STEP 2 trial evaluated the effect of s.c. semaglutide 2.4 mg vs placebo on physical function in adults with overweight or obesity and type 2 diabetes.

Materials and methods: The STEP 2 trial was a clinical trial in adults with BMI ≥27 kg/m^2 and type 2 diabetes. Patients were randomised 1:1:1 to 68 weeks' once-weekly semaglutide 2.4 mg (N=404), 1.0 mg (N=403; data not shown), or placebo (N=403), plus lifestyle changes. Endpoints included change in physical function (baseline to week 68) using the SF-36v2® Health Survey acute version (SF-36) and Impact of Weight on Quality of Life-Lite Clinical Trials Version (IWQOL-Lite-CT), and achievement of meaningful within-person physical function improvements (≥3.7 points [SF-36]; ≥14.6 points [IWQOL-Lite-CT]). SF-36 scores are norm-based scores (transformed to a scale where the 2009 US general population has a mean of 50 and an SD of 10).

Results: Patients were 51% female, with a mean age of 55 years, body weight 99.8 kg and BMI 35.7 kg/m^2. Body weight change at week 68 with semaglutide 2.4 mg vs placebo was -9.6% vs -3.4%. Semaglutide significantly improved physical function vs placebo on SF-36 and IWQOL Lite CT (Table). SF-36 and IWQOL-Lite-CT physical function improvements correlated with weight loss during the trial. Proportions of patients with clinically meaningful physical function improvements were greater with semaglutide vs placebo (Table).

Conclusion: In adults with overweight or obesity and type 2 diabetes, semaglutide 2.4 mg improved physical function vs placebo and led to greater proportions of patients with clinically meaningful physical function changes in SF-36 and IWQOL-Lite-CT.

Table. Physical functioning results

ETD in SF-36 and IWQOL-Lite-CT physical functioning scores from baseline to week 68 (all patients)			
PRO	**Placebo baseline mean ± SD**	**Semaglutide 2.4 mg baseline mean ± SD**	**ETD [95% CI]; P-value**
SF-36 physical functioning	49.6 ± 8.3	49.2 ± 8.8	1.52 [0.44, 2.61]; 0.0061
IWQOL-Lite-CT physical function	69.2 ± 24.0	67.1 ± 25.2	4.83 [1.79, 7.86]; 0.0018
Proportions of patients achieving meaningful within-person improvements in physical functioning from baseline to week 68			
PRO responder threshold	**Placebo, %**	**Semaglutide 2.4 mg, %**	**OR [95% CI]; P-value**
SF-36 (≥3.7 points)	27.9	42.0	1.84 [1.30, 2.61]; 0.0006*
IWQOL-Lite-CT (≥14.6 points)	31.0	42.6	1.57 [1.12, 2.19]; 0.0089*

*Not adjusted for multiplicity as the endpoint was not included in the testing hierarchy in the study protocol.

ETD, estimated treatment difference; IWQOL-Lite-CT, Impact of Weight on Quality of Life-Lite Clinical Trials Version; PRO, patient-reported outcome; SF-36, SF-36v2® Health Survey acute version.

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Semaglutide 2.4 mg improves health-related quality of life in adults with overweight or obesity and type 2 diabetes in the STEP 2 trial

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Background and aims: Effects of s.c. semaglutide 2.4 mg vs placebo on health-related quality of life (HRQoL) were evaluated in the STEP 2 trial in adults with overweight or obesity and type 2 diabetes.

Materials and methods: STEP 2 was a randomised controlled trial in adults with BMI ≥27 kg/m^2 and type 2 diabetes. Patients were randomised 1:1:1 to 68 weeks' once-weekly semaglutide 2.4 mg (N=404), 1.0 mg (N=403; results not shown) or placebo (N=403), plus lifestyle intervention. Changes in scores at week 68, and achievement of clinically meaningful within-person improvements (see Table for meaningful change thresholds), in weight-related quality of life (Impact of Weight on Quality of Life-Lite Clinical Trials Version [IWQOL-Lite-CT]) and HRQoL (SF-36v2® Health Survey acute version [SF-36]) were assessed. For the SF-36, anchor-based, obesity-specific meaningful improvements have been established for the physical functioning domain; data for other domains/component summaries are not presented.

Results: Body weight change at week 68 with semaglutide 2.4 mg vs placebo was -9.6% vs -3.4%. Changes in IWQOL-Lite-CT scores (Physical Function, Physical, Psychosocial and Total) and SF-36 scores (Physical Functioning, General Health, Mental Health and Physical Component Summary) were consistently in favour of semaglutide vs placebo (Table). Observed changes in SF-36 for the domains role-physical, bodily pain, vitality, social functioning, role emotional and mental component summary were not significantly different between semaglutide vs placebo. The proportions of patients with clinically meaningful improvements were greater with semaglutide vs placebo for all IWQOL-Lite-CT scores and SF-36 physical functioning score (Table).

Conclusion: In adults with overweight or obesity and type 2 diabetes, semaglutide 2.4 mg improved both physical and mental health components vs placebo and led to greater proportions of patients with clinically meaningful changes in weight-related quality of life and physical functioning.

Table. A) ETD in SF-36 and IWQOL-Lite-CT scores from baseline (mean ± SD) to week 68 (all patients) B) Proportions of patients achieving meaningful improvements, baseline to week 68

IWQOL-Lite-CT	Placebo	Semaglutide 2.4 mg	ETD [95% CI]; P-value	PRO responder threshold	Placebo, %	Semaglutide 2.4 mg, %	OR [95% CI]; P-value
Physical function	89.2 ± 24.0	97.1 ± 25.2	4.8 [1.8, 7.9]; 0.0018	≥14.6 points	31.0	42.6	1.6 [1.1, 2.2]; 0.0089*
Physical	89.0 ± 22.6	96.4 ± 24.2	4.9 [2.0; 7.8]; 0.0010*	≥13.5 points	28.5	40.2	1.5 [1.1, 2.2]; 0.011**
Psychosocial	77.0 ± 19.8	74.8 ± 21.6	2.9 [0.7, 5.1]; 0.0094*	≥16.2 points	18.6	26.3	1.5 [1.0, 2.2]; 0.0786*
Total score	74.2 ± 19.2	71.9 ± 20.9	3.6 [1.2, 5.9]; 0.0031*	≥16.6 points	17.5	26.3	1.5 [1.0, 2.3]; 0.0484*
SF-36	**Placebo**	**Semaglutide 2.4 mg**	**ETD [95% CI]; P-value**	**PRO responder threshold**	**Placebo, %**	**Semaglutide 2.4 mg, %**	**OR [95% CI]; P-value**
Physical functioning	49.6 ± 8.3	49.2 ± 8.8	1.5 [0.4; 2.6]; 0.0061	≥3.7 points	27.9	42.0	1.8 [1.3, 2.6]; 0.0006*
General health	51.2 ± 7.8	51.2 ± 7.6	1.6 [0.5; 2.6]; 0.0033*				
Mental health	55.2 ± 5.7	54.9 ± 6.4	1.1 [0.0; 2.2]; 0.0460*				
Physical component summary	49.9 ± 8.0	49.8 ± 8.2	1.1 [0.1; 2.2]; 0.0359*				

*Not adjusted for multiplicity. ETD, estimated treatment difference; IWQOL-Lite-CT, Impact of Weight on Quality of Life-Lite Clinical Trials Version; PRO, patient-reported outcome; SF-36, SF-36v2® Health Survey acute version.

Clinical Trial Regiatration Number: NCT03552757

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Treatment with once-weekly semaglutide 2.4 mg improves cardiometabolic risk factors in adults with overweight or obesity and type 2 diabetes: STEP 2 post-hoc analysis

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Background and aims: This analysis evaluated the effect of semaglutide 2.4 mg vs placebo or semaglutide 1.0 mg on cardiometabolic risk in STEP 2.

Materials and methods: STEP 2 was a double-blind, placebo-controlled trial in which 1210 patients with overweight or obesity and type 2 diabetes were randomised 1:1:1 to receive 68 weeks' once-weekly semaglutide 2.4 mg, 1.0 mg or placebo, plus lifestyle intervention. Primary endpoints included percent change in body weight. Secondary endpoints included change in cardiometabolic risk factors. Results are presented regardless of treatment adherence or use of other anti-obesity therapies. The table presents baseline values as mean ± SD or geometric mean (coefficient of variation in %).

Results: Mean body weight at baseline was 99.9 kg, 99.0 kg and 100.5 kg in the semaglutide 2.4 mg, 1.0 mg and placebo groups, respectively. Mean body weight change from baseline to week 68 was −9.6% with semaglutide 2.4 mg vs −3.4% with placebo (estimated treatment difference [ETD]: −6.2%; 95% CI: −7.3, −5.2; p<0.0001) and -7.0% with semaglutide 1.0 mg (ETD: −2.7%; 95% CI: −3.7, −1.6; p<0.0001). Semaglutide 2.4 mg improved other cardiometabolic parameters vs placebo (Table): HbA_{1c}, waist circumference, systolic blood pressure, levels of triglycerides, C-reactive protein, fasting plasma glucose and fasting insulin (p<0.01 for all ETDs/estimated treatment ratios). Improvements were similar with semaglutide 1.0 mg except for change in body weight and waist circumference. In all patients, cardiovascular risk factors improved more in those losing ≥10% body weight than in those losing <10% body weight (data not shown).

Conclusion: Overall, in patients with overweight or obesity and type 2 diabetes, semaglutide 2.4 mg improved cardiometabolic risk vs placebo, indicating favourable effects of semaglutide 2.4 mg in addition to body weight loss. Higher weight loss was associated with greater improvements in cardiovascular risk factors.

Table: Changes in selected cardiometabolic risk parameters from baseline to week 68 (full analysis set)

	Semaglutide 2.4 mg (N=404)		Semaglutide 1.0 mg (N=403)		Placebo (N=403)		Semaglutide 2.4 mg vs placebo	Semaglutide 2.4 mg vs 1.0 mg
Parameter	Baseline	Change	Baseline	Change	Baseline	Change	ETD (95% CI)[a]	ETD (95% CI)[a]
HbA_{1c}, %	8.1 (0.8)	−1.00 %-points	8.1 (0.8)	−1.40 %-points	8.1 (0.8)	−0.37 %-points	−1.23 (−1.42, −1.05); p<0.0001	+0.10 (+0.01, 0.19); p=0.0218*
Waist circumference, cm	114.5 ± 14.3	−9.40	113.9 ± 14.0	−6.72	115.5 ± 13.9	−4.52	−4.88 (−5.97, −3.79); p<0.0001	−2.69 (−3.72, −1.65); p<0.0001*
Systolic blood pressure, mmHg	130 ± 13	−3.92	130 ± 14	−2.89	130 ± 13	−0.49	−3.43 (−5.57, −1.30); p=0.0016	−1.04 (−3.33, 1.24); p=0.3713*
Triglycerides, mg/dL[a]	154.90 (53.4)	−22%	160.83 (54.0)	−17%	159.48 (52.9)	−9%	−14% (−19%, −8%); p<0.0001*	−6% (−11%, 0%); p=0.0558*
Non-HDL cholesterol, mg/dL[a]	123.55 (31.1)	−5%	120.58 (32.5)	−5%	124.54 (31.1)	−2%	−3% (−7%, 1%); p=0.1068*	0% (−3%, 4%); p=0.8915*
LDL cholesterol, mg/dL[a]	90.10 (37.2)	0%	89.16 (40.7)	+1%	90.07 (37.8)	0%	0% (−4%, 5%); p=0.8749*	1% (−3%, 6%); p=0.6331*
CRP, mg/L[a]	3.48 (159.3)	−49%	3.36 (143.8)	−42%	3.36 (157.0)	−17%	−39% (−46%, −30%); p<0.0001*	−12% (−23%, 1%); p=0.0621*
Fasting plasma glucose, mg/dl	157.7 ± 40.5	-38.0	155.7 ± 41.5	-32.3	157.9 ± 42.1	-1.4	−36.6 (−43.2, −30.0); p<0.0001*	−5.7 (−12.4, 0.9); p=0.0853*

[a]Values in % are the ratio to baseline or estimated treatment ratio expressed as percent; *not adjusted for multiplicity. CRP, C-reactive protein; ETD, estimated treatment difference.

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Time spent in glycaemic control after initiating treatment with oral semaglutide vs empagliflozin: an exploratory analysis of the PIONEER 2 trial

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Background and aims: A standard objective in the management of type 2 diabetes is the achievement and maintenance of HbA_{1c} targets, but the duration of time that patients spend within glycaemic control targets has not been previously reported for oral semaglutide. In this exploratory analysis, the duration of time that patients were in glycaemic control (HbA_{1c} <7.0% [53 mmol/mol] and <6.5% [48 mmol/mol]) during the 52-week PIONEER 2 trial was assessed.

Materials and methods: Patients with uncontrolled type 2 diabetes (n=822; HbA_{1c} 7.0–10.5% [53-91 mmol/mol]) were randomised to oral semaglutide 14 mg once daily or empagliflozin 25 mg once daily. Both drugs underwent dose escalation, with oral semaglutide starting at 3 mg, increasing to 7 mg after 4 weeks and 14 mg after 8 weeks. Empagliflozin was initiated at 10 mg and escalated to 25 mg after 8 weeks. For this analysis, outcomes were evaluated using the on-treatment without rescue medication observation period, in all randomised patients.

Results: Baseline characteristics were similar between treatment arms. Mean baseline HbA_{1c} for both arms was 8.1% (65 mmol/mol). A greater proportion of patients receiving oral semaglutide vs empagliflozin

achieved HbA_{1c} <7.0% and <6.5% at any point during the study (HbA_{1c} <7.0%: 77.9% vs 60.5%; HbA_{1c} <6.5%: 54.0% vs 29.3%) (Table). Greater proportions of patients receiving oral semaglutide vs empagliflozin maintained HbA_{1c} <7.0% for ≥14 weeks, ≥26 weeks, and ≥38 weeks (Table). During treatment, the overall mean duration of time spent at HbA_{1c} <7.0% and <6.5% was 26.6 weeks and 16.2 weeks, respectively, for oral semaglutide, and 19.0 weeks and 6.6 weeks for empagliflozin (Table). The odds of patients achieving HbA_{1c} <7.0% at both week 26 and 52 were significantly greater with oral semaglutide vs empagliflozin (estimated odds ratio 4.12 [95% CI 2.94, 5.76]; p<0.0001) (Table).

Conclusion: Despite an 8-week dose-escalation schedule and a mean baseline HbA_{1c} of 8.1%, nearly half of patients receiving oral semaglutide achieved glycaemic control (HbA_{1c} <7.0%) for more than 70% of the 52-week treatment duration. These data suggest that patients spent more time in glycaemic control during treatment with oral semaglutide than with empagliflozin.

Table. Time spent in glycaemic control with oral semaglutide vs empagliflozin.

HbA_{1c} target		Oral sema 14 mg	Empa 25 mg
	Full analysis set	n=411	n=410
HbA_{1c} <7.0% (53 mmol/mol)	Patients spending any time in control	77.9%	60.5%
	In control for ≥14 wks	65.2%	47.6%
	In control for ≥26 wks	56.2%	37.6%
	In control for ≥38 wks	46.2%	27.6%
	Mean ± SD | median time in control, wks	26.6 ± 19.7 | 33.7	19.0 ± 19.9 | 10.9
	Odds of control at wks 26 and 52	**EOR 4.12 [95% CI 2.94, 5.76]; p<0.0001**	
HbA_{1c} <6.5% (48 mmol/mol)	Patients spending any time in control	54.0%	29.3%
	Mean | median time in control, wks	16.2 ± 18.2 | 6.2	6.6 ± 13.4 | 0.0

Empa, empagliflozin; EOR (trial product estimand), estimated odds ratio for oral sema vs empa; sema, semaglutide; wks, weeks.

Clinical Trial Registration Number: NCT02863328

Supported by: Novo Nordisk A/S

Disclosure: **F.K. Knop:** Employment/Consultancy; Carmot Therapeutics, Eli Lilly, Novo Nordisk (all consultancy only). Grants; AstraZeneca, Boehringer Ingelheim, Gubra, Novo Nordisk, Sanofi, Zealand Pharma. Honorarium; AstraZeneca, Boehringer Ingelheim, Carmot Therapeutics, Eli Lilly, Gubra, MSD/Merck, Mundipharma, Novo Nordisk, Sanofi, Zealand Pharma. Lecture/other fees; AstraZeneca, Boehringer Ingelheim, Eli Lilly, MSD/Merck, Mundipharma, Novo Nordisk, Sanofi. Non-financial support; AstraZeneca, Mundipharma, Novo Nordisk, Sanofi. Stock/Shareholding; Antag Therapeutics.

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Reduced glycaemic variability with once-weekly semaglutide vs active comparators in post hoc analysis of the SUSTAIN programme

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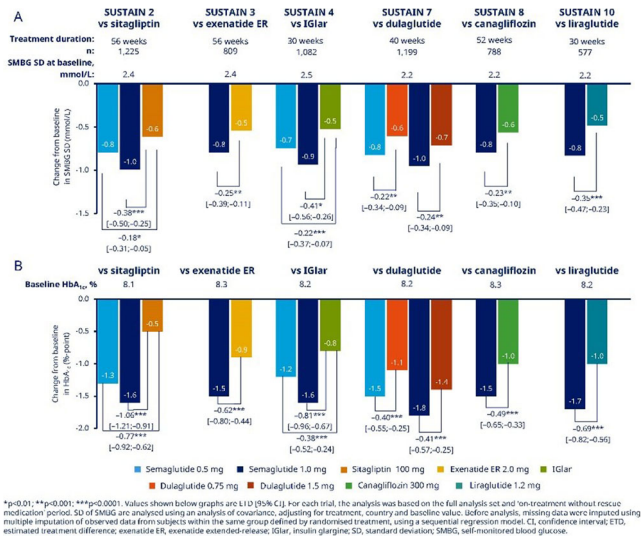
Background and aims: High glycaemic variability is independently associated with adverse clinical outcomes such as micro- or macrovascular complications. As glucose-dependent action of once-weekly semaglutide may translate to reduced glycaemic variability, we compared within-day glycaemic variability of once-weekly semaglutide vs active comparators (sitagliptin, exenatide extended release, insulin glargine, dulaglutide, canagliflozin and liraglutide) in people with type 2 diabetes (T2D).

Materials and methods: This *post hoc* analysis was based on individual subject data in the full analysis set from the on-treatment without rescue medication period in the SUSTAIN 2 (n=1,225), 3 (n=809), 4 (n=1,082), 7 (n=1,199), 8 (n=788) and 10 (n=577) trials. Glycaemic variability was assessed as change from baseline to end of treatment in standard deviation (SD) of the self-measured blood glucose (SMBG) over the day if measured ≥6 times, using an analysis of covariance, adjusting for treatment, country and baseline value. Before analysis, missing data were imputed using multiple imputation of observed data from subjects within the same group defined by randomised treatment, using a sequential regression model.

Results: The average baseline SD of the SMBGs was 2.2-2.5 mmol/L across all treatment arms in the trials. Significantly greater reductions in SD of SMBGs from baseline to end of treatment were observed with semaglutide 0.5 mg (estimated treatment difference range -0.18 to -0.22 mmol/L) and 1.0 mg (-0.23 to -0.41 mmol/L), vs comparators (all comparisons, p<0.01; **Figure A**). Changes in SD of SMBGs were closely aligned with changes in HbA_{1c} from baseline (**Figure B**).

Conclusion: The reduction of within-day glycaemic variability from baseline to end of treatment was significantly greater with once-weekly semaglutide vs all active comparators, providing supporting evidence for the improvement in clinically relevant outcomes with semaglutide treatment for T2D management.



*p<0.01; **p<0.001; ***p<0.0001. Values shown below graphs are LSD (95% CI). For each trial, the analysis was based on the full analysis set and 'on-treatment without rescue medication' period. SD of SMBG are analysed using an analysis of covariance, adjusting for treatment, country and baseline value. Before analysis, missing data were imputed using multiple imputation of observed data from subjects within the same group defined by randomised treatment, using a sequential regression model. CI, confidence interval; ETD, estimated treatment difference; exenatide ER, exenatide extended-release; IGlar, insulin glargine; SD, standard deviation; SMBG, self-monitored blood glucose.

Clinical Trial Registration Number: NCT01930188; NCT01885208; NCT02128932; NCT02648204; NCT03136484; NCT03191396

Disclosure: **E. Jodar:** Grants; Amgen, AstraZeneca, Boehringer, GSK, Janssen, Lilly, MSD, Novo Nordisk, Pfizer, Sanofi. Honorarium; Amgen, AstraZeneca, FAES, GSK, Italfármaco, Lilly, MSD, Novo Nordisk, Shire, UCB. Lecture/other fees; Amgen, AstraZeneca, FAES, Lilly, MSD, Novo Nordisk.

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Derived time in glycaemic range with once-weekly semaglutide vs active comparator: post hoc analysis from the SUSTAIN clinical trial programme

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Background and aims: Time in range (TIR) is the time an individual spends in the target glucose range of 3.9-10.0 mmol/L (70-180 mg/dL), calculated from continuous glucose monitoring (CGM) data. TIR correlates with HbA_{1c}, provides insight on variability in daily plasma glucose and predicts long-term complications. Improvements in TIR of 5% have been associated with meaningful benefits in glycaemic control for individuals with T2D. Derived TIR (dTIR), an alternative to TIR if CGM data are unavailable, is the proportion of 7-point self-measured blood glucose (SMBG) measurements within glycaemic range. We investigated the effect of once-weekly (OW) semaglutide vs active comparators on dTIR, as a complementary, informative measure of glycaemic control, using SUSTAIN clinical trial data.

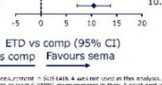
Materials and methods: This *post hoc* analysis was based on individual subjects in full analysis sets from the on-treatment without rescue medication period across the SUSTAIN 2–4, 7, 8 and 10 trials (N=5,680). Subjects with ≥6 SMBG measurements in their 7-point profile at baseline were included. dTIR for semaglutide (0.5 and 1.0 mg) and comparators (Fig) was calculated per trial, at baseline and end of treatment (EOT) using individual patient SMBG profiles (before and after breakfast, lunch, and dinner and at bedtime). Missing dTIR values at EOT were imputed.

Results: Overall, dTIR improved from baseline to EOT with semaglutide and comparators. dTIR at EOT was greater with semaglutide vs active comparators from multiple drug classes. Increases in dTIR from baseline to EOT were statistically greater with semaglutide vs comparators (estimated treatment differences [95% confidence interval] ranged from 6.8% [2.8;10.8] to 11.8% [8.2;15.4]), except for semaglutide 0.5 mg vs insulin glargine in SUSTAIN 4 (Fig). Semaglutide appeared to increase dTIR for both pre- and post-prandial states (data not shown).

Conclusion: Consistent with the superior HbA_{1c} reductions seen in the SUSTAIN clinical trials, OW semaglutide was associated with greater improvements in glucose control based on dTIR vs active comparators in subjects with T2D. This likely reflects more effective glycaemic control where both pre- and post-prandial glucose levels are better kept in range by OW semaglutide.

Figure: Derived time in range (i.e. proportion of SMBG measurements within 3.9–10.0 mmol/L [70–180 mg/dL]) at baseline and end of treatment with once-weekly semaglutide vs active comparators in the SUSTAIN 2–4, 7, 8 and 10 clinical trials

Trial	Comparisons	% dTIR at baseline	% dTIR at EOT		ETD vs comp, absolute % [95% CI]†	p-value
			Sema	Comp		
SUSTAIN 2	Sema 0.5 mg vs sita 100 mg	47.4	82.9	75.0	7.9 [4.2;11.6]	<0.0001
	Sema 1.0 mg vs sita 100 mg		86.7		11.8 [8.2;15.4]	<0.0001
SUSTAIN 3	Sema 1.0 mg vs exenatide ER	44.7	85.1	78.3	6.8 [2.8;10.8]	0.0009
SUSTAIN 4*	Sema 0.5 mg vs IGlar	42.9	76.6	74.4	2.2 [-1.6;6.0]	0.2482
	Sema 1.0 mg vs IGlar		83.1		8.7 [4.9;12.4]	<0.0001
SUSTAIN 7	Sema 0.5 mg vs dula 0.75 mg	50.6	87.3	78.3	9.0 [5.2;12.9]	<0.0001
	Sema 1.0 mg vs dula 1.5 mg		91.5	83.8	7.7 [3.8;11.5]	0.0001
SUSTAIN 8	Sema 1.0 mg vs cana 300 mg	51.4	86.0	78.4	7.6 [3.8;11.4]	0.0001
SUSTAIN 10	Sema 1.0 mg vs lira 1.2 mg	53.2	90.9	80.4	10.5 [7.2;13.8]	<0.0001



Clinical Trial Registration Number: SUSTAIN 2: NCT01930188; SUSTAIN 3: NCT01885208; SUSTAIN 4: NCT02128932; SUSTAIN 7: NCT02648204; SUSTAIN 8: NCT03136484; SUSTAIN 9: NCT03086330

Supported by: Novo Nordisk A/S, Søborg, Denmark

Disclosure: **V.R. Aroda:** Employment/Consultancy; Employment (Spouse) – Merck, Janssen, Consultancy (Self) – Applied Therapeutics, Duke, Novo Nordisk, Pfizer, Sanofi. Grants; Research Contracts (institutional contracts): Applied Therapeutics/Medpace; Eli Lilly; Premier/Fractyl, Novo Nordisk, Sanofi/Medpace.

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Effect of once-weekly semaglutide on insulin use in subjects with type 2 diabetes: a post hoc analysis of SUSTAIN 6

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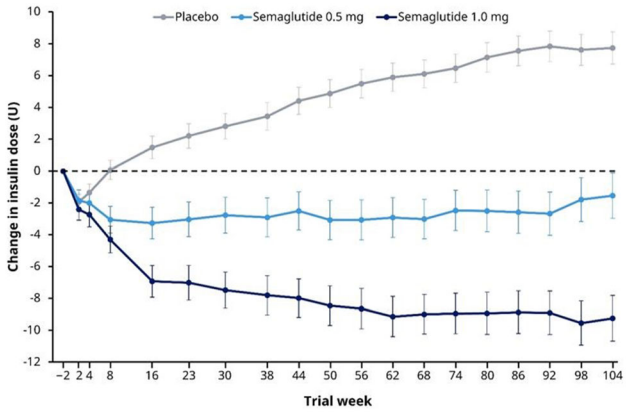
Background and aims: The SUSTAIN 6 cardiovascular outcomes trial demonstrated a 26% reduction in the risk of major adverse cardiovascular events with once-weekly (OW) subcutaneous semaglutide vs placebo, both as add-on to standard care, with many subjects receiving insulin. Prior studies indicate that use of OW semaglutide may reduce the need for insulin. This *post hoc* analysis of SUSTAIN 6 explored insulin use in subjects receiving semaglutide.

Materials and methods: The following were assessed in this analysis of the SUSTAIN 6 trial: the proportion of insulin-naïve subjects who initiated insulin treatment during the trial; the proportion of subjects on insulin at baseline who were on insulin at the end of the treatment period; and the absolute change in mean daily insulin dose in subjects on insulin at baseline. Intensification with other anti-hyperglycaemic treatments was not accounted for.

Results: With semaglutide 0.5 mg, 1.0 mg and placebo, 12.5%, 8.4% and 32.8% of insulin-naïve subjects (n=1,384), respectively, received insulin treatment for ≥21 consecutive days during the trial; of those on insulin at baseline (n=1,913), 93.7%, 92.0% and 98.2%, respectively, were on insulin at the end of the treatment period. At baseline the overall mean insulin dose in subjects on insulin was 55.8 U. The reduction in insulin dose from baseline at end of treatment, in subjects on insulin at baseline, was significant in favour of both semaglutide 0.5 and 1.0 mg vs placebo (estimated treatment differences: -9.3 and -17.0 U, respectively; both $p<0.0001$). In this group, compared with baseline, mean (standard deviation) reduction in daily insulin dose was 1.5 (1.4) U and 9.3 (1.4) U with semaglutide 0.5 and 1.0 mg, respectively, whereas insulin dose was increased by 7.7 (1.0) U with placebo at end of treatment (Figure). Despite reduced insulin use, in the overall trial population, reductions in HbA_{1c} were greater with semaglutide 0.5 and 1.0 mg vs placebo, with similar hypoglycaemia rates.

Conclusion: With OW semaglutide, the need for insulin was lower compared with placebo; insulin initiation was less common and insulin elimination more common at end of treatment, and greater reductions in daily insulin dose from baseline were observed. This reduction in insulin use following initiation of semaglutide, while still achieving clinically relevant reductions in HbA_{1c}, may have benefits for patients.

Figure: Change from baseline in insulin dose in subjects receiving insulin at baseline in SUSTAIN 6



Estimated mean changes based on an MMRM with treatment and stratification (cardiovascular disease status [established cardiovascular or chronic kidney disease or cardiovascular risk factors only], insulin treatment [none, basal insulin only, or premixed insulin], and estimated glomerular filtration rate [≤30 ml or >30 ml/min/1.73 m²] at screening) as fixed factors and baseline insulin dose as covariate. Error bars are +/−SEM. Baseline is defined as the latest assessment before randomisation.
MMRM, mixed-model for repeat measurements; SEM, standard error of the mean.

Clinical Trial Registration Number: NCT01720446

Supported by: Novo Nordisk

Disclosure: **J. Seufert:** Honorarium; Novo Nordisk. Lecture/other fees; Novo Nordisk.

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Achievement of near-normal HbA_{1c} with early initiation of oral semaglutide: an exploratory subgroup analysis of PIONEER 1

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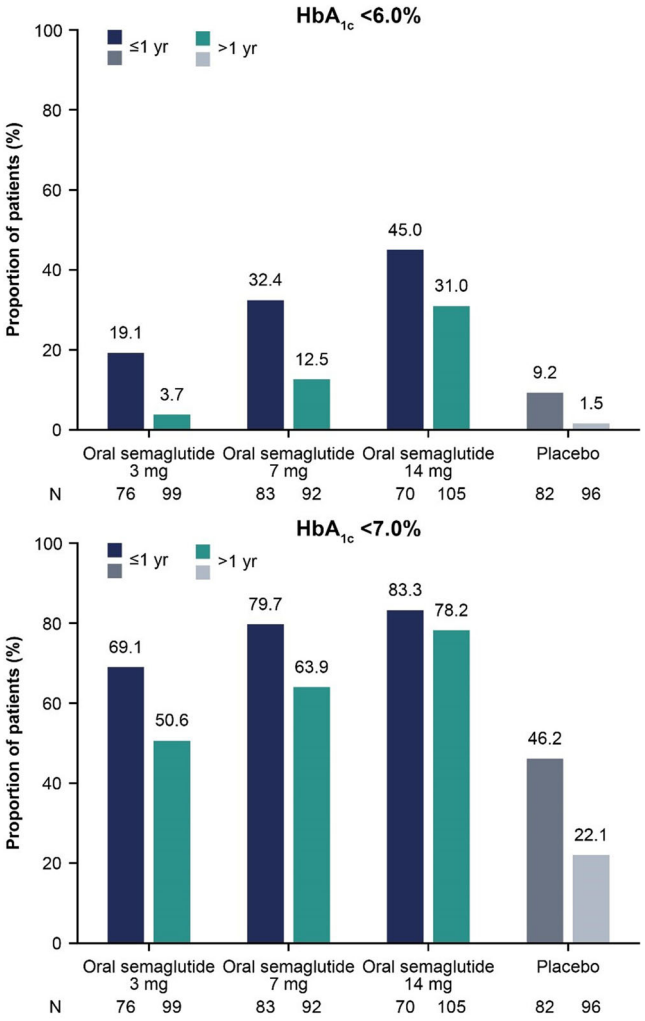
Background and aims: Early achievement of near-normal HbA_{1c} is associated with a reduced risk of future complications in type 2 diabetes (T2D) and may help motivate patients to maintain treatment. We conducted a post-hoc analysis of the PIONEER 1 study to look at the impact of early initiation of oral semaglutide on glycaemic efficacy, body weight and achievement of glycaemic targets.

Materials and methods: Patients on diet and exercise were randomised to oral semaglutide 3, 7 or 14 mg once daily, or placebo. HbA_{1c} and body weight reductions, and achievement of HbA_{1c} targets (<7.0%, ≤6.5%, <6.0%), were assessed at 26 weeks in patients with T2D duration ≤1 year and >1 year for comparison.

Results: At baseline, patients with T2D duration ≤1 vs >1 year were younger (mean age 51.4 vs 55.7 years for oral semaglutide 14 mg, 51.4 vs 55.8 years for placebo) with higher body weight (94.2 vs 84.1 kg and 91.8 vs 85.9 kg, respectively), but similar HbA_{1c} (8.0 vs 7.9% and 7.9 vs 7.9%, respectively). Greater HbA_{1c} and body weight reductions were seen for oral semaglutide 14 mg versus placebo for both duration ≤1 year (-1.6% vs -0.4%; -4.3 kg vs -1.6 kg) and >1 year (-1.4% vs 0.2%; -4.0 kg vs -1.4 kg); the subgroup interaction (≤1 vs >1 year) was significant for HbA_{1c} (p=0.04) but not body weight. A high proportion of patients initiating oral semaglutide within ≤1 year of T2D diagnosis reached glycaemic targets, including HbA_{1c} <6.0% in 45% of patients on oral semaglutide 14 mg (vs 31% in the >1-year group; Figure); subgroup interactions were not significant.

Conclusion: Initiation of oral semaglutide in patients within ≤1 year of T2D diagnosis resulted in robust HbA_{1c} and body weight reductions, with a high proportion of patients attaining glycaemic targets including near-normal HbA_{1c}. These observations support early initiation of therapy and further study.

Figure. Proportions of patients with diabetes duration ≤1 year and >1 year in PIONEER 1 who achieved HbA_{1c} <6.0% and <7.0 after 26 weeks of treatment with oral semaglutide 3, 7 and 14 mg, and placebo.



Observed data from the on-treatment without rescue medication period. Tests for interaction between treatment and T2D duration subgroup were not significant (trial product estimand). HbA_{1c}, glycated haemoglobin; N, number of patients contributing to the analyses; T2D, type 2 diabetes; yr, year.

Clinical Trial Registration Number: NCT02906930

Supported by: Novo Nordisk A/S

Disclosure: **E.C. Morales-Villegas:** Employment/Consultancy; Boehringer Ingelheim, Bristol Myers Squibb/AstraZeneca, Janssen-Cilag, Eli Lilly, Novo Nordisk, Takeda (all consultancy only). Grants; Boehringer Ingelheim, Bristol Myers Squibb/AstraZeneca, Janssen-Cilag, Eli Lilly, Novo Nordisk, Takeda. Honorarium; Novo Nordisk, Takeda. Lecture/other fees; Novo Nordisk, Takeda.

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Incorporating treatment pauses, dosing flexibility and education to support GLP-1RA therapy persistence: data from PIONEER 6

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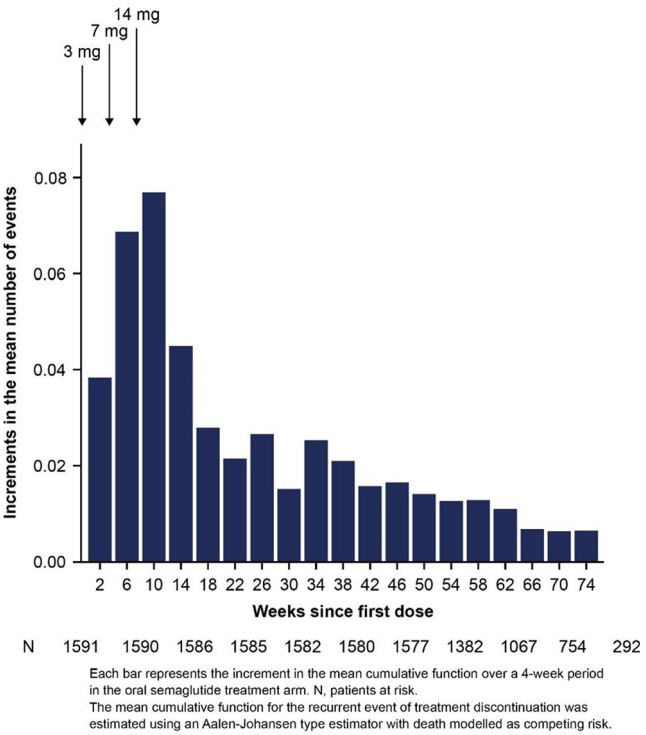
Background and aims: As the first oral glucagon-like peptide-1 receptor agonist (GLP-1RA), oral semaglutide may facilitate increased access to the benefits of GLP-1RA therapy in broader care settings. We aimed to explore flexible management of GLP-1RA therapy when tolerability issues arise, including gastrointestinal (GI) adverse events (AEs), as a potentially important part of overcoming barriers to treatment persistence.

Materials and methods: The PIONEER 6 trial (N=3183) examined the efficacy and safety of oral semaglutide in patients with type 2 diabetes either ≥50 years with established cardiovascular (CV) or kidney disease, or ≥60 years with CV risk factors. We evaluated medication management strategies within PIONEER 6 to assess their role in supporting treatment continuation; the mean number of treatment discontinuations (mean cumulative function) was estimated using an Aalen-Johansen type estimator.

Results: Patients on oral semaglutide underwent dose escalation starting at 3 mg, increasing to 7 mg after 4 weeks and 14 mg after 8 weeks. Discontinuation of oral semaglutide (temporary and permanent) mostly occurred during the initial dose-escalation period (**Figure**). Investigators were permitted to reduce the dose if AEs developed and to re-escalate the dose once symptoms had resolved or diminished. Patients were educated to address GI tolerability issues throughout the trial. Patients who discontinued treatment because of an AE were encouraged to resume treatment once willing or once the AE had ceased. In total, 27% of patients stopped taking oral semaglutide at least once during the trial due to an AE, but only 12% permanently discontinued due to an AE. Importantly, 75% of patients restarted oral semaglutide after the first AE-related treatment discontinuation. Any time off oral semaglutide (for AEs or any other reason) was considered a treatment pause. If treatment pauses were >21 days, re-escalation from a lower dose was recommended to mitigate GI AEs. In total, 23% of patients receiving oral semaglutide had ≥1 treatment pause for any reason, the majority of whom (72%) had just one pause. The median duration of treatment pause was 21 (interquartile range 7-51) days.

Conclusion: These data highlight the role of treatment pauses, flexibility and education in mitigating potential AEs to support treatment persistence on GLP-1RAs.

Figure. Mean number of treatment discontinuations (increase by time) in the oral semaglutide treatment arm in PIONEER 6.



Clinical Trial Registration Number: NCT02692716

Supported by: Novo Nordisk A/S

Disclosure: **S. Bain:** Grants; Health & Care Research Wales. Honorarium; AstraZeneca, Boehringer Ingelheim, Eli Lilly, Novo Nordisk, Sanofi-Aventis. Stock/Shareholding; Glycosmedia.

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Neutrophil-to-lymphocyte ratio predicts cardiovascular events in patients with type 2 diabetes: post hoc analysis of SUSTAIN 6 and PIONEER 6

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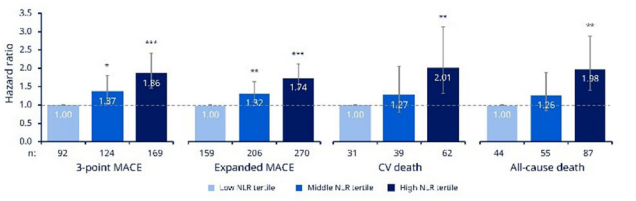
Background and aims: Inflammation plays an important role in atherosclerosis. The neutrophil-to-lymphocyte ratio (NLR) may serve as a clinically useful biomarker of inflammation and cardiovascular (CV) disease, although this relationship has not been studied in people with type 2 diabetes (T2D). This *post hoc* analysis investigated the relationship between NLRs and CV outcomes in T2D CV outcomes trials for two formulations of semaglutide, a glucagon-like peptide-1 receptor agonist.

Materials and methods: In pooled analyses of the SUSTAIN 6 and PIONEER 6 trials, 6,480 patients with T2D at high CV risk received placebo or semaglutide (once-weekly subcutaneously up to 1.0 mg, or once-daily orally up to 14 mg). NLRs were calculated from complete blood counts at randomisation. Adjudicated outcomes included 3-point major adverse CV events (MACE: composite of CV death, non-fatal myocardial infarction [MI] or non-fatal stroke; primary outcome), expanded MACE, CV death and all-cause death (secondary outcomes). Patient characteristics and CV outcomes were analysed according to baseline NLR tertiles using pooled trial data. Estimation of HRs for all outcomes across NLR tertiles used a Cox proportional hazards model

Results: Overall, baseline NLR was recorded in 6,364 patients. Mean baseline NLRs were 1.5, 2.2 and 3.6 in the low, middle and high tertiles, respectively. Patients in the high NLR tertile were older (66.6 years), more likely to be male (70.0%), had longer duration of diabetes (15.3 years), higher body weight (93.3 kg), lower diastolic blood pressure (75.5 mmHg) and eGFR (70.4 mL/min/1.73m^2) vs those in the lower NLR tertiles (all p<0.0001). Higher NLR was associated with an increased risk of MACE (HR [95% CI]: 1.37 [1.05;1.80; p=0.02] and 1.86 [1.45;2.41; p<0.0001] for the middle and high tertiles, respectively, vs the low tertile). The high NLR tertile was also associated with a 74% increased risk of expanded MACE and twofold risk for CV death and all-cause death vs the low NLR tertile (**Figure**). Further analysis of NLR and MACE by tertiles showed a more pronounced association in patients without prior MI and/or stroke (HR [95% CI]: 1.64 [1.07;2.56]; p=0.03 and 2.09 [1.38;3.21]; p=0.0006 in the middle and high tertiles, respectively, vs the low tertile).

Conclusion: Baseline NLR predicts MACE, CV death and all-cause death in patients with T2D and high CV risk. NLR is readily accessible from routinely obtained and inexpensive blood counts; it could offer a convenient, clinically useful inflammatory biomarker for CV risk prediction in this population.

Figure: Hazard ratios for 3-point MACE (primary outcome), expanded MACE, CV death and all-cause death (secondary outcomes) by NLR tertiles



*p<0.05; **p<0.01; ***p<0.0001 vs the low BL NLR tertile. Error bars represent 95% confidence intervals. Low NLR tertile: patients with NLR ≤1.88 at BL (mean 1.5); middle NLR tertile: patients with NLR >1.88 to ≤2.65 at BL (mean 2.2); high NLR tertile: patients with NLR >2.65 at BL (mean 3.6). The dashed line shows the reference value (low NLR tertile). 3-point MACE included a composite of CV death, non-fatal MI or non-fatal stroke, and was the primary outcome in both SUSTAIN 6 and PIONEER 6. Expanded MACE (CV death, non-fatal MI, non-fatal stroke, hospitalisation for unstable angina, hospitalisation for heart failure, and, in SUSTAIN 6 only, revascularisation [coronary or peripheral]). CV death and all-cause death were secondary outcomes. All outcomes were adjudicated by an external CEC except for peripheral revascularisation. Adjusted hazard ratios across NLR tertiles were computed using a Cox regression model with treatment (semaglutide, placebo) and NLR tertiles as fixed factors, stratified by trial.
BL, baseline; CV, cardiovascular; CEC, event adjudication committee; MACE, major adverse cardiovascular events; MI, myocardial infarction; NLR, neutrophil-to-lymphocyte ratio.

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Disclosure: **S. Verma:** Employment/Consultancy; AdBoard participation Amgen, AdBoard participation AstraZeneca, AdBoard participation Bayer, AdBoard participation Boehringer Ingelheim, AdBoard participation Eli Lilly, AdBoard participation HLS Therapeutics, AdBoard participation Janssen, AdBoard participation Merck, AdBoard participation Novo Nordisk, AdBoard participation Sanofi. Grants; Amarin, Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly, HLS Therapeutics, Janssen, Merck, Novo Nordisk, Pfizer, PhaseBio. Honorarium; AstraZeneca, Bayer, Boehringer Ingelheim, Eli Lilly, EOCI, HLS Therapeutics, Janssen, Merck, Novartis, Novo Nordisk, Sanofi, Sun Pharmaceuticals, TKTWG. Other; President of the Canadian Medical and Surgical Knowledge Translation Research Group.

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Empagliflozin reduces inflammation and alters bioenergetic metabolism in proinflammatory human macrophages: Clues to mechanisms of vascular protection?

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Background and aims: SGLT-2 inhibitors (SGLT2i) reduce cardiovascular (CV) risk in subjects with type 2 diabetes and atherosclerotic CV diseases, however the mechanisms underlying this CV benefit are still unclear. Macrophages (Mφ), key components of the innate immune response, play a pivotal role in the atherosclerotic process. Mφ inflammatory function is strictly dependent on their bioenergetic metabolism. Aim of this study was to determine whether in proinflammatory primary human Mφ (M1) empagliflozin (EMPA) 1. reduces inflammation and oxidative stress; and 2. modifies bioenergetic metabolism.

Materials and methods: M1 Mφ were differentiated from healthy donors' (n=10) monocytes and incubated with/without EMPA 100 μM for 16h. Biomarkers of inflammation and oxidative stress were quantified by qPCR. Steady-state bioenergetic metabolism (fluxes: pmol/min for 5x10^4 cells) was measured with an innovative method of indirect microcalorimetry, based on the integration of the flows of four independent primary measures [O_2 consumption (OCR) and H^+ by Agilent Seahorse XFp, lactate and NH_4 by microfluorimetric methods in the supernatant] with the stoichiometric equations of the main metabolic pathways. The following fluxomic parameters were calculated: lipid (LipOX,) glucose and amino acid oxidation; anaerobic glycolysis (AnaerGlyc); tricarboxylic acid cycle (TCA); succinate (SDH) and pyruvate (PDH) dehydrogenase; energy expenditure (EPR) and efficiency; maximum (ATPmax) and anaerobic (AnaerATP) estimates of ATP production.

Results: In M1, EMPA reduces (≈50%) inflammation (IL1-β, IL-8, TNF-α and MCP-1; p<0.05-0.001) and oxidative stress (GPX and HO-1; p <0.05) vs control. EMPA lowers OCR (133±9.7 vs 153±10.3, p<0.001), H^+ (197±15 vs 240±23 p <0.05) and lactate (424±46 vs 539 ±48; p <0.01) releases. Fluxomic analyses revealed that EMPA reduced LipOX (1.15±0.1 vs 1.33±0.1; p<0.005), TCA (45±3 vs 52±4; p<0.001) and SDH (46±3 vs 53±4; p<0.001) fluxes, ATPmax (1097±94 vs 1313 ±94; p <0.005) and AnaerATP (421±47 vs 537±48; p<0.01), and EPR (83 ±7 vs 98±7 μJ/min; p<0.001) compared to control. After dissipating mitochondrial membrane potential with FCCP to force maximal OCR, TCA (74±11 vs 97±12, p=0.001), SDH (78±11 vs 102±11; p<0.005), PDH (24±3 vs 33±4; p<0.01), LipOX (1.7±0.3 vs 2.2±0.4, p<0.005) and EPR (125±17 vs 153±17; p<0.005), but not anaerobic glycolysis and ATP, were still inhibited by EMPA.

Conclusion: EMPA reduced M1 inflammation along with cytosolic (AnaerGlyc) and - to a greater extent - mitochondrial (TCA) bioenergetics. EMPA-induced metabolic dysfunction of M1 *in vivo* may result into reduced metaflammation, thereby contributing to SGLT2i-dependent CV protection.

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Conceptual health economic modelling study of treatment sequencing for SGLT2is

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Background and aims: Type 2 diabetes (T2D) treatment guidelines are often guided by health economic modelling to assess the cost-effectiveness of pharmacological therapies. The T2D treatment algorithm, as recommended by the 2019 ADA-EASD consensus, consists of sequential initiation of additional T2D therapies when patients' HbA1c becomes uncontrolled. The *status quo* modelling approach used to inform guidelines prior to the publication of cardiovascular outcome trials for SGLT2is in T2D, typically assumed subsequent lines of therapy to be independent of prior therapy lines. This approach may not capture important cardiorenal benefits experienced by patients receiving SGLT2is beyond first intensification. The aim of this conceptual modelling study was to describe a *best practice* modelling approach that models clinically relevant T2D treatment sequences, thereby capturing the full benefits of SGLT2is throughout a patient's lifetime.

Materials and methods: The published Cardiff diabetes cost-effectiveness model was adapted to evaluate the lifetime cost-effectiveness of a commonly used standard of care (SoC) treatment sequence (metformin [MET], MET+DPP4i+SU, followed by MET+insulin) with a treatment sequence involving SGLT2i at first intensification. The SGLT2i algorithm was defined as MET+SGLT2i, MET+DPP4i+SU, followed by MET+insulin when using the *status quo* modelling approach and MET+SGLT2i, MET+SGLT2i+SU, followed by SGLT2i+insulin when using the *best practice* modelling approach. The HbA1c therapy escalation threshold was assumed to be 7.5%.

Results: Results from the two alternative modelling approaches are presented in Table 1. The increased cost-savings and QALY gain predicted by the *best practice vs*. *status quo* approach reflect the modelling of a more clinically relevant treatment sequence that captures weight benefits and reductions in heart failure hospitalisation and end-stage renal disease associated with SGLT2is over a patient's lifetime. A scenario analysis assuming a higher HbA1c therapy escalation threshold reflective of clinical practice, showed that the *status quo* modelling approach is associated with even more uncaptured benefits.

Conclusion: In clinical practice, patients are likely to stay on an SGLT2i over lifetime once initiated, including in combination with insulin. It is important for health economic modelling of pharmacological T2D therapies that inform national treatment guidelines to capture the cardiorenal benefits and treatment costs of SGLT2is throughout a patient's lifetime. Our modelling study demonstrated that the *status quo* modelling approach underestimates the benefits and cost-savings associated with SGLT2is, by at least 0.59 QALYs and -£3,799 over a patient's lifetime. When health economic modelling is conducted to inform treatment guidelines, the *best practice*-approach should be used in preference of the *status quo* approach to more accurately estimate the cost-effectiveness of T2D therapies.

		SoC sequence	SGLT2i sequence	Δ Costs§	Δ QALY§
***Status quo* modelling approach**	1st intensification	MET+DPP4i	MET+SGLT2i	-£3,032	0.12
	2nd intensification	MET+DPP4i+SU	MET+DPP4i+SU		
	Insulin rescue	MET+insulin	MET+insulin		
***Best practice* modelling approach**	1st intensification	MET+DPP4i	MET+SGLT2i	-£6,831	0.70
	2nd intensification	MET+DPP4i+SU	MET+SGLT2i +SU		
	Insulin rescue	MET+insulin	SGLT2i+insulin		

Abbreviations: MET=metformin, SU=sulfonylurea, DPP4i=dipeptidyl peptidase 4 inhibitors, SGLT2i= sodium-glucose co-transporter 2 inhibitor, §-incremental difference (SGLT2i– SoC)

Table 1. Incremental costs and QALYs over lifetime (SGLT2i – SoC)

Disclosure: **G. Chen:** Employment/Consultancy; GC, RLM, and RH are employees of AstraZeneca, VF, RB, PC, and PM are employed by HEOR Ltd who provide research and dissemination services to AstraZeneca and other pharmaceutical companies.

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Empagliflozin reduced the total burden of events leading to or prolonging hospitalisation in EMPA-REG OUTCOME

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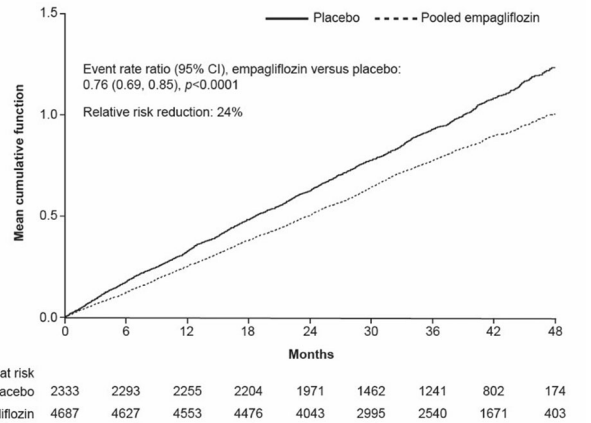
Background and aims: In EMPA-REG OUTCOME, empagliflozin (EMPA) reduced the risk of all-cause mortality (ACM) and total (first plus recurrent) events leading to all-cause hospitalisation in patients with type 2 diabetes and established atherosclerotic cardiovascular disease (ASCVD). We assessed the effect of EMPA on the total burden of events leading to or prolonging all-cause hospitalisation (ACPH) as well as the composite of ACPH and ACM.

Materials and methods: Patients were randomised to EMPA 10 mg, 25 mg, or placebo. *Post hoc,* we assessed the effect of pooled EMPA vs placebo on total events of ACPH, as reported by investigators, and an ACPH/ACM composite, using a negative binomial model. For hospitalisations, the model considered one event per day and events that also lead to death were counted only once.

Results: Among 7,020 patients, there were 5,256 ACPH events (5,031 leading to and 225 prolonging hospitalisation) and 5,617 ACPH/ACM events. EMPA reduced the risk of total events of ACPH by 22% vs placebo (rate ratio [95% CI]: 0.78 [0.70, 0.87]) and ACPH/ACM by 24% vs placebo (0.76 [0.69, 0.85]) (Figure). The estimated number of ACPH/ACM events prevented with EMPA vs placebo was 67.7 per 1000 patient years; the number needed to treat (NNT) (95% CI) over the three years of the trial to prevent one event was 4.9 (3.5, 8.4).

Conclusion: EMPA showed a sizeable reduction in the total burden of mortality and events leading to or prolonging hospitalisation in patients with type 2 diabetes and ASCVD, with a clinically relevant number of events prevented and a low NNT.

Figure. The effect of empagliflozin on the number of total (first plus recurrent) events of the composite of events leading to or prolonging hospitalisation for any cause and all-cause mortality



Event rate ratio, 95% CI, and *p*-value analysed by a negative binomial model. The cumulative mean function shows the population cumulative mean number of events up to time *t*. Events leading to or prolonging hospitalisation for any cause (n=5256) plus mortality (n=463) is larger than total events (n=5617) because events leading to or prolonging hospitalisation that also led to death were counted only once in the composite. CI, confidence interval.

Clinical Trial Registration Number: NCT01131676

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Disclosure: **S.E. Inzucchi:** Honorarium; AstraZeneca, Boehringer Ingelheim, Novo Nordisk, Sanofi/Lexicon, Merck, vTv Therapeutics, Abbott/Alere. Lecture/other fees; AstraZeneca, Boehringer Ingelheim, Novo Nordisk, Sanofi/Lexicon, Merck, vTv Therapeutics, Abbott/Alere.

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Angiotensin profiles in patients with type 2 diabetes and combination therapy of empagliflozin and linagliptin versus metformin and insulin glargine

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Background and aims: The early stage of type 2 diabetes mellitus is characterized by a systemic and local activation of the renin-angiotensin system (RAS). Whereas the classic RAS comprises the ACE-Ang II AT1R axis which promotes vasoconstriction among other damaging effects the ACE2-Ang (1-7)-Mas axis is an endogenous negative regulatory pathway of the classical RAS with protective components. In our IIS "Effects of Empagliflozin + Linagliptin (E+L) vs Metformin + Insulin Glargine (M+I) on Renal and Vascular Changes in Type 2 Diabetes" we showed vasodilation of the renal vas efferens after treatment with E+L compared to M+I. To better understand the underlying pathophysiological mechanism, we determined Angiotensin profiles in patients from the ELMI clinical trial.

Materials and methods: The angiotensin equilibrium levels of Ang I, Ang II, Ang-(1-7), Ang-(1-5) were determined in 101 patients with T2DM from the ELMI clinical trial at baseline and three months after application of either E+L or M+I. An LC-MS/MS based approach was used to simultaneously quantify individual angiotensin metabolites (Attoquant Diagnostics, Vienna, Austria). This allowed us to directly determine the medication induced effect on the RAS-System. Moreover, angiotensin-based markers for renin PRA-S (AngI+AngII) and angiotensin-converting-enzyme (ACE-S:Ang II/Ang I) were calculated.

Results: In the E+L group medication induced a rise in Ang II, Ang I as well as PRA-S between baseline and treatment, which was not present in the M+I group (table 1). In the E+L group 19 patients showed an increase in Ang-(1-7) between baseline and treatment, 2 patients showed a decrease and 30 patients had values below LLoQ, whereas in the M+I group 9 patients showed an increase in Ang-(1-7), 9 patients presented a decrease and 28 patients showed values below LLoQ (p-value for chi-quadrat-test: 0.020). There were no changes between baseline and treatment for Ang-(1-5) and ACE-S in both treatment groups (table1). We performed a subgroup-analysis in the 44 patients (19 from the M+I and 25 from the E+L group) without previous RAS inhibiting medication. Again, in the E+L group we found a rise in Ang II (p=0.025), Ang I (p=0.049) and PRA-S (p=0.027), but not for Ang-(1-5) (p=0.149) between baseline and treatment. No significant changes in angiotensin profiles occurred in the M+I group.

Conclusion: Three months of therapy with E+L led to a rise in Ang II, Ang I and PRA-S compared to baseline, which was not present in the M+I group. Likewise, Ang-(1-7) increased in a substantial portion of patients treated with empagliflozin, which was not the case in the M+I group. These changes indicate an activation of the RAS with increases of damaging and protective components. The biological consequence of this complex RAS activation remains unclear and further to be elucidated.

	Emplagliflozin + Linagliptin			**Metformin + Insulin**		
	Baseline	Treatment	p-value	Baseline	Treatment	p-value
Ang II (pmol/L)	43.6 (18.9-69.7)	51.5 (21.9-103.8)	0.003	55.8 (18.1-113.9)	57.9 (20.6-114.3)	0.906
Ang I (pmol/L)	19.5 (7.2-69.8)	35.1 (12.0-221.6)	< 0.001	30.1 (11.0-149.8)	21.4 (9.4-75.1)	0.359
Ang-(1-5) (pmol/L)	2.0 (2.0-6.2)	2.7 (2.0-6.0)	0.150	4.1 (2.0-8.1)	3.5 (2.0-8.5)	0.188
PRA-S (pmol/L)	74.7 (40.3-136.7)	140 (62.1-343.4)	< 0.001	124 (60.4-383.9)	95.3 (51.8-225.1)	0.645
ACE-S (pmol/L)/ (pmol/L)	3.4 (0.2-5.8)	3.4 (0.08-5.2)	0.437	2.9 (0.24-5.0)	4.2 (0.16-5.9)	0.238

Data are presented as median and interquartile range, Ang – Angiotensin, PRA-S – marker for renin activity, ACE-S - marker for ACE-Inhibitor activity.

Clinical Trial Registration Number: NCT02752113

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Disclosure: **A. Bosch:** Grants; Boehringer Ingelheim.

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Efficacy and safety of sotagliflozin in patients with type 2 diabetes: meta-analysis of randomised controlled trials

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Background and aims: Sotagliflozin, a novel, first-in-class dual SGLT1/2 inhibitor, increases urine glucose excretion and reduces intestinal glucose absorption. We performed a meta-analysis of randomised controlled trials to assess its efficacy and safety in patients with type 2 diabetes.

Materials and methods: We searched Pubmed, Embase, the Cochrane Library, and international clinical trial registries up to January 2021 for randomised controlled trials comparing sotagliflozin with placebo or active antidiabetic agents in adults with type 2 diabetes. Efficacy outcomes included change from baseline in HbA1c, body weight, and blood pressure. Safety outcomes included cardiovascular endpoints, serious adverse events, discontinuation due to any adverse event, hypoglycaemia, diabetic ketoacidosis, diarrhoea, volume depletion, and genital infections. We performed random effects meta-analyses and calculated mean differences (MDs) for continuous outcomes and odds ratios (ORs) for dichotomous outcomes, alongside 95% confidence intervals (CIs).

Results: We included 10 trials with 16231 patients. Mean trial duration was 50 weeks, while patient's mean age at baseline was 63.8 years, mean HbA1c was 8.2% and mean systolic blood pressure was 135.6 mmHg. Nine trials compared sotagliflozin with placebo, two with empagliflozin and one with glimepiride. Compared with placebo, sotagliflozin reduced HbA1c by 0.40 % (95% CI -0.54 to -0.26), body weight by 1.32 kg (95% CI -1.59 to -1.05), and systolic blood pressure by 2.44 mmHg (95% CI -2.81 to -2.07). No difference was evident versus active comparators. Compared with placebo, sotagliflozin reduced incidence of hospitalisation for heart failure (OR 0.67, 95% CI 0.57 to 0.78) and myocardial infarction (OR 0.73, 95% CI 0.54 to 0.98), and had a neutral effect on all-cause mortality, cardiovascular mortality and stroke. Treatment with sotagliflozin was safe regarding incidence of serious adverse events, severe hypoglycaemia, and diabetic ketoacidosis. However, it was associated with increased incidence of diarrhoea (OR

1.47, 95% CI 1.26 to 1.71), volume depletion (OR 1.27, 95% CI 1.08 to 1.50) and genital infections (OR 2.98, 95% CI 2.16 to 4.13).

Conclusion: Sotagliflozin effectively and safely reduces HbA1c, body weight, systolic blood pressure, hospitalisation for heart failure, and myocardial infarction. Nevertheless, it is associated with increased incidence of diarrhoea, volume depletion, and genital infections.

Table. Summary of meta-analysis results for efficacy and cardiovascular outcomes against placebo.

Outcome	Studies included, n	Participants analysed, n	Effect estimate (WMD/OR)*	95% CI	I^2, %
Change in HbA1c (%)	8	14341	-0.40	-0.54 to -0.26	73
Change in body weight (kg)	8	14341	-1.32	-1.59 to -1.05	27
Change in systolic BP (mm Hg)	8	14341	-2.44	-2.81 to -2.07	0
All-cause mortality	9	15561	0.96	0.82 to 1.13	0
Cardiovascular mortality	7	14582	0.90	0.75 to 1.10	0
Hospitalisation for heart failure	8	15030	0.67	0.57 to 0.78	0
Myocardial infarction	8	15094	0.73	0.54 to 0.98	0
Stroke	8	14991	0.72	0.52 to 1.01	0

For change in HbA1c, body weight and systolic blood pressure, the effect estimate is the weighted mean difference along with 95% confidence interval. For all-cause mortality, cardiovascular mortality, hospitalisation for heart failure, myocardial infarction and stroke, the effect estimate is odds ratio along with 95% confidence interval.
Abbreviations: BP, blood pressure; CI, confidence interval; OR, odds ratio; WMD, weighted mean difference.

Disclosure: **P. Kakotrichi:** None.

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Glycaemic variability of oral semaglutide vs empagliflozin: a post-hoc analysis of PIONEER 2

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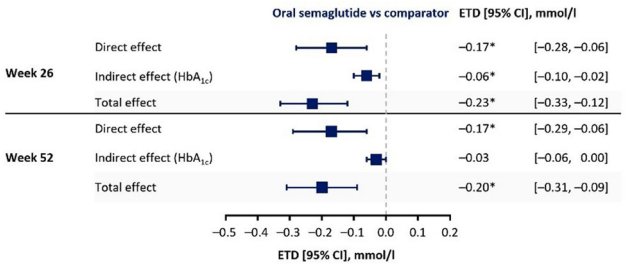
Background and aims: Glycaemic variability is associated with markers of microvascular and macrovascular complications and impacts quality of life in patients with type 2 diabetes. Glucagon-like peptide-1 receptor agonists (GLP-1RAs) have been shown to both improve HbA_{1c} concentration and reduce glycaemic variability in patients with type 2 diabetes. GLP-1RAs increase insulin secretion and suppress glucagon in a glucose-dependent manner, which may partly explain their ability to reduce glycaemic variability. However, it is not clear if HbA_{1c} influences the effect of GLP-1RAs on glycaemic variability. Oral semaglutide, the first GLP-1RA available as an oral formulation, is effective at reducing HbA_{1c} vs a range of comparators, but its effect on glycaemic variability needs to be understood. Therefore, we have assessed the change in glycaemic variability and its possible relationship with HbA_{1c} in a 52-week randomised, open-label trial (PIONEER 2) comparing oral semaglutide with the sodium-glucose co-transporter-2 (SGLT2) inhibitor empagliflozin in patients with type 2 diabetes uncontrolled on metformin.

Materials and methods: The seven-point self-measured blood glucose profile (7-point SMBG) was assessed at baseline and at weeks 26 and 52 in the PIONEER 2 trial. The standard deviation (SD) of the measurements in the 7-point SMBG profile was used as a measure of glycaemic variability. An additional post-hoc mediation analysis was performed to explore the indirect effect of HbA_{1c} concentration on the direct relationship between treatment and glycaemic variability (the SD of the 7-point SMBG) at weeks 26 and 52.

Results: The SD of the 7-point SMBG at baseline for the once-daily oral semaglutide 14 mg (N=411) and empagliflozin 25 mg (N=410) treatment arms was 2.06 and 2.05 mmol/l, respectively. The change from baseline in the SD of the 7-point SMBG in the oral semaglutide and empagliflozin arms was -0.67 and -0.44 mmol/l, respectively, at week 26, and -0.69 and -0.49 mmol/l, respectively, at week 52. The treatment differences (95% CI) for the SD of the 7-point SMBG for oral semaglutide vs empagliflozin at weeks 26 and 52 were -0.23 (-0.33, -0.12; p<0.0001) and -0.20 (-0.31, -0.09; p=0.0003) mmol/l, respectively. The mediation analysis showed that the indirect effect of HbA_{1c} accounted for -0.06 (-0.10, -0.02; p=0.0029) and -0.03 (-0.06, 0.00; p=0.08) mmol/l of the treatment difference for the SD of the 7-point SMBG at weeks 26 and 52, respectively (Figure).

Conclusion: Oral semaglutide significantly reduces glycaemic variability, as assessed by the SD of the 7-point SMBG, compared with empagliflozin, and most of this effect was not explained by an indirect effect of HbA_{1c}.

Figure. Contribution of direct and indirect effects to the estimated treatment differences in the SD of the 7-point SMBG (mmol/l) for oral semaglutide vs empagliflozin at weeks 26 and 52.



*p<0.05 vs comparator.

Data are from the on-treatment without rescue medication period. Missing values for both outcome and mediator were imputed using an analysis of variance based sequential multiple imputation model with region, post-baseline measurements prior to the time point in question, and baseline value as covariate. The mediation analysis assessed change from baseline in glycaemic variability (SD of the 7-point SMBG) using HbA_{1c} at the visit in question as a continuous mediator, treatment and region as factor, interaction between treatment and mediator, and baseline value as covariate.

ETD, estimated treatment difference; SMBG, self-measured blood glucose.

Clinical Trial Registration Number: NCT02863328

Supported by: Novo Nordisk A/S

Disclosure: **E. Montanya:** Employment/Consultancy; MSD, Sanofi (all consultancy only). Grants; Menarini. Lecture/other fees; MSD, Novo Nordisk.

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Anaplerotic therapy counters ketosis induced by empagliflozin in diabetic sheep

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Background and aims: Type 1 diabetes (T1D) patients rely on exogenous insulin therapy. Poor adherence to insulin treatments, strenuous physical activity, intercurrent illness or use of drugs that affect carbohydrate metabolism may precipitate diabetic ketoacidosis (DKA); a life-threatening metabolic crisis characterized by extreme hyperglycemia, hyperketonemia, and anion-gap acidosis. Inhibitors of sodium-glucose cotransporters (SGLTi) represent a new class of antidiabetic agents that reduce blood glucose through excretion in the urine. As such, they improve glycemic control and confer cardiometabolic benefits.

However, SGLTi have been associated with increased rates of DKA and thus have not been approved by the FDA for use in T1D. We **hypothesized** that the continuous depletion of carbohydrate-based energy induced by SGLTi therapy limits the dynamic range for hormonal regulation of ketogenesis by insulin and thus the capacity to withstand DKA stressors. Accordingly, suitable energetic support to replenish the hepatic TCA-cycle oxidative capacity should reduce ketogenesis both by decreasing the substrate abundance and by enabling higher insulin doses in the SGLTi setting. Here, we used insulin-dependent diabetic sheep to investigate the potential of glycerol to protect against DKA induced by SGLTi therapy with empagliflozin.

Materials and methods: Diabetes was induced with alloxan (50 μg/kg) in yearling sheep (n=6). Ketosis (1.5 < β-hydroxybutyrate [BHB] <3.0mM) was induced by partial insulin withdrawal and subcutaneous administration of 10 mg empagliflozin. Animals were randomly assigned to intravenous treatments with either 1L 5% glycerol in physiological saline (GLY) followed by one day of wash time and then treatment with 1L saline (SAL) as control or to the reverse sequence. To assess the potential benefit of the combined therapy of insulin together with glycerol, a similar experiment was conducted but instead with infusions of 0.5L of either 5% GLY or SAL containing 10 IU of human insulin. Statistical analysis was performed in JMP using the mixed-model approach with Treatment as a within-subject fixed factor, Sequence as a between-subject fixed factor, Treatment x Sequence interaction, and sheep as a random effect nested within Sequence.

Results: GLY resulted in a maximal decrease of 28.5% in ketosis compared to 11.5% for SAL ($P < 0.03$). Expressed by AUC, the ketosis-reducing effect was 1.55 h x mM for GLY compared to a negative effect of 2.52 h x mM for SAL ($P < 0.006$). GLY had a higher maximal increasing effect on blood glucose compared to SAL (63.3% vs. 10.5%; $P < 0.04$). Similarly, treatments containing insulin revealed a substantial improvement in ketosis for GLY compared to SAL (AUC of -8.2 h x mM Vs. -5.0 h x mM; $P < 0.003$); GLY also had a higher increasing effect than SAL on glucose levels (AUC of 114 h x mg/dL vs. -317 h x mg/dL; $P < 0.03$) indicating the utilization of glycerol for gluconeogenesis.

Conclusion: This study provides robust evidence for the capacity of glycerol to counter diabetic ketosis and hypoglycemia associated with empagliflozin therapy. Therefore, glycerol may offer a new opportunity for the management and prevention of DKA. Moreover, proper routine energetic support with glycerol may allow T1D patients to benefit from the metabolic value of SGLTi therapy at a reduced DKA risk.

Disclosure: **H. Dvir:** None.

SO 33 Non-insulin treatment in type 1 and type 2 diabetes

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Six-day sc GIP infusion increases plasma NEFA without altering the adipose tissue transcriptome, GIPR levels or plasma markers of inflammation in patients with type 1 diabetes

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Background and aims: Glucose-dependent insulinotropic polypeptide (GIP) has been proposed to increase adipose tissue lipolysis (at low insulin levels), adipokine secretion and circulating levels of inflammation markers via the GIP receptor (GIPR) in adipose tissue. We investigated the effect of a 6-day sc GIP infusion on circulating lipids and inflammation markers, the adipose tissue transcriptome and the presence of GIPR mRNA in adipose tissue in patients with type 1 diabetes.

Materials and methods: In a randomised, placebo-controlled, double-blind, crossover study, 20 men with type 1 diabetes (age [mean ± SD] 26 ± 8 years, BMI 23.8 ± 1.8 kg/m^2, HbA1c 51 ± 10 mmol/mol (6.8 ± 3.1%) underwent 2 × 6 days of continuous sc GIP (6 pmol/kg/min) infusion and placebo (saline) infusion, with an interposed 7-day washout period. Fasting blood samples were drawn frequently within the initial 3 hours and after 1 and 6 days of infusion. Adipose tissue biopsies were collected before and after 6 days of infusion.

Results: During the initial 3 hours of infusion, but not at day 1 and 6, GIP increased baseline-subtracted AUC of plasma NEFA compared to placebo (16.8 ± 10.4 vs 4.4 ± 9.2 min × mmol/l, $p < 0.001$) without affecting plasma glycerol and triglycerides. A panel of 23 circulating inflammation markers were unaffected by the GIP infusion. Adipose tissue transcriptomics did not show differentially expressed genes after 6 days of GIP infusion compared to placebo. In situ hybridization showed GIPR mRNA in the adipocyte and not in connective tissue. In situ hybridization and qPCR verified that GIPR mRNA levels were not altered by 6 days of sc GIP infusion.

Conclusion: Compared to placebo, a 6-day sc GIP infusion in patient with type 1 diabetes temporarily increases circulating levels of NEFA without altering the adipose tissue transcriptome, GIPR mRNA levels in adipocytes or circulating inflammation markers.

Clinical Trial Registration Number: NCT03734718

Supported by: grants from The Leona M. and Harry B. Helmsley Charitable Trust and Aase og Ejnar Danielsens Fond

Disclosure: **S.M.N. Heimbürger:** Grants; The Leona M. and Harry B. Helmsley Charitable Trust, Aase og Ejnar Danielsens Fond.

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Effects of liraglutide in type 1 diabetes by baseline anthropometrics in ADJUNCT ONE and TWO

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Background and aims: We re-evaluated the efficacy and safety of liraglutide, a glucagon-like peptide-1 (GLP-1) analogue, for use as adjunctive therapy in people with type 1 diabetes (T1D). Results from the two ADJUNCT trials suggested that residual beta-cell function is associated with more pronounced benefits and less risk of hypoglycaemia. Here we analyse results of the two trials according to other baseline anthropometrics.

Materials and methods: ADJUNCT ONE and TWO were randomised controlled phase 3 trials in broad T1D populations (1398 and 835 participants, respectively) treated with liraglutide (1.8, 1.2 or 0.6 mg) or placebo. ADJUNCT ONE was a 52-week trial with a treat-to-target design and no limits on insulin titration; ADJUNCT TWO was a 26-week trial with an individual maximal insulin dose. In a post hoc analysis, we evaluated certain estimated placebo-adjusted treatment effects across clinically relevant participant subgroups.

Results: At week 26 in ADJUNCT ONE, placebo-adjusted reductions in HbA_{1c}, body weight (BW) and daily insulin dose (up to -0.30 %-points, -5.0 kg and -12%, respectively, with liraglutide 1.8 mg) were significant ($p<0.05$) and greater than at week 52. In both trials, the placebo-adjusted reductions in HbA_{1c}, BW and daily insulin dose with liraglutide did not depend ($p>0.05$) on baseline HbA_{1c} or BMI at neither week 26 nor 52. The risk of clinically significant hypoglycaemia (symptomatic episodes with blood glucose <3.1 mmol/L) or hyperglycaemia with ketosis did not differ significantly ($p>0.05$ vs placebo) by baseline HbA_{1c}, BMI or insulin regimen.

Conclusion: In ADJUNCT ONE and TWO, the efficacy and glycaemic safety of liraglutide did not depend on key baseline anthropometrics. This leaves residual beta-cell function the only identified parameter impacting the treatment effect of the GLP-1 analogue used as an adjunct to insulin in T1D. Together with recent progress in the understanding of T1D and of the benefits of GLP-1 receptor agonism, these findings suggest a role for GLP-1 receptor agonists in T1D, warranting further study.

Clinical Trial Registration Number: NCT01836523; NCT02098395

Supported by: Novo Nordisk A/S

Disclosure: **T. Dejgaard:** Employment/Consultancy; Boehringer Ingelheim. Grants; Research support: AstraZeneca, Novo Nordisk. Honorarium; Speakers bureau: AstraZeneca, Novo Nordisk, Boehringer Ingelheim. Other; Advisory panel: Novo Nordisk.

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Multicenter, open-label, two-arm, pilot trial for safe reduction of basal insulin dose combined with SGLT2 inhibitor in type 1 diabetes: RISING-STAR trial

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Background and aims: The safe method of instructing insulin dose reduction in combination with SGLT2 inhibitors, dapagliflozin for patients with type 1 diabetes mellitus has not been clarified. In this study, we conducted a stratified, two-arm, parallel comparative study with the primary endpoint of decreasing the frequency of hypoglycemia by instructing basal insulin dose reduction.

Materials and methods: The basal insulin dose to total daily insulin dose (TDD) ratio was defined as less than 40% in group A and more than 40% in group B. Group B was instructed to reduce basal insulin dose, while group A was not. The study protocol was reported at "https://europepmc.org/article/ppr/ppr196934".

Results: Twenty-nine patients in group A and 28 patients in group B were included in the analysis due to withdrawal of consent before the start of the study. The frequency of hypoglycemia before and after the intervention was 0.23 times/day in group A to 0.26 times/day and 0.19 times/day in group B to 0.23 times/day, with no significant difference between the two groups (P=0.69). The frequency of ketosis (fasting serum β-hydroxybutyric acid ≥600 μM), a secondary endpoint, also increased significantly after the intervention, from 0.013 times/day to 0.086 times/day in Group A (p=0.013) and from 0.013 times/day to 0.059 times/day in Group B (p=0.011). There was no significant difference between the two groups (p=0.40). The total insulin dose reduction with dapagliflozin was about 10% in both groups. Time In Range (TIR: 70-180 mg/dL) on FGM improved from 63.6% to 69.7% in group A and from 59.0% to 69.1% in group B. Mean amplitude of glycemic excursion, MAGE improved from 121.7 mg/dL to 105.0 mg/dL in group A and from 123.4 mg/dL to 108.0 mg/dL in group B. HbA1c decreased by an average of 0.3% in both groups during the 4-week intervention, and the DTSQ showed a significant improvement in question 2 regarding hyperglycemia in both groups after the intervention, from 3.6±1.2 to 2.5±1.4 (p=0.008) in group A and from 3.7±1.3 to 2.8±1.2 (p=0.017) in group B.

Conclusion: In the patients who were able to self-adjust their insulin dose, there was no significant difference in the number of hypoglycemia occurrences after concomitant use of dapagliflozin with or without instruction to reduce basal insulin dose. Concomitant use of dapagliflozin resulted in less glycemic variability, prolonged TIR and improved patient satisfaction regarding with hyperglycemia. Concomitant use of dapagliflozin with adequate attention to ketosis is expected to improve glycemic control and patient satisfaction.

Clinical Trial Registration Number: jRCTs051190114

Supported by: AstraZeneca K.K. and Ono Pharmaceutical Co.

Disclosure: **M. Hamaguchi:** Grants; AstraZeneca K.K. and Ono Pharmaceutical Co., Ltd.

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Real-world efficacy and safety of SGLT2 inhibitors in type 1 diabetes: a two-center cohort retrospective study

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Background and aims: Despite substantial advances in insulin formulations, insulin delivery technologies and glucose monitoring devices, achieving recommended glycaemic targets to avoid diabetes related complications remains challenging for individuals with type 1 diabetes mellitus (T1DM). An unmet need exists to evaluate effectivity and safety of co-adjunctive treatment options to insulin replacement. SGLT2 inhibitors recently have shown promising results on glycaemic control, weight loss and renal function in phase 3 clinical trials in T1DM. Our aim was to

evaluate real-world efficacy and safety of SGLT2 inhibitor use in people with T1DM.

Materials and methods: We conducted a retrospective cohort European two-center study. Data on patient characteristics, HbA1c, weight, insulin use, renal function and adverse events were collected from 199 individuals with T1DM who initiated SGLT2 inhibitors adjunct to insulin therapy. Subgroup analysis was performed to identify subgroups who benefited most and those who were more at risk for adverse events.

Results: In our population (35,7% from Belgium; 64,3% from Spain) 113 (56,8%) patients were female, mean age was 48,1 ±10,1 years and diabetes duration was 12,0 ±13,2 years. Multiple daily injections (MDI) was used by 165 (82,9%) patients while 34 (17,1%) used insulin pumps (CSII). After 12 months of SGLT2i use, we observed a reduction of HbA1c (from 8,2±0,9% to 7,7±0,7%, p=0,000), weight (83,6±14,8 kg to 80,7±15,0 kg, p=0,000) and mean insulin daily dose (0,67±0,27IU/kg to 0,61±0,22 IU/kg, p=0,000) (Table). Seventy-eight patients (39,2%) reported adverse events: 59 (29,6%) genital infections, 11 (5,5%) diabetic ketoacidosis (DKA), 8 (4,0%) elevated ketone levels without acidosis and 9 (4,5%) severe hypoglycaemia episodes. Twenty-four individuals (12,1%) discontinued SGLT-2 inhibitors due to adverse events. Of the 11 patients who suffered DKA, 9 were female (8% vs 2,3% in those without DKA) and 6 used CSII (17,6% vs 3,0% in those without DKA).

Conclusion: Real-world data on SGLT2 inhibitors show promising results in reduction in HbA1c, weight and insulin daily dose. Benefits were more pronounced in individuals with higher baseline HbA1c and BMI. Adverse events were frequent, despite additional educational measures.

Table. Change in mean HbA1c, weight and total daily insulin dose.

	All patients (n=199)	Baseline HbA1c, %			Baseline BMI, Kg/m²	
		<7.0 (n=10)	7.0-8.0 (n=79)	>8.0 (n=110)	≤27 (n=63)	>27 (n=136)
HbA1c, %						
Baseline	8.2 ±0.9	6.7 ±0.2	7.6 ±0.3	8.8±0.7	8.1 ±0.8	8.3 ±0.9
At 12 months	7.7±0.9 (p=0.000)	6.6±0.3 (p=0.525)	7.4±0.5 (p=0.001)	8.1±0.7 (p=0.000)	7.8±0.7 (p=0.000)	7.7±0.8 (p=0.000)
Difference at 12 months	-0.5 (-6.1%)	-0.1 (-1.5%)	-0.2 (-2.6%)	-0.7 (-8.0%)	-0.3 (-3.7%)	-0.5 (-6.1%)
Weight, Kg						
Baseline	83.6 ±14.8	85.0 ±13.4	82.5 ±15.0	84.2±14.8	71.1 ±8.3	89.4 ±13.4
At 12 months	80.7 ±15.0 (p=0.000)	84.1±12.8 (p=0.692)	79.4±15.0 (p=0.000)	81.3±15.2 (p=0.000)	69.4 ±9.4 (p=0.000)	85.9±14.4 (p=0.000)
Difference at 12 months	-2.9 (-3.5%)	-0.9 (-1.1%)	-3.1 (-3.8%)	-2.9 (-3.4%)	-1.7 (-2.4%)	-3.5 (-3.9%)
Total daily insulin, IU/Kg						
Baseline	0.67±0.27	0.63 ±0.29	0.65 ±0.23	0.69±0.30	0.62 ±0.22	0.69±0.29
At 12 months	0.61±0.22 (p=0.000)	0.58±0.33 (p=0.211)	0.57±0.18 (p=0.000)	0.64±0.24 (p=0.002)	0.57±0.21 (p=0.000)	0.62±0.23 (p=0.000)
Difference at 12 months	-0.06 (-8.5 %)	-0.05 (-8.4 %)	-0.08 (-12.5 %)	-0.05 (-7.3 %)	-0.05 (-7.9 %)	-0.07 (-10.2 %)

Disclosure: **F. van Nes:** None.

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A pre-exercise low-carbohydrate-high-protein meal stabilises plasma glucose during and after exercise in persons with type 1 diabetes

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Background and aims: Physical exercise can cause large undulations in plasma glucose (PG) in persons with type 1 diabetes. High glycemic and/or high carbohydrate amounts are commonly advocated to rapidly raise PG ahead of exercise. Although low glycemic index carbohydrates have been explored, the impact of other macronutrient combinations on glycemic stability during exercise have been less studied. The aim of this study was to compare the effect of a pre-exercise low-carbohydrate-high protein (LCHP) meal versus a high-carbohydrate-low-protein (HCLP) meal on PG during and after exercise in persons with type 1 diabetes.

Materials and methods: Fourteen persons with insulin pump-treated type 1 diabetes (11 female, age 47 (range 25-65) years, HbA1c 51 (43-59) mmol/l, diabetes duration 30 (12-55) years, BMI 24.1 (19.3-27.1) kg/m^2) completed a 2-arm randomised cross-over study. On each visit participants arrived in the morning and ingested either a LCHP (carbohydrate 21E%, protein 52E%, fat 27E%) or HCLP (carbohydrate 52E%, protein 21E%, fat 27E%) breakfast. Meals were isocaloric (bodyweight <75 kg: 425 Kcal; ≥75 kg: 520 Kcal). Basal insulin rate and meal bolus were reduced by 35% and 25%, respectively, according to international guidelines. After the meal, participants rested for 90 minutes and then cycled at 60% VO_2peak for 45 min. Thereafter, participants rested for 180 minutes. During each study visit PG (figure 1), plasma insulin and plasma glucagon were measured at regular intervals.

Results: Average PG concentration was similar between LCHP and HCLP (9.64 ± 2.02 vs. 8.66 ± 2.03 mmol/l, P=0.142), but there was less PG variability after LCHP compared with HCLP (CV 14.81 ± 5.78 vs. 25.44 ± 8.23%, P=0.001). PG peak was similar between LCHP and HCLP (11.79 ± 2.21 vs 12.34 ± 2.57 mmol/l, P=0.488) but nadir was lower for HCLP (7.49 ± 1.88 vs. 5.51 ± 2.13 mmol/l, P=0.011). PG change from start to end exercise was less in LCHP compared with HCLP (1.56 ± 1.87 vs. 3.78 ± 2.03, P=0.002). In HCLP four episodes of PG<3.9 mmol/l were treated with 15 g carbohydrate and one episode of PG>15.0 mmol/l was treated with insulin bolus administration. In LCHP there was one hypoglycemia and one hyperglycemia rescue. PG values after rescue treatment were censored.

Conclusion: Compared with HCLP a LCHP pre-exercise meal lowers PG variability, diminishes the decrease in PG from start to end exercise, and reduces treatment-requiring hypoglycemia. This study shows the importance of taking into account the pre-exercise meal composition in the PG management during and after exercise in type 1 diabetes.

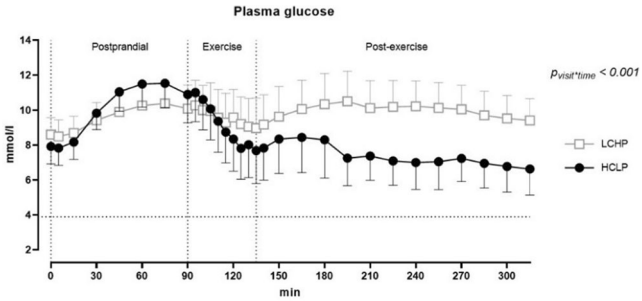

Figure 1: Plasma glucose responses to ingestion of a low-carbohydrate-high-protein (LCHP) or high-carbohydrate-low-protein meal (HCLP) before, during and after cycling (n=14). Comparison over time between study visits (time x visit) were analysed with a repeated measurement ANOVA

Clinical Trial Registration Number: NCT04472962

Disclosure: **K.B. Kristensen:** None.

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A pragmatic low carbohydrate diet intervention changes neither carbohydrate consumption nor glycaemia appreciably in adolescents and young adults with type 1 diabetes

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Background and aims: Low carbohydrate diets (LCDs) have gained popularity as a purported tool to mitigate postprandial hyperglycemia among highly motivated patients with type 1 diabetes (T1DM). Despite this enthusiasm, no randomized, prospective study has quantified how much an LCD improves glycemic outcomes compared with a standard carbohydrate diet (SCD) in pediatric T1DM. Moreover, no study to date has addressed the feasibility and acceptability of an LCD intervention in this population. We aimed to quantify the efficacy and feasibility of an LCD intervention in adolescents and young adults with T1DM.

Materials and methods: We randomized 29 patients with T1DM aged 13-21 years who were using both an insulin pump and continuous glucose monitor (CGM) to 1 of 3 interventions: an LCD (25-35% of daily caloric intake from carbohydrates, n=11), an equicaloric SCD (45-65% carbohydrate, n=9), or a general diabetes education program with no specific dietary recommendations (control, n=9). The 12-week intervention included 3 telephone sessions to reiterate the nutrition or general diabetes education and reinforcing text messages 2-4 times weekly. Glycemic outcomes included change in HbA1c and CGM parameters.

Results: Carbohydrate consumption was similar at baseline between LCD and SCD groups, then decreased by a median of 69 grams/day in the LCD group and increased by 3 grams/day in the SCD group over 12 weeks (Δ = 72 grams/day, 95% CI □ -38 to 125 grams/day, figure 1A). Carbohydrate consumption decreased by 11 grams/day in the control group. Adherence to diet varied widely, and there was no statistical difference in the change in carbohydrate consumption between groups (p=0.27). There was no clinically or statistically significant difference between groups for change in HbA1c (p=0.46, figure 1B), CGM average blood glucose (p=0.59), CGM time in range (p = 0.41), CGM coefficient of variation (p=0.36), or total daily dose of insulin (p=0.82).

Conclusion: Although participants received significant nutritional instruction and ongoing education, possibly reflected in a trend towards decreased carbohydrate consumption in the LCD group, adherence to the prescribed diet varied so widely that carbohydrate consumption was not appreciably different between groups, and all glycemic measures remained unchanged. These findings suggest that nutrition education alone is insufficient to mitigate dysglycemia in adolescent patients with T1DM. While small, uncontrolled studies have promoted LCDs as a means to improve glycemia, it is possible that these highly motivated participants not only consistently restricted carbohydrate consumption but also excelled in all aspects of diabetes self-care. This pragmatic study suggests that clinic-based LCD interventions are unlikely to improve glycemia in the general adolescent T1DM population.

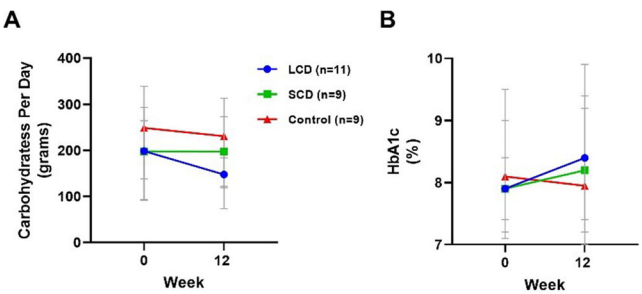

Figure 1: Median carbohydrate intake by group (grams per day, A) and median HbA1c by group (%, B) at baseline (week 0) and post-intervention (week 12). Data are summarized as medians with 95% CI.

Clinical Trial Registration Number: NCT03997409
Supported by: NIH and NIDDK T32DK007061
Disclosure: **S.H. Duffus:** None.

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Exploring a suitable marker of residual beta cell function associated with glycaemic response to dulaglutide in patients with type 2 diabetes

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Background and aims: Glucagon-like peptide-1 receptor agonists (GLP-1RAs) improve glycemic control via potentiation of endogenous β-cell insulin secretion. Previous studies showed poor residual β-cell function leads to reduced treatment response to several GLP-1RAs. However, clinical markers for β-cell function can be confounded by glucose toxicity, and appropriate markers for the residual β-cell function remain elusive. In addition, the association between clinical β-cell function markers and glycemic response to dulaglutide is yet to be fully understood in clinical settings. In the present study, we explored a suitable clinical marker of β-cell function and evaluated its association with glycemic response to dulaglutide in patients with type 2 diabetes (T2D).

Materials and methods: We retrospectively studied 141 patients with T2D who initiated once-weekly dulaglutide 0.75 mg after a meal tolerance test (MTT) with a standardized meal from September 2015 to December 2019 at our hospital. Prior to initiation of dulaglutide, baseline β-cell function was assessed with fasting and 2-h postprandial serum C-peptide immunoreactivity (FCPR and PCPR, respectively) as well as increment of CPR (ΔCPR) measured in the MTT. Glycemic response to dulaglutide (HbA_{1c} change 0-6 months) was assessed in the patients who continued dulaglutide for more than 6 months. We analyzed the results in a two-step manner. First, we explored a marker of β-cell function which was least affected by baseline HbA_{1c}. Second, we evaluated its association with glycemic response to dulaglutide using linear and logistic regression with adjustment for baseline HbA_{1c}.

Results: In 141 patients, PCPR and ΔCPR showed significant negative correlation with baseline HbA_{1c} (r = -0.25 and -0.33, respectively; P < 0.01 for both), whereas FCPR did not (r = 0.02; P = 0.853), suggesting confounding effects of high baseline HbA_{1c} on PCPR and ΔCPR. Therefore, we adopted FCPR as a clinical marker for β-cell function in the following analysis. We then evaluated the relationship between FCPR and glycemic response to dulaglutide. Of the 141 patients, 59 patients were followed up and continued dulaglutide without commencing any additional glucose-lowering medications for more than 6 months. The baseline mean ± SD HbA_{1c}, fasting plasma glucose, and FCPR of the 59 patients were 8.9 ± 1.2%, 9.2 ± 2.3 mmol/L (166.1 ± 42.2 mg/dL), 0.50 ± 0.29 nmol/L (1.52 ± 0.86 ng/mL), respectively. The mean ± SE HbA_{1c} change 0-6 months was -1.2 ± 0.2%. Notably, FCPR was significantly correlated with HbA_{1c} change 0-6 months (P = 0.031). Moreover, FCPR was a significant predictor for achieving a reduction in HbA_{1c} ≥1% over 6 months (OR, 1.40; 95% CI, 1.14-1.71; P = 0.039) with the area under the receiver operating characteristics curve of 0.83.

Conclusion: The present study illustrates that FCPR is a suitable marker of the residual β-cell function less influenced by baseline HbA_{1c} than PCPR and ΔCPR are. This is likely due to the confounding effects of chronic hyperglycemia-induced glucotoxicity on meal-stimulated endogenous insulin secretion. Our findings also suggest that the HbA_{1c}-lowering effect of dulaglutide is significantly associated with FCPR, highlighting the usefulness of FCPR as a simple and effective β-cell function marker of glycemic response to dulaglutide.

Disclosure: **M. Hasebe:** None.

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Fixed-ratio combination of insulin glargine plus lixisenatide (iGlarLixi) improves beta cell function in people with type 2 diabetes

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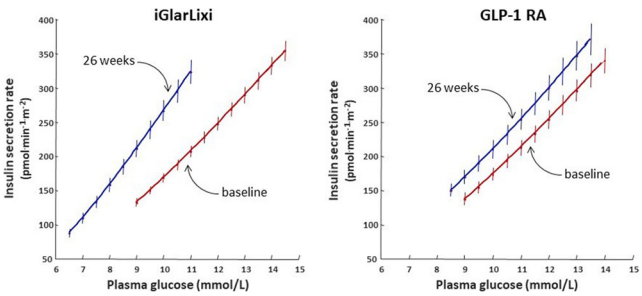
Background and aims: Multiple studies have shown the efficacy of combining a glucagon-like peptide 1 receptor agonist (GLP-1 RA) with basal insulin (BI) in people with T2D inadequately controlled on dual/triple oral therapy. Fixed-ratio combinations (FRCs) of BI + a GLP-1 RA represent an advance to facilitate management. FRCs combine the complementary mechanisms of action of their individual components into one formulation. However, it is not known whether the FRC drug combination affects ß-cell dysfunction, which is the main defect of T2D. We therefore set out to measure endogenous insulin secretion and ß-cell function in patients with T2D randomised to single GLP-1 RA therapy or FRC therapy comprising GLP-1 RA + BI treatment.

Materials and methods: We analysed data from 251 participants randomised to receive an FRC of insulin glargine 100 U/mL plus lixisenatide (iGlarLixi, mean daily dose = 17 µg Lixi and 44 U insulin) or to continue daily or weekly GLP-1 RA both on top of metformin (~2 g/day) (exploratory analysis from the LixiLan-G study). Each participant received a 2-hour mixed meal test before randomisation and at study end (26 weeks) with timed plasma glucose and C-peptide determinations. ß-cell function parameters were resolved using mathematical modelling.

Results: In the GLP-1 RA group (n=162), body weight and HbA_{1c} both decreased at week 26 (by 1.4 ± 3.1 kg and 4 ± 4 mmol/L, respectively, mean ± SD, both $p<0.0001$ vs baseline by Wilkinson test), yet none of the insulin secretion/ß-cell function parameters changed significantly. In contrast, in the iGlarLixi group (n=189), HbA_{1c} decreased (by 11 ± 5 mmol/mol) significantly more than in the GLP-1 RA group ($p<0.0001$ by 2-way repeated-measures ANOVA) despite an increase in body weight with iGlarLixi (by 1.7 ± 3.9 kg, $p<0.0001$). Both fasting and post-glucose insulin secretion decreased at week 26 (by 47 [46] $pmol \cdot min^{-1} \cdot m^{-2}$ and 3.5 [12.4] $nmol \cdot m^{-2}$, respectively, both $p<0.0001$ vs the other group) while ß-cell glucose sensitivity increased by a median 35% (from 33.7 [25.2] to 47.6 [48.8] $pmol \cdot min^{-1} \cdot m^{-2} \cdot mM^{-1}$, $p=0.0032$ vs the other group) (**Figure**). Glucose rate sensitivity - an index of early insulin secretion - and potentiation ratio - a metric for potentiation of endogenous insulin release - were also significantly increased with iGlarLixi than GLP-1 RA ($p=0.0073$ and $p=0.0007$, respectively). In a bivariate regression model of the pooled data, the change in ß-cell glucose sensitivity was related to the improvement in HbA_{1c} (by 12 ± 2 $pmol \cdot min^{-1} \cdot m^{-2} \cdot mM^{-1}$ per each 1% decrement, $p<0.0001$) and the age of diabetes onset (by 5 $pmol \cdot min^{-1} \cdot m^{-2} \cdot mM^{-1}$ per each 10 years of later onset, $p=0.026$).

Conclusion: In people with T2D on metformin, 26-week treatment with iGlarLixi, a once daily titrable FRC is associated with a marked improvement in ß-cell function concomitant with sparing of endogenous insulin release. The effect is accentuated in people with later disease onset.



Clinical Trial Registration Number: NCT02787551
Supported by: Sanofi US

Disclosure: **E. Ferrannini:** Employment/Consultancy; Lexicon; and Boehringer Ingelheim/Lilly&Co. Grants; J&J; AZ; and Boehringer Ingelheim. Honorarium; Boehringer Ingelheim/Lilly&Co.; and Sanofi. Lecture/other fees; Boehringer Ingelheim/Lilly&Co.; and Sanofi.

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Cellular internalisation and localisation of once weekly basal insulin Fc

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Background and aims: Basal Insulin Fc (BIF, LY3209590) is an insulin Fc-fusion protein in clinical testing as a once weekly treatment for diabetes mellitus. BIF is comprised of a human single-chain insulin fused to a human IgG2 Fc domain through a peptide linker. The cellular internalization characteristics of BIF compared to native insulin using U2OS cells expressing human insulin receptor (hIR) are reported in this work.

Materials and methods: Internalization and sub-cellular localization were visualized by immuno-fluorescent confocal microscopy in U2OS cells expressing human insulin receptor (IR).

Results: Internalization of BIF was observed, and the insulin and Fc moieties remained co-localized. Co-localization studies using an antibody to the early endosomal marker Early Endosome Antigen 1 (EEA1) showed that human insulin and BIF (insulin and Fc moieties) were rapidly internalized and co-localized with EEA1. During ligand washout, the remaining insulin and Fc moieties of BIF remained largely co-localized with EEA1 and a concomitant loss of immunostaining was observed, similar to human insulin. Studies using an antibody to the lysosomal marker Lysosomal Associated Membrane Protein-1 (LAMP-1) revealed a low level of lysosomal localization for human insulin and BIF (insulin and Fc moieties), indicating a similar intracellular trafficking pattern of human insulin and BIF to the lysosomal degradative pathway. The co-localization of remaining ligand decreased over time. IR internalization and transport to early endosomes was measured using an enzyme complementation assay. The EC50 for BIF was 4.7 ± 2.1 nM (n = 3), and the EC50 for human insulin was 0.06 ± 0.01 nM (n = 3).

Conclusion: BIF stimulated IR internalization to a similar maximum level; however, with decreased potency versus human insulin. The subcellular trafficking pattern of BIF is similar to human insulin. BIF undergoes rapid internalization and transport to early endosomes with limited transport to the lysosomes and undergoes loss of cellular immunostaining during ligand washout.

Supported by: Eli Lilly and Company

Disclosure: **J. Moyers:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

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Ultra Rapid Lispro (URLi) accelerates insulin lispro absorption and insulin action vs lispro in healthy Chinese subjects

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Background and aims: URLi is a novel insulin lispro formulation developed to more closely match physiological insulin secretion. This study assessed the pharmacokinetics (PK), glucodynamics (GD) and tolerability following administration of URLi or Lispro in healthy Chinese subjects.

Materials and methods: This was a randomized, subject-blind, crossover study conducted in 15 healthy Chinese subjects. The PK and GD were evaluated during a euglycemic clamp up to 10 hours post SC injection of 7U or 15U dose of URLi or 15U dose of Lispro.

Results: After a 15U dose, the early 50% tmax occurred 15.3 min ($p<0.0001$) sooner with URLi (8.1 min) vs Lispro (23.4 min). This resulted in greater early insulin lispro exposure: 5.3-fold in the first 15 min and 2.8-fold in the first 30 min after injection vs Lispro (both $p<0.0001$). Onset of insulin action was faster (8.57 vs 17.1 min; $p=0.0008$) and early insulin action was increased by 2.5-fold ($p=0.0002$) in the first 30 min of the clamp for URLi vs Lispro. Late insulin action (glucose infused from 4 h to the end of the clamp) was reduced by 56% ($p=0.0076$) and duration of action was 67.7 min shorter with URLi vs Lispro. Total insulin lispro exposure and glucose infused during the clamp did not differ between URLi vs Lispro. URLi is well tolerated in Chinese subjects.

Conclusion: In healthy Chinese subjects, URLi accelerated insulin lispro absorption with a reduced late exposure vs. Lispro, resulting in faster onset of action, reduced late insulin action and overall shorter duration. This study confirmed the faster PK and GD profile of URLi in this population.

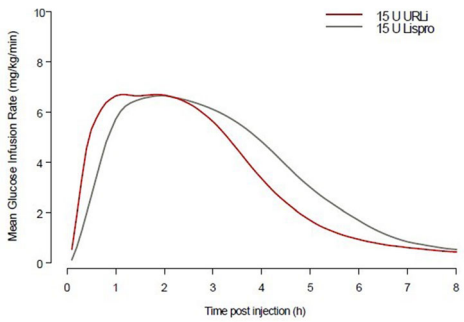

Figure. Mean locally weighted scatterplot smoothing fits of weight normalized glucose infusion rate versus time following a 15U dose of URLi compared to a 15U dose of Lispro in Healthy Chinese subjects

Clinical Trial Registration Number: NCT04049123

Supported by: Eli Lilly and Company

Disclosure: **Y. Yu:** None.

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Faster recovery from hyperglycaemia with ultra rapid lispro vs lispro in patients with type 1 diabetes on continuous subcutaneous insulin infusion

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Background and aims: Ultra Rapid Lispro (URLi) is a novel insulin lispro formulation developed to more closely match physiological insulin secretion. The aim of the study was to assess the time of recovery from hyperglycemia following administration of URLi compared to Lispro.

Materials and methods: This randomized, double-blind, 2-period, crossover study compared pharmacokinetics and glucodynamics of URLi vs Lispro during recovery from hyperglycaemia (plasma glucose [PG] >13.3 mmol/L) due to missed meal bolus or basal insulin suspension in 32 adults with type 1 diabetes on continuous subcutaneous insulin infusion (CSII).

Results: Following a missed meal bolus, a correction dose of URLi reduced maximum PG (-0.72 mmol/L; $p=0.02$), produced more rapid decline in PG (1.3 mmol $L^{-1}hr^{-1}$; $p=0.07$), and achieved recovery (PG 7.8 mmol/L) 23 min earlier ($p=0.1$) compared to Lispro. Similar results were observed during recovery of hyperglycaemia due to basal

suspension: a correction dose of URLi reduced maximum PG (-0.3 mmol/L; p=0.02), produced faster PG decline (1.3 mmol $L^{-1}hr^{-1}$; p<0.001), and achieved recovery 16 min earlier (p=0.001) compared to Lispro. The early 50% t_{max} for insulin lispro occurred sooner (-6 or -12 min; p<0.001), and early insulin exposure was greater (2.5 or 4.3 fold, $AUC_{0\text{-}15min}$; 1.7 or 2.5 fold $AUC_{0\text{-}30min}$; both p<0.001), with URLi compared to Lispro after correction bolus for a missed meal bolus or basal insulin suspension, respectively.

Conclusion: During episodes of hyperglycaemia commonly experienced by patients with type 1 diabetes, a correction dose of URLi provided faster recovery compared to Lispro, reflective of the faster insulin absorption.

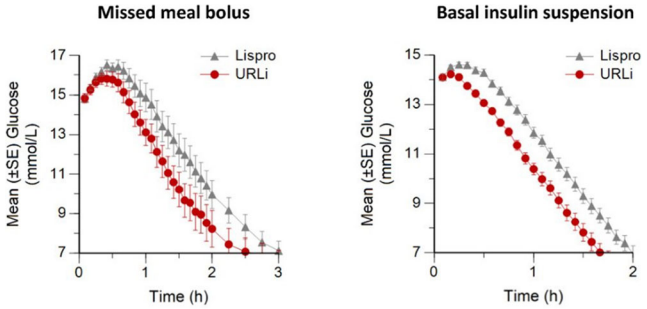

Figure: Mean plasma glucose concentration (± standard error [SE]) vs time after correction bolus of Ultra Rapid Lispro (URLi) or Lispro for hyperglycaemia due to a missed meal bolus (left) or due to basal suspension (right) in patients with type 1 diabetes.

Clinical Trial Registration Number: NCT04276207

Supported by: Eli Lilly and Company

Disclosure: **J. Leohr:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

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Achieving HbA_{1c} <7% in insulin naive people with type 2 diabetes on insulin glargine 300 U/mL in interventional vs observational European studies: REALI pooled database

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Background and aims: Initiation of people with type 2 diabetes (PWT2D) with basal insulin (BI) aims at improving glycaemic control. This pooled analysis assessed achievement of recommended glycaemic target HbA_{1c} <7% in interventional (IS) or observational (OS) studies of European patients initiated on insulin glargine 300 U/mL (Gla-300).

Materials and methods: PWT2D insulin naive at baseline (BL) pooled from the large REALI database were categorized according to type of study: IS or OS. A descriptive analysis compared patients' BL characteristics and glycaemic control as per HbA_{1c} target <7% achievement (A1cT) or not (non-A1cT), after 24 weeks of treatment (24 W) with Gla-300. Among those non-A1cT, similar analysis compared the two subgroups defined based on recommended fasting plasma glucose (FPG) target <7.2 mmol/L (130 mg/dL) achievement at 24 W. Continuous variables are shown as mean±SD; missing data were not imputed.

Results: The analysis included 654 patients from IS and 2709 from OS, of whom 39% and 27% were A1cT, respectively. Patients were comparable for age and BMI in all subgroups, with a higher proportion of men in A1cT in both IS and OS. Median duration of diabetes was shorter for A1cT vs non-A1cT and in OS than IS (7 vs 8 and 10 vs 11 years, respectively). Overall, diabetes complications and CV risk factors were balanced except for fewer ischemic CV events in A1cT vs non-A1cT in IS. BL HbA_{1c} was lower for A1cT vs non-A1cT but overall lower in IS vs OS (8.63±0.8% vs 8.91±0.8% and 9.12±1.8% vs 9.33±1.4%). Drop in HbA_{1c} from BL to 24 W was higher in A1cT than non-A1cT (2.16±0.8% vs 1.22±0.9% in IS; 2.67±1.9% vs 1.24±1.5% in OS). Hypoglycaemic events were overall low, yet slightly more symptomatic events were reported in A1cT in OS. No notable difference was observed regarding change in body weight (≤1 kg from BL to 24 W). Starting Gla-300 dose in OS was lower than recommended in both A1cT and non-A1cT (0.14 ±0.08 to 0.16±0.10 U/kg/day) and compared to IS (0.19±0.03 U/kg/day). Dose increase at 24 W was higher in IS (0.16±0.17 in A1cT and 0.17±0.17 U/kg/day in non-A1cT) than OS (0.08±0.12 and 0.11±0.13 U/kg/day, respectively). In non-A1cT group, more patients achieved FPG <7.2 mmol/L (130 mg/dL) in IS (55%) than OS (36%) (see Table).

Conclusion: In those PWT2D achieving FPG but not HbA_{1c} targets, tighter prandial glycaemic control could be considered. Comparison of IS with OS indicates that BI may not be optimally used and titrated in real world setting, leading to underachievement in HbA_{1c} and FPG control.

Table. Baseline characteristics and outcomes in insulin naive PWT2D who did not achieve HbA_{1c} target <7% (N=1907)

FPG Target, mmol/L (mg/dL)	Interventional (N=374)*		Observational (N=1533)*	
	FPG <7.2 (130) N=195	FPG ≥7.2 (130) N=160	FPG <7.2 (130) N=275	FPG ≥7.2 (130) N=483
Age, year	65.2 (8.4)	64.9 (8.5)	65.7 (10.6)	63.6 (11.3)
BMI, kg/m^2	30.4 (5.0)	31.7 (5.4)	31.2 (5.1)	31.9 (5.3)
T2D duration, median (Q1;Q3), year	11.0 (7.0;15.0)	10.0 (6.0;14.0)	10.0 (5.0;14.0)	7.0 (5.0;12.0)
HbA_{1c} at BL, %	8.88 (0.73)	8.94 (0.83)	8.56 (0.86)	8.89 (1.15)
HbA_{1c} change from BL to 24 W, %	-1.34 (0.82)	-1.06 (0.88)	-0.98 (0.84)	-0.65 (1.21)
Gla-300 dose at BL, U/kg/day	0.19 (0.03)	0.19 (0.03)	0.16 (0.07)	0.17 (0.11)
Gla-300 dose at 24 W, U/kg/day	0.37 (0.18)	0.35 (0.16)	0.27 (0.13)	0.29 (0.18)

*Data presented as mean (SD) unless otherwise specified. *Missing data 19/374 patients in interventional and 775/1533 in observational studies. Abbreviations: 24 W, Week 24 of treatment; BL, baseline, BMI, body mass index; FPG, Fasting plasma glucose; Gla-300, insulin glargine 300 U/mL; HbA_{1c}, haemoglobin A1c; PWT2D, people with type 2 diabetes; Q, quartile, SD, standard deviation.*

Supported by: Sanofi

Disclosure: **P. Gourdy:** Employment/Consultancy; Consultancy fees from Sanofi.

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Real-world effectiveness and safety of insulin glargine 300 U/mL in insulin-naive people with type 2 diabetes and renal impairment: a subgroup analysis of the ATOS study

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[1]Division of Endocrinology, Diabetes and Metabolism, Chaim Sheba Medical Center, and Tel-Aviv University, Tel Hashomer, Israel, [2]Imperial College London Diabetes Centre, Al Ain, United Arab Emirates, [3]Division of Endocrinology and Metabolism, Department of Internal Medicine, Universidad del Cauca, Popayan-Cauca, Colombia, [4]Sanofi, Singapore, Singapore, [5]Sanofi, Paris, France, [6]Endocrinology Research Centre of Health Care Ministry of Russian Federation, Dmitriya Ulyanova, Moscow, Russian Federation.

Background and aims: ATOS, a prospective, 12-month observational study in 18 countries outside US and Western Europe, showed that initiation of insulin glargine 300 U/mL (Gla-300) in people with type 2 diabetes (T2D) resulted in improved glycemic control with

low rates of hypoglycemia and minimal weight change. This subgroup analysis of the ATOS study evaluated the effectiveness and safety of Gla-300 in participants with T2D and a history of renal impairment (RI).

Materials and methods: Participants were stratified by history of RI (≈70% microalbuminuria, with RI: N=581; without RI: N=3841). The primary endpoint was achievement of individualised HbA_{1c} target at 6 months.

Results: Patients with RI were older (62.2 ± 10.3 vs 56.4 ± 10.7 years), had a longer duration of T2D (mean: 12.7 ± 6.9 vs 9.8 ± 6.0 years) and more comorbidities than patients without RI. Baseline HbA_{1c} was comparable between groups (9.3 % [78.1 mmol/mol]). Physician-set individualised HbA_{1c} (%) goals at baseline in the RI vs. non-RI groups were <7: 7.2 vs 14.6%; 7 to <7.5: 60.4 vs 72.0%; 7.5 to <8: 25.0 vs 9.8%; ≥8: 7.4 vs 3.6%. HbA_{1c} target achievement at Month 6 was 27.5% (95% CI 23.7, 31.6) in RI group and 24.8% (95% CI 23.4, 26.3) in the non-RI group. There was no difference between groups in HbA_{1c} reductions (least square (LS) mean change) from baseline to Month 6 (-1.50 [95% CI -1.59, -1.41] vs -1.50 % [95% CI -1.54, -1.47]) and 12 (-1.84 [95% CI -1.91, -1.76] vs -1.88 % [95% CI -1.91, -1.85]); **Table**. Change in fasting plasma glucose from baseline to Month 6 (LS Mean: -3.36 [95% CI -3.54, -3.19] vs -3.43 mmol/L [95% CI -3.49, -3.36]; and 12 (-3.79 [95% CI -3.94, -3.64] vs -3.96 mmol/L [95% CI -4.02, -3.90]) was similar between groups. At Month 12, increments in the Gla-300 dose was similar across the groups (RI vs. non-RI: 0.12 vs 0.10 U/kg); and body weight changes were minimal (95% CI -0.7, 0.1 vs. -0.3 to 0.0). The reported hypoglycaemia incidence was low although higher in the RI group (**Table**). Incidence of treatment-emergent adverse events was low in both RI (9.8%) and non-RI (5.9%) groups.

Conclusion: Initiation of Gla-300 is effective with low risk of hypoglycaemia in T2D patients with and without RI.

Note: Abstract accepted for presentation at ADA, 81st Scientific sessions, Virtual, 25-29 June 2021.

	Baseline		Month 6[a]		Month 12[a]	
	Renal Impairment	No renal impairment	Renal Impairment	No renal impairment	Renal Impairment	No renal impairment
HbA_{1c} (%)	9.32 ± 0.97	9.27 ± 1.00	7.82 ± 1.04	7.76 ± 1.06	7.47 ± 0.86	7.37 ± 0.98
Change from baseline (95% CI)[b]			−1.50 (−1.59, −1.41)	−1.50 (−1.54, −1.47)	−1.84 (−1.91, −1.76)	−1.88 (−1.91, −1.85)
HbA_{1c} (mmol/mol)	78.35 ± 10.60	77.81 ± 10.92	62.00 ± 11.33	61.37 ± 11.58	58.16 ± 9.41	57.05 ± 10.71
Change from baseline (95% CI)[b]	–	–	−16.39 (−17.35, −15.44)	−16.43 (−16.79, −16.07)	−20.07 (−20.90, −19.24)	−20.55 (−20.92, −20.19)
Body weight (kg)	84.1 ± 17.5	80.1 ± 16.1	84.1 ± 16.7	80.9 ± 15.3	84.3 ± 16.4	80.9 ± 15.0
Change from baseline (95% CI)[b]			−0.2 (−0.6, 0.2)	0.0 (−0.1, 0.1)	−0.3 (−0.7, 0.1)	−0.1 (−0.3, 0.0)
Documented symptomatic hypoglycaemia (≤3.9 mmol/L), % (event rate PPY)	..	..	1.72 (0.066)	0.73 (0.021)	1.89 (0.050)	1.17 (0.017)
Documented symptomatic hypoglycaemia (<3.0 mmol/L), % (event rate PPY)	–	–	0.34 (0.007)	0.08 (0.002)	0.34 (0.004)	0.18 (0.002)
Severe hypoglycaemia, % (event rate PPY)	–	–	0.52 (0.014)	0.05 (0.001)	0.52 (0.007)	0.08 (0.001)

Data shown are mean ± SD unless otherwise specified. Safety analyses were undertaken in the eligible population (RI: [N=581]; non-RI: [N=3841] those meeting the inclusion/exclusion criteria who started Gla-300 ± 31 days from study start). Efficacy analyses were undertaken in the evaluable population (RI: [N=512]; non-RI: [N=3419] all eligible patients with an HbA_{1c} assessment at Month 6, of whom RI: [N=489]; non-RI: [N=3259] had an HbA_{1c} assessment at Month 12)

Renal impairment subgroup is defined as "Yes" (as reported by the investigator) if the patient was diagnosed with renal impairment, nephropathy or chronic Kidney disease; and "No" in other case. eGFR data was not available

[a]6-month period for hypoglycemia defined as from the first treatment administration to visit 9 (Month 6) or treatment discontinuation, whichever occurred first. [a]12-month period for hypoglycemia defined as from the first treatment administration to visit 4 (Month 12) or treatment discontinuation, whichever occurred first. [b]LS mean change from a MMRM approach

CI, confidence interval; LS, least-square; MMRM, mixed model for repeated measurements; PPY, per patient-year; RI, renal impairment; SD, standard deviation

Clinical Trial Registration Number: NCT03703869

Supported by: Sanofi

Disclosure: **A. Tirosh:** Grants; Medtronic. Honorarium; Sanofi, Novo Nordisk, MSD, AstraZeneca, Boehringer Ingelheim. Lecture/other fees; Sanofi, Novo Nordisk, MSD, AstraZeneca, Medtronic.

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Assessing infusion site reactions with ultra rapid lispro in continuous subcutaneous insulin infusion

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Background and aims: In phase 3 trials, ultra rapid lispro (URLi) showed superior postprandial glucose control and non-inferior HbA_{1c} reduction compared to insulin lispro. However, infusion site reactions (combined; ISRs) were more frequent with URLi.

Materials and methods: We examined whether occurrence of ISRs was related to any intrinsic or extrinsic factors by analysing data from the 16-week PRONTO-Pump-2 trial in patients with type 1 diabetes (URLi, N=215; insulin lispro, N=217) between randomisation and end of treatment period.

Results: The most frequently reported adverse events (AEs) were infusion site reaction (URLi, n=41 [19.1%]; insulin lispro, n=15 [6.9%]) and infusion site pain (URLi, n=34 [15.8%]; insulin lispro, n=6 [2.8%]). ISRs were reported by 37.7% of patients on URLi and 10.1% on insulin lispro. Most ISRs with URLi started in the first 4 weeks after randomisation, were primarily mild in severity (~76%) and lasted a median of 2-6 days, depending on the type of reaction. Individual ISRs resolved during the study, but another event could occur with a different infusion set/site. Overall, 7 (3.3%) patients discontinued URLi treatment due to ISRs, 6 within the first 4 weeks after randomisation and 1 at week 6. Sex, age, BMI, ethnicity, race, infusion set model, bolus speed, total daily dose, duration of diabetes and duration of insulin pump use did not impact incidence of ISRs between treatments. However, significant treatment-by-subgroup interactions were observed for cannula length (6mm vs >6mm; p=0.070) and region (US vs non-US; p=0.046): incidence of ISRs with cannula length >6mm and incidence of ISRs in US was similar between treatments, but a higher incidence of ISRs was observed with URLi when cannula length was 6 mm or in non-US regions.

Conclusion: Infusion site reaction and infusion site pain were the most frequently reported AEs, although most were mild and resolved in less than a week. Differential treatment effects on ISR occurrence were seen with cannula length or region. Further studies are needed to confirm these findings.

Clinical Trial Registration Number: NCT03830281

Supported by: Eli Lilly and Company

Disclosure: **D. Ignaut:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

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CGM-based parameters for once-weekly insulin icodec vs once-daily insulin glargine U100 in insulin-naive patients with type 2 diabetes

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Background and aims: Insulin icodec (icodec) is a once-weekly basal insulin in development. This post hoc analysis used continuous glucose monitoring (CGM) data from a phase 2 treat-to-target trial to investigate CGM-based time in, above, below range (TIR, TAR, TBR) and glycaemic variability measured as coefficient of variation (CV%).

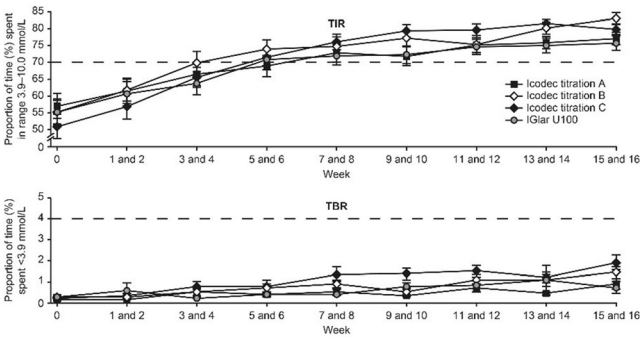
Materials and methods: Insulin-naïve patients with type 2 diabetes (T2D) (N = 205) received insulin glargine U100 (IGlar U100; pre-breakfast self-measured blood glucose target 4.4-7.2 mmol/L, adjusted ±4 U/day), icodec titration A (4.4-7.2 mmol/L, ±21 U/week), B (4.4-7.2 mmol/L, ±28 U/week; most relevant comparator to IGlar U100), or C (3.9-6.0 mmol/L, ±28 U/week). Treatments were titrated weekly. TIR (3.9-10 mmol/L), TAR (>10 mmol/L), TBR (<3.9 and <3.0 mmol/L) and CV% were calculated during the 2-week screening and the 16-week treatment periods using double-blinded Dexcom G6® CGM data.

Results: During the trial, across arms, TIR increased to >70% from weeks 7&8, TAR decreased to <25% from weeks 11&12, $TBR_{<3.9\ mmol/L}$ and $TBR_{<3.0\ mmol/L}$ remained below the recommended targets (<4% and <1%). At weeks 15&16, the proportion of patients achieving

>70% TIR and $TBR_{<3.9\ mmol/L}$ <4% was 63.3% for titration A, 80.0% for B, 66.0% for C and 64.0% for IGlar U100.

Conclusion: TIR increased during the trial across treatments, and similar or numerically higher proportion of patients achieved >70% TIR and $TBR_{<3.9\ mmol/L}$ <4% with icodec vs IGlar U100 at weeks 15&16.

Figure: TIR (3.9–10 mmol/L) and TBR (<3.9 mmol/L) for insulin icodec titration A, B, C, and insulin glargine U100 throughout the study by 14-day (2-week) intervals. Full analysis set. Observed data. Data are mean (symbol) ± standard error of the mean (error bars). The dotted lines represent the recommended targets of >70% for TIR and <4% for TBR.



Clinical Trial Registration Number: NCT03951805

Disclosure: **I. Lingvay:** Employment/Consultancy; Valeritas, Zealand Pharma, Janssen, TARGETPharma. Grants; Novo Nordisk, Sanofi, Merck, Pfizer, Novartis, GI Dynamics, Mylan. Honorarium; Novo Nordisk, Eli Lilly, Sanofi, AstraZeneca, Boehringer Ingelheim, Intercept, Intarcia, Bayer, and Zealand Pharma. Non-financial support; Novo Nordisk, Eli Lilly, Sanofi, AstraZeneca, Boehringer Ingelheim, Merck, Pfizer.

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CGM-based measurements for once-weekly insulin icodec vs once-daily insulin glargine U100 in insulin-treated patients with type 2 diabetes: a post hoc analysis

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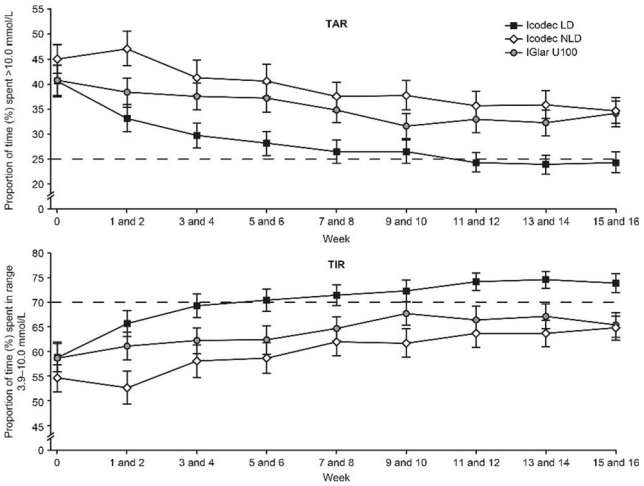
Background and aims: Insulin icodec (icodec) is a once-weekly basal insulin in clinical development. In a phase 2 treat-to-target trial in patients with type 2 diabetes (T2D) switching from daily insulin (N = 154), icodec with an initial 100% loading dose (LD; doubling the first dose) showed greater time in range (TIR, 3.9-10 mmol/L) vs once-daily insulin glargine U100 (IGlar U100) at weeks 15&16 (primary endpoint), as measured by double-blinded Dexcom G6® continuous glucose monitoring (CGM).

Materials and methods: This post hoc analysis compared TIR, time above range (TAR, >10 mmol/L) and below range (TBR, <3.9 and <3.0 mmol/L) for icodec LD and icodec with no loading dose (NLD) vs IGlar U100, calculated using CGM data from the 2-week screening and the 16-week treatment periods.

Results: Over the 16 weeks, overall observed mean TIRs were 71.4% for icodec LD, 60.7% for icodec NLD and 64.3% for IGlar U100. $TBR_{<3.9\ mmol/L}$ and $TBR_{<3.0\ mmol/L}$ remained below the internationally recommended targets (<4% and <1%, respectively) in all groups over the 16 weeks. At weeks 15&16, the proportion of patients achieving a combination of >70% TIR and <4% $TBR_{<3.9\ mmol/L}$ was 64.2% for icodec LD, 40.0% for icodec NLD and 45.8% for IGlar U100. Icodec LD prevented a mild transient increase in TAR observed during the switch with icodec NLD and appeared to lead to a lower TAR than IGlar U100 over the 16-week treatment period.

Conclusion: Overall, switching to icodec LD appeared to result in higher TIR and lower TAR than IGlar U100 through 16 weeks. TBR remained within the recommended targets.

Figure: TAR (>10 mmol/L) and TIR (3.9–10 mmol/L) for insulin icodec LD, insulin icodec NLD and insulin glargine U100 throughout the study by 14-day (2-week) intervals. Full analysis set. Observed data. Data are mean (symbol) ± standard error to the mean (error bars). The dotted lines represent the recommended targets of >70% for TIR and <25% for TAR.



Clinical Trial Registration Number: NCT03922750

Disclosure: **H.S. Bajaj:** Lecture/other fees; Investigator fee for trial conduct. Non-financial support; Conference registration.

SO 35 More on insulins

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Proposed biosimilar insulin aspart (GL-ASP) shows pharmacokinetic (PK) and pharmacodynamic (PD) bioequivalence to US-licenced and EU-authorised insulin aspart

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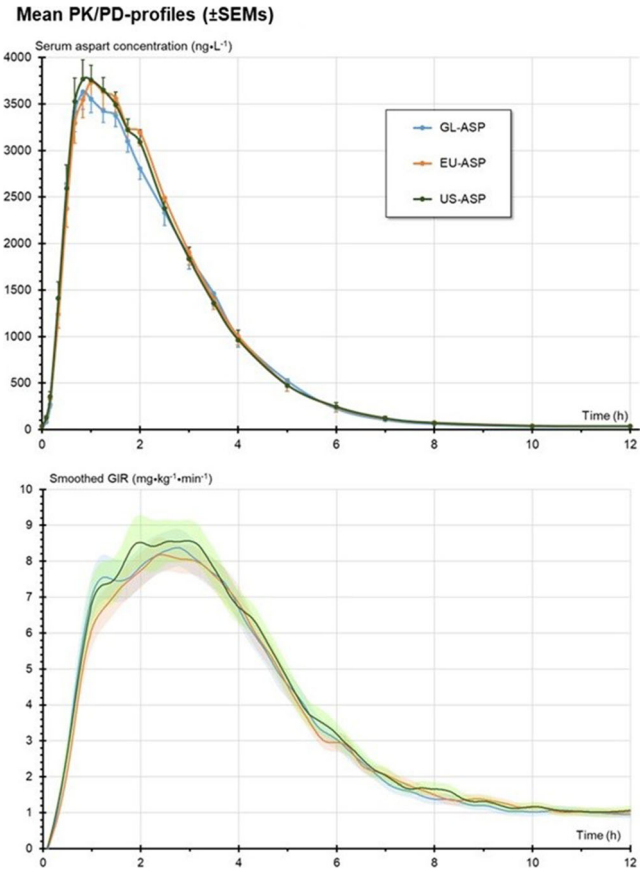
Background and aims: This trial aimed to evaluate PK and PD bioequivalence of proposed biosimilar GL-ASP compared to US-licenced ASP and EU- authorised ASP (US-ASP and EU-ASP). PK and PD parameters, single-dose safety, and local tolerability of the three ASPs were also compared.

Materials and methods: This Phase 1, single-center, randomized, double-blind, 3-treatment, 3-period crossover bioequivalence trial compared single doses of GL-ASP with US-ASP and EU-ASP in healthy male subjects (18-64 years old, fasting plasma glucose concentration ≤5.50 mmol/L, BMI 18.5-29.0 kg/m²). Each subject received a single 0.2 U/kg dose of either GL-ASP, US-ASP, or EU-ASP in each of the 3 euglycaemic glucose clamp periods (ClampArt [automated computer-controlled glucose clamp device], clamp level 81 mg/dL, clamp duration 12 h post-dose, 3-14-day washout period between dosing visits). Dosing was based on the body weight measured prior to first ASP dose. Doses were administered using a prefilled pen. Primary PK endpoints were serum ASP concentration point estimates for C_{max} and $AUC_{0\text{-}12h}$. Primary PD endpoints were glucose infusion rate (GIR) point estimates for C_{max} and $AUC_{0\text{-}12h}$. Secondary outcomes included $AUC_{ASP.0\text{-}2h}$, $AUC_{GIR.0\text{-}2h}$, adverse events, and injection-site tolerability. Equivalence margins of 80-125% for PK and 80-120% for PD, log-transformed PK point estimates, and 90% confidence intervals (CIs; 95% CI for PD GL-ASP/EU-ASP point estimates) were prespecified.

Results: Overall, 36 subjects (mean±SD age 36±12 years, BMI 24.3±2.7 kg/m²) were randomised. GL-ASP and comparator insulins showed superimposable ASP concentration and GIR profiles (Figure). PK bioequivalence was demonstrated between GL-ASP and US-ASP, with $C_{ASP.max}$ = 96.4% (90% CI: [90.7; 102.5]) and $AUC_{ASP.0\text{-}12h}$ = 97.5% (90% CI: [95.0; 100.2]), and EU-ASP $C_{ASP.max}$ = 97.7% (90% CI: [91.9;103.9]) and $AUC_{ASP.0\text{-}12h}$ = 97.1% (90% CI: [94.5; 99.7]). These CIs were well contained within the pre-defined equivalence margins. Likewise, the primary PD endpoints met bioequivalence criteria with point estimates for GL-ASP compared to US-ASP $C_{GIR.max}$ = 97.4% (90% CI: [91.5; 104.0]) and $AUC_{GIR.0\text{-}12h}$ = 96.4% (90% CI: [92.5; 100.6]); and compared to EU-ASP $C_{GIR.max}$ = 104.0% (95% CI: [98.4; 109.8]) and $AUC_{GIR.0\text{-}12h}$ = 101.6% (95% CI: [96.9; 106.3]). Secondary PK/PD endpoints also supported bioequivalence. Sensitivity analyses with untransformed data or exclusion of profiles with C-peptide increases confirmed PD bioequivalence. Adverse events were rare with all insulins (7-14 events per insulin, only 1 injection-site reaction [mild erythema] with GL-ASP).

Conclusion: GL-ASP demonstrated both PK and PD bioequivalence and similar safety profile to US-ASP and EU-ASP formulations.



Clinical Trial Registration Number: NCT04237129

Supported by: Gan & Lee Pharmaceuticals

Disclosure: **L. Plum-Mörschel:** Employment/Consultancy; Profil. Grants; Eli Lilly and Company, Novo Nordisk. Lecture/other fees; Eli Lilly and Company, Novo Nordisk.

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Proposed biosimilar insulin glargine (GL-GLA) shows pharmacokinetic (PK) and pharmacodynamic (PD) bioequivalence to US-licenced and EU-authorised insulin glargine

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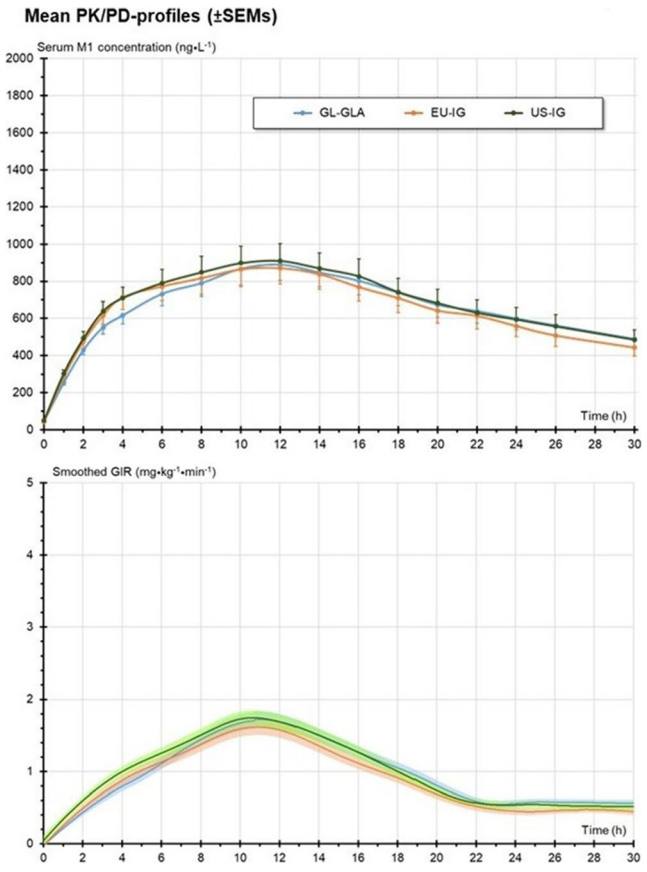
Background and aims: This trial aimed to evaluate PK and PD bioequivalence of the proposed biosimilar GL-GLA compared to US-licenced and EU-authorised IG (US-IG and EU-IG). PK and PD parameters, single-dose safety, and local tolerability of the three IGs were also compared.

Materials and methods: This Phase 1, two-center, randomised, double-blind, 3-way crossover, 3-treatment, euglycaemic glucose clamp trial compared single doses of GL-GLA with US-IG and EU-IG in male subjects with type 1 diabetes mellitus (18-64 years old, total insulin dose <1.2 U/kg/day, HbA1c ≤9.0%, BMI 18.5-29.0 kg/m²). Each subject received a single 0.5 U/kg dose of either GL-GLA, EU-IG, or US-IG in each of the 3 euglycaemic glucose clamp periods (ClampArt [automated computer-controlled glucose clamp device], clamp level 100 mg/dL, clamp duration 30 h post-dose, 5-21-day washout period between dosing visits). Dosing was based on the body weight measured prior to first IG

dose. Doses were administered using a prefilled pen. Primary PK endpoints were measured metabolite M1 (the mainly absorbed IG metabolite) point estimates for C_{max} and $AUC_{0\text{-}24h}$. Primary PD endpoints were glucose infusion rate (GIR) point estimates for GIR_{max} and $AUC_{0\text{-}24h}$. Secondary outcomes included $AUC_{M1.0\text{-}12h}$, $AUC_{M1.12\text{-}24h}$, $AUC_{GIR.0\text{-}12h}$, and $AUC_{GIR.12\text{-}24h}$, adverse events, and tolerability. Equivalence margins of 80-125% for PK and 80-120% for PD, log-transformed PK point estimates, and 90% confidence intervals (CIs; 95% CI for PD GL-GLA/EU-IG point estimates) were prespecified.

Results: Overall, 114 subjects (mean±SD age 42±8 years, BMI 25.8±2.0 kg/m²) were randomised. GL-GLA and comparator insulins showed superimposable profiles for PK and GIRs (Figure). PK bioequivalence was demonstrated between GL-GLA and US-IG, with $C_{M1.max}$ = 98.6% (90% CI: [93.4; 104.1]) and $AUC_{M1.0\text{-}24h}$ = 98.4% (90% CI: [93.7; 103.3]), and EU-IG, with $C_{M1.max}$ = 101.5% (90% CI: [96.0; 107.3]) and $AUC_{M1.0\text{-}24h}$ = 102.5% (90% CI: [97.5; 107.6]). These CIs were well contained within the prespecified equivalence margins. Likewise, the primary PD endpoints met bioequivalence criteria, with GL-GLA compared to US-IG reporting GIR_{max} = 94.9% (90% CI: [86.1; 104.5]) and $AUC_{GIR.0\text{-}24h}$ = 95.3% (90% CI: [87.3; 103.7]); and compared to EU-IG GIR_{max} = 107.2% (95% CI: [96.0; 119.7]) and $AUC_{GIR.0\text{-}24h}$ = 106.6% (95% CI: [96.2; 118.4]). Moreover, secondary PK/PD endpoints also supported bioequivalence. All insulins were well tolerated with adverse event rates of 19%, 26% and 21% for GL-GLA, US-IG and EU-IG respectively, and only a few injection-site reaction events per insulin (1 [GL-GLA]; 2 [US-IG]; 2 [EU-IG]).

Conclusion: GL-GLA demonstrated both PK and PD bioequivalence and similar safety profile to US-IG and EU-IG formulations.



Clinical Trial Registration Number: NCT04236895

Supported by: Gan & Lee Pharmaceuticals

Disclosure: **T. Heise:** Grants; Adocia, Afon Technology, AstraZeneca, Biocon, Boehringer Ingelheim, Eli Lilly, Gan & Lee Pharmaceuticals, Johnson & Johnson, Julphar, Mylan, Nestlé, Neuraly, Nordic Bioscience, Novo Nordisk, Sanofi, Zealand Pharma. Honorarium; Novo Nordisk. Other; Member of advisory panels for Novo Nordisk and Valbiotis.

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Star.ro - real world data on effectiveness and safety of iglarlixi in people with type 2 diabetes uncontrolled on oral antidiabetic drugs ± basal insulin treatment

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Background and aims: Current guidelines recommend a combination of Glucagon-Like Peptide 1 Receptor Agonists (GLP-1 RA) and basal insulin (BI) in people with type 2 diabetes (T2D) if actual HbA1c is 1.5-2% higher than individualized target. Multiple randomized clinical trials indicated the benefit of advancing treatment with a fixed-ratio combination (FRC) of GLP-1 RA and BI in subjects inadequately controlled with BI and/or oral anti-diabetic drugs (OADs). The aim of our study was to assess on a large cohort of T2D subjects the effectiveness and safety of iGlarLixi, a fixed-ratio combination of insulin glargine 100 U/mL and lixisenatide in a daily clinical practice setting.

Materials and methods: This was a multicenter, prospective, non-interventional, open label, 24 weeks, product registry trial performed in 65 study sites. The study group included 808 adults with T2D (57.2% F) uncontrolled with previous OAD ± BI treatment, started on iGlarLixi upon the investigator decision. Main outcome was change in HbA1c at week 24. Comparison between quantitative variables was made with the paired sample T test using the SPSS v21 software.

Results: Mean age was 62.5±8.3 years and mean duration of diabetes was 10.3±5.7 years. Baseline diabetes treatments included metformin (100% of patients), sulphonylureas (27.3%), dipeptidilpeptase-4 inhibitors (2.8%), sodium-glucose co-transporter-2 inhibitors (2%) and BI (58.6%). Majority (58.2%) of participants received metformin + BI. Mean HbA1c at baseline was 9.2±1.4% (79±4.2 mmol/mol) with a mean fasting plasma glucose (FBG) of 10.8±2.9 mmol/L. Mean initial dose was 19.5 IU - 0.2 IU/kg/day (iGlarLixi 100 IU/50 ug/ml pen) and 30.1 IU - 0.3 IU/kg/day (100 IU/33 ug/ml pen). Overall, mean HbA1c change from baseline was -1.4% ($p<0.0001$) at week 24, higher in subjects previously treated only with OADs (-1.8%, $p<0.0001$) compared with previous BI (0.9%, $p<0.0001$). Mean FBG decreased to 8±2 mmol/L (week 12) and 7.7±1.9 mmol/L (week 24). Overall, 42.3% of study participants reached the HbA1c target of 7.5% at week 24. Mean weight change was -1.6 kg over 24 weeks. Mean iGlarLixi dose increased to 30.2 IU - 0.4 IU/kg/day (ratio 2/1 pen) and 45 IU - 0.5 IU/kg/day (ratio 3/1 pen) at week 24. Adverse events (AEs) were reported by 4.9% of subjects with 0.5% reporting SAEs. Incidence of hypoglycemia was low, with only 1.3% of subjects reporting at least one event of symptomatic or confirmed (< 3.9 mmol/L) hypoglycemia. Only one episode of severe hypoglycemia was reported. A total of 2.1% of subjects reported gastro-intestinal (GI) AEs.

Conclusion: In a real-world setting, 24 weeks iGlarlixi treatment provided a significant reduction of HbA1c with low hypoglycemia risk and

body weight loss in people with T2D inadequately controlled with OADs with or without BI.

Supported by: Sanofi Romania

Disclosure: **C. Guja:** Lecture/other fees; Sanofi Romania.

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Phase 3 confirmatory study comparing efficacy and safety of proposed biosimilar and reference insulin aspart, combined with metformin, in patients with diabetes

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Background and aims: Insulin aspart (ASP) is a rapid-acting human insulin analog approved for pre-prandial use in patients with type 1 and type 2 diabetes mellitus and may be combined with concomitant oral antidiabetic treatment. The aim of this phase 3, confirmatory study was to compare the efficacy and safety of proposed biosimilar ASP (GL-ASP) and reference ASP (NN-ASP), in patients with diabetes mellitus, when administered in combination with metformin.

Materials and methods: This confirmatory, phase 3, 24-week, multicenter, randomized, open-label, controlled clinical study was conducted at 21 hospitals in China. Patients diagnosed with type 2 diabetes mellitus whose blood sugar glucose was inadequately controlled by oral antidiabetic drugs were randomized (ratio 3:1) to receive daily mealtime subcutaneous injections of GL-ASP or NN-ASP, in combination with metformin. All other oral anti-hypoglycemic drugs were discontinued. The primary objectives were to demonstrate non-inferiority (NI, margin of 0.4%) in efficacy (assessed by HbA1c change from baseline) of GL-ASP versus NN-ASP, and to compare safety profiles, after 24 weeks. Secondary outcomes included changes in 2-h postprandial plasma glucose (PPG), fasting plasma glucose (FPG), and percentage of patients achieving HbA1c <7.0% and ≤6.5%.

Results: 590 patients were randomized to receive GL-ASP (n = 441) or NN-ASP (n = 149), making up the full analysis set; the safety set included all patients who received ≥1 dose of study medication, GL-ASP (n = 439) and NN-ASP (n = 149). Baseline characteristics were similar between treatment groups (mean age 56 years, BMI 25.7 kg/m^2, disease duration 8.4 years, HbA1c 9.5%). There were relatively more males in the GL-ASP group compared with the NN-ASP group (56.2% vs 44.3%, respectively). After 24 weeks, HbA1c decrease was demonstrated in GL-ASP (mean: -2.20%) and NN-ASP (mean: -2.32%), meeting NI criteria for GL-ASP versus NN-ASP (treatment difference 95% confidence interval: -0.17, 0.26, which was within NI margin). Comparable proportions of patients reported adverse events (AE; GL-ASP: 43.7%; NN-ASP: 41.6%; p = 0.6509) and serious AE (GL-ASP: 3.87%; NN-ASP: 0.67%; p = 0.0543). Positive rates of ASP-specific antibodies were comparable between the two groups, with no statistical difference (odds ratio [95% CI]: 0.7281 [0.2751, 1.9273]; p > 0.05). There were no differences in hypoglycemic event rates between the two groups (GL-ASP: 59.0%; NN-ASP: 59.1%; p = 0.9893) and the majority of events were mild. Secondary outcomes were comparable, with no statistically significant differences between groups; mean change in 2-h PPG (GL-ASP: -6.14 mmol/L; NN-ASP: -6.29 mmol/L; p = 0.3527), FPG (GL-ASP: -2.02 mmol/L; NN-ASP: -1.70 mmol/L; p = 0.2906), and percentage of patients achieving HbA1c values of <7.0% (GL-ASP: 52.6%; NN-ASP: 51.0%) and ≤6.5% (GL-ASP: 34.2%; NN-ASP: 30.9%).

Conclusion: The efficacy, safety, and immunogenicity of GL-ASP matched that of NN-ASP in patients with type 2 diabetes mellitus also receiving metformin over 24 weeks.

Clinical Trial Registration Number: ChiCTR200031290

Supported by: Gan & Lee Pharmaceuticals

Disclosure: **J. Yao:** None.

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Proposed biosimilar insulin lispro (GL-LIS) shows pharmacokinetic (PK) and pharmacodynamic (PD) bioequivalence versus US-licenced and EU-authorised insulin lispro

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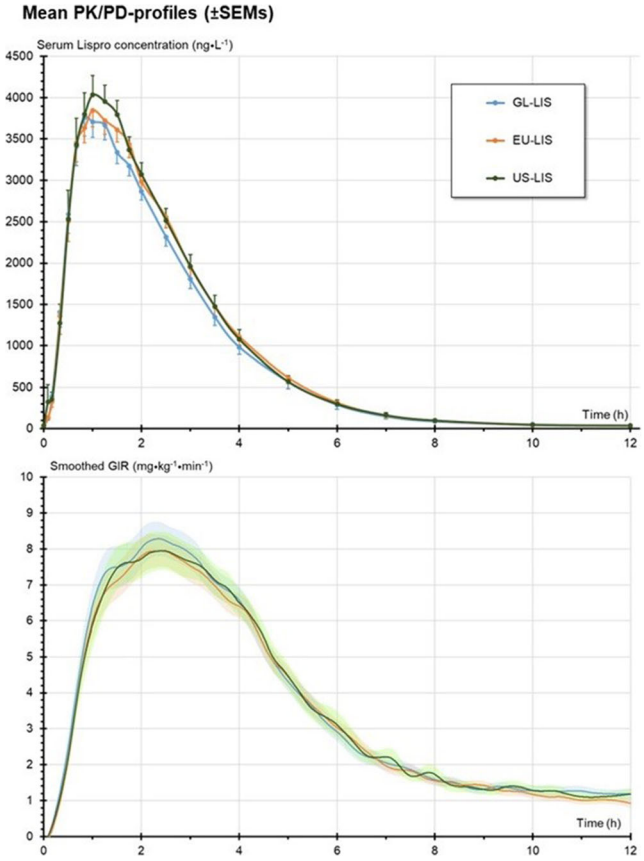
[1]Profil Neuss, Neuss, Germany, [2]Profil Mainz, Mainz, Germany, [3]Gan & Lee Pharmaceuticals, New Jersey, USA.

Background and aims: This trial aimed to evaluate the PK and PD bioequivalence of proposed biosimilar GL-LIS compared to US-licenced and EU-authorised LIS (US-LIS and EU-LIS). PK and PD parameters, single-dose safety, and local tolerability of all three LISs were also compared.

Materials and methods: This Phase 1, single-center, randomised, double-blind, 3-treatment, 3-period crossover bioequivalence trial compared single doses of GL-LIS with US-LIS and EU-LIS in healthy male subjects (18-64 years old, fasting plasma glucose concentration ≤5.50 mmol/L, BMI 18.5-29.0 kg/m²). Each subject received a single 0.2 U/kg dose of either GL-LIS, US-LIS, or EU-LIS in each of the 3 euglycaemic glucose clamp periods (ClampArt [automated computer-controlled glucose clamp device], clamp level 81 mg/dL, clamp duration 12 h post-dose, 3-14-day washout period between dosing visits). Dosing was based on the body weight measured prior to first LIS dose. Doses were administered using a prefilled pen. Primary PK endpoints were serum LIS concentration point estimates for C_{max} and $AUC_{0\text{-}12h}$. Primary PD endpoints were glucose infusion rate (GIR) point estimates for GIR_{max} and $AUC_{0\text{-}12h}$. Secondary outcomes included $AUC_{LIS.0\text{-}2h}$, $AUC_{LIS.0\text{-}4h}$, $AUC_{LIS.0\text{-}6h}$, $AUC_{GIR.0\text{-}2h}$, $AUC_{GIR.0\text{-}4h}$, $AUC_{GIR.0\text{-}6h}$, and $AUC_{GIR.6\text{-}12h}$, adverse events, and injection-site tolerability. Equivalence margins of 80-125% for PK and 80-120% for PD, log-transformed PK point estimates, and 90% confidence intervals (CIs; 95% CI for PD GL-LIS/EU-LIS point estimates) were prespecified.

Results: Overall, 38 subjects (mean±SD age 41±15 years, BMI 24.4±2.8 kg/m²) were randomised. GL-LIS and comparator insulins showed superimposable serum LIS concentration and GIR profiles (Figure). PK bioequivalence was demonstrated between GL-LIS and US-LIS, with $C_{LIS.max}$ = 89.8% (90% CI: [83.3-96.8]) and $AUC_{LIS.0\text{-}12h}$ = 94.6% (90% CI: [91.8-97.5]), and EU-LIS, with $C_{LIS.max}$ = 97.0% (90% CI: [90.0-104.6]) and $AUC_{LIS.0\text{-}12h}$ = 95.3% (90% CI: [92.5-98.2]). These CIs were well contained within the pre-specified equivalence margins. Likewise, the primary PD endpoints met bioequivalence criteria, with point estimates for GL-LIS compared to US-LIS GIR_{max} = 100.8% (90% CI: [95.6; 106.3]) and $AUC_{GIR\ .0\text{-}12h}$ = 101.5% (90% CI: [96.8; 106.3]), and compared to EU-LIS GIR_{max} = 103.6% (95% CI: [97.4; 110.1]) and $AUC_{GIR.0\text{-}12h}$ = 103.3% (95% CI: [97.3; 109.6]). Moreover, secondary PK/PD endpoints also supported bioequivalence. Several sensitivity analyses confirmed bioequivalence. All insulins had a low incidence of adverse events (5-11 events per insulin). No injection-site reactions were observed.

Conclusion: GL-LIS demonstrated both PK and PD bioequivalence and similar safety profile to US-LIS and EU-LIS formulations.



Clinical Trial Registration Number: NCT04235439

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Efficacy and safety of iGlarLixi vs insulin glargine 100 U/ml in Chinese people with type 2 diabetes (T2D) inadequately controlled on basal insulin (BI): LixiLan-L-China trial

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Background and aims: Efficacy and safety of iGlarLixi, a fixed-ratio combination of BI glargine 100 U/ml (iGlar) and the glucagon-like peptide-1 receptor agonist lixisenatide, has been demonstrated in Caucasian and Japanese populations. The LixiLan-L-China trial compared efficacy and safety of iGlarLixi vs iGlar in Chinese people with T2D inadequately controlled on BI ± oral antihyperglycaemic drugs (OADs).

Materials and methods: This 30-week, open-label, active-controlled study recruited Chinese people with T2D and HbA_{1c} 53-91 mmol/mol (7.0-10.5 %) despite BI treatment (10-25 U/day) ± ≤2 OADs. Participants were randomised 1:1 to iGlarLixi or iGlar, both titrated to fasting plasma glucose <5.6 mmol/l (<100 mg/dl) to a maximum dose of 40 U/day, both ± metformin. Primary endpoint was change in HbA_{1c} from baseline to Week (W) 30.

Results: 426 individuals were enrolled (mean diabetes duration 12.3 years; duration of previous BI use 2.7 years; BMI 25.2 kg/m^2; HbA_{1c} 65 mmol/mol [8.1 %]) without major differences in characteristics at screening/baseline. At W30, iGlarLixi demonstrated statistically superior HbA_{1c} reduction from baseline vs iGlar (**Table**), to a mean final level of 50 mmol/mol (6.7 %) vs 57 mmol/mol (7.4 %). iGlarLixi also showed significantly greater reductions in 2-h postprandial glucose (PPG) and bodyweight vs iGlar. Significantly greater proportions of participants achieved HbA_{1c} target (<53 mmol/mol [<7.0 %]) and HbA_{1c} target without weight gain at W30. Greater proportions of participants achieved HbA_{1c} target without weight gain at W30 and without Level 2 hypoglycaemic events (documented <3.0 mmol/l [<54 mg/dl]) during the 30-week treatment period with iGlarLixi vs iGlar. From a mean value of 18 U before randomisation in both groups, mean daily insulin dose increased during treatment to similar values for iGlarLixi (29 U [0.4 U/kg]) and iGlar (28 U [0.4 U/kg]) at W30. Level 2 hypoglycaemic events were similar for both groups (**Table**), and few severe hypoglycaemic events (Level 3) were seen (n=2 in each group). Apart from an expected higher incidence of gastrointestinal (GI) adverse events (AEs) with iGlarLixi (26% vs 17% with iGlar), safety profiles were generally similar for both treatments. No GI AEs were graded severe and no unexpected safety findings were identified.

Conclusion: Switching to iGlarLixi provided superior glycaemic control, with weight benefit, no additional risk of hypoglycaemia and relatively few GI AEs vs iGlar. iGlarLixi could offer an efficacious and well-tolerated option for Chinese people with T2D intensifying from BI ± OAD therapy.

Table

	Mean ± SD at baseline		Mean ± SD at Week 30		LS mean change ± SE at Week 30		
	iGlarLixi	iGlar	iGlarLixi	iGlar	iGlarLixi	iGlar	LS mean difference (95% CI)
HbA_{1c}, mmol/mol[a]	65.3 ± 8.6	65.0 ± 8.9	49.8 ± 8.2	57.0 ± 9.7	-15.6 ± 0.6	-7.7 ± 0.6	-7.9 (-9.5, -6.4)*
HbA_{1c}, %	8.13 ± 0.79	8.10 ± 0.81	6.71 ± 0.75	7.37 ± 0.89	-1.43 ± 0.06	-0.70 ± 0.06	-0.72 (-0.87, -0.59)*
Body weight, kg	69.3 ± 10.8	69.8 ± 10.7	69.2 ± 10.8	70.7 ± 11.2	-0.25 ± 0.17	+0.67 ± 0.17	-0.92 (-1.38, -0.46)*
2-hr PPG, mmol/l	16.2 ± 2.9	15.8 ± 3.2	10.0 ± 3.6	14.4 ± 3.2	-6.33 ± 0.25	-1.66 ± 0.25	-4.67 (-5.28, -4.07)*
					n (%) at Week 30		Proportion difference (95% CI)
HbA_{1c} target[b] achievement					133 (63.3)	63 (29.9)	35.3 (27.0, 43.7)*
HbA_{1c} target[b] without weight gain[c]					85 (40.5)	35 (16.6)	24.7 (16.6, 32.8)*
HbA_{1c} target[b] without weight gain[c] or hypoglycaemia[d]					73 (34.8)	30 (14.2)	21.3 (13.6, 29.1)*
	n (%)		OR (95% CI)		Events (PPY)		RR (95% CI)
Level 2 hypoglycaemia[d]	25 (11.8)	24 (11.3)	1.05 (0.58, 1.91)		31 (0.26)	33 (0.28)	0.94 (0.52, 1.69)

*p<0.0001; †p=0.0001; ‡p-value not assessed.

[a]HbA_{1c} values converted from values in %; [b]HbA_{1c} target <53 mmol/mol (<7.0 %); [c]at Week 30; [d]documented hypoglycaemia with plasma glucose <3.0 mmol/l (<54 mg/dl) during the 30-week open-label treatment period.

iGlar, insulin glargine 100 U/ml; iGlarLixi, a fixed-ratio combination of insulin glargine 100 U/ml and the glucagon-like peptide-1 receptor agonist, lixisenatide; LS, least squares; PPG, postprandial glucose, PPY, per-participant year; RR, rate ratio; SD, standard deviation; SE, standard error; U, unit.

Clinical Trial Registration Number: NCT03798080

Supported by: Sanofi

Disclosure: **X. Guo:** None.

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Effect of insulin degludec vs insulin glargine U100 on occurrence of nocturnal hypoglycaemia assessed by plasma glucose profiles in people with type 1 diabetes: HypoDeg trial

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Background and aims: The risk of nocturnal hypoglycaemia (NH) is a limiting factor for achieving good glycemic control in people with type 1 diabetes (T1D). Insulin degludec (IDeg) is proven to lower the risk of self-reported NH. As most episodes of NH are asymptomatic, we assessed differences in occurrence of NH by hourly plasma glucose (PG) measurements at treatments with IDeg or insulin glargine U100 (IGlar) in people with T1D suffering from recurrent nocturnal severe hypoglycaemia.

Materials and methods: These are the results of a pre-defined optional substudy of the HypoDeg trial, a 2-year investigator-initiated, 10-center, randomized, cross-over trial where 149 participants, with T1D and one episode or more of nocturnal severe hypoglycaemia within the last 2 years, were randomized to treatment with IDeg or IGlar. After one year of treatment (3 months wash-out and 9 months maintenance) the participants crossed over to the other treatment. Fifty-one participants (mean (SD) age 58 (13) years, diabetes duration 28 (14) years and HbA1c 7.8 (0.8) %, 62 (9) mmol/mol were admitted for two nights for hourly blinded plasma glucose measurements for a minimum of one night (23:00h to 07:00h) during each 1-year treatment period. The primary endpoints were NH at level 1 (PG ≤ 3.9 mmol/L) and level 2 (PG < 3.0 mmol/L).

Results: We collected data from 196 nights. A total of 57 nights in 33 participants were hypoglycaemic (at least ≤ 3.9 mmol/L). The incidence of NH at level 1 was lower when treated with IDeg [16 events in 97 nights (16%)] as compared to IGlar [36 events in 99 nights (36%)], corresponding to a hazard ratio (HR) of 0.39 (95% CI: 0.22-0.71; p=0.002) during treatment with IDeg. At level 2, the incidence of NH was also lower during treatment with IDeg [8 events in 97 nights (8%)] as compared to IGlar [22 events in 99 nights (22%)], corresponding to an HR of 0.36 [95% CI: 0.16-0.80; p=0.013] during treatment with IDeg.

Conclusion: In people with T1D and recurrent nocturnal severe hypoglycaemia, the treatment with IDeg as compared to IGlar results in a clinically significant lower rate of NH at both levels of hypoglycaemia as assessed by hourly plasma glucose measurements during the night.

Clinical Trial Registration Number: NCT02192450

Supported by: Novo Nordisk unrestricted grant

Disclosure: **J.M.B. Brøsen:** None.

SO 36 Determinants and consequences of hypoglycaemia

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Prolonged effect of hypoglycaemia on circulating immune cell composition in people with type 1 diabetes

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Background and aims: Iatrogenic hypoglycaemia activates the immune system and is associated with an increased risk for atherosclerotic disease. Since atherosclerosis is driven by vascular inflammation and mainly regulated by monocytes, we set out to determine the duration of the pro-inflammatory response to hypoglycaemia in people with type 1 diabetes.

Materials and methods: 39 adults with type 1 diabetes (M/F 21/18, age 47.0±18.9 years, diabetes duration 22.9±14.0 years, HbA1c 7.6±0.8 %) underwent a hyperinsulinemic-normoglycaemic (5.0±0.5 mmol/L) hypoglycaemic (2.8±0.1 mmol/L) glucose clamp. At the end of normoglycaemia, during hypoglycaemia and 24 hours, 72 hours and one-week later blood was drawn to determine immune cell composition using flow cytometry. In 21 participants of the study, an in-depth analysis of the circulating immune cell composition was performed.

Results: Acute hypoglycaemia increased granulocyte, lymphocyte and monocyte counts from 3.71 to $4.24 \cdot 10^3$/μL, from 1.56 to $2.40 \cdot 10^3$/μL and from 0.43 to $0.59 \cdot 10^3$/μL (all p<0.001), respectively. Granulocyte counts fell below baseline levels ($3.28 \cdot 10^3$/μL, p=0.016) after 72 hours before normalising, whereas levels of lymphocytes and monocytes also subsequently decreased, but remained elevated after a week (both p<0.001). Hypoglycaemia acutely led to a shift from classical monocytes (phagocytizing) towards intermediate (ROS producing and pro-inflammatory) and non-classical (patrolling endothelium and cytokine production) monocytes, which normalized after 24 hours. This pattern was also seen in CD36 (foam cell formation) and CCR2 (pro-inflammatory) positive monocytes. In the CD11b (pro-inflammatory) positive monocytes shift from classical towards intermediate and non-classic monocytes remained till after 72 hours (p=0.012) and in the CD41 (coagulation) positive monocytes till after a week (p=0.001).

Conclusion: Hypoglycaemia induces an early and prolonged increase in circulating immune cells. Phenotypically, hypoglycaemia induces a shift from classical monocytes to intermediate and non-classical monocytes. Future research is needed to determine the long-term consequences of these changes.

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Development and validation of a risk prediction model for hypoglycaemia in inpatients with type 2 diabetes during intensive insulin therapy

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Background and aims: Hypoglycemia is one of the most frequent adverse events associated with intensive insulin therapy. Few studies have reported predictive and risk factors for hypoglycemia in hospitalized patients with Type 2 diabetes mellitus (T2DM) received intensive insulin therapy. Therefore, we aimed to establish a hypoglycemia risk model for hospitalized patients with T2DM treated with intensive insulin therapy in China, identify the risk factors of hypoglycemia as soon as possible, and take active measures to reduce the incidence of hypoglycemia.

Materials and methods: We performed a retrospective study utilizing the electronic database of the department of Endocrinology and Metabolism of our university hospital. Data of 802 patients with type 2 diabetes undergoing intensive insulin therapy (insulin pump or multiple daily injection) were analyzed. Logistic regression analysis was used to derive the clinical prediction rule with hypoglycemia (defined as blood glucose ≤ 3.9mmol/L) as the main result, 10-fold cross-validation was used to internally validate the risk prediction model. The receiver operating characteristic curve was used to verify the discrimination of the model.

Results: In the derivation cohort, the incidence of hypoglycemia was 44.9%. The final model included eight variables: body mass index (BMI), diabetes duration, history of hypoglycemia within 1 year, treatment time, standard deviation of first day peripheral blood glucose (SDBG), natural logarithm of homeostasis model assessment of insulin resistance (lnHOMA-IR), estimated glomerular filtration rate (GFR) and triglycerides (TG)(Table 1). The area under the curve (AUC) of this model and the 10-fold cross-validation model for predicting incidence of hypoglycemia was 0.712 (0.677~0.748) and 0.697, respectively. By using this model for predicting incidence of hypoglycemia, the sensitivity is 68.3%, the specificity is 66.1%, and the Youden index is 0.344.

Conclusion: The model showed fair accuracy to predict hypoglycemia in inpatients with T2DM received intensive insulin therapy. It may be a useful tool for prevention of hypoglycemia in guiding intensive insulin therapy.

Table 1 Prediction model for hypoglycemia in inpatients with type 2 diabetes received intensive insulin therapy

variables	B	Wald value	p	OR (95%CI)
BMI	-0.073	8.839	0.003	0.93(0.886～0.976)
Diabetes duration	0.285	2.994	0.084	1.33(0.963～1.838)
Hypoglycemia history (within 1 year)	1.091	5.137	0.023	2.976(1.159～7.644)
treatment time (day)	0.1	16.477	<0.001	1.105(1.053～1.16)
SDBG of 1st day	0.086	4.292	0.038	1.089(1.005～1.181)
ln_HOMAIR	-0.549	26.858	<0.001	0.578(0.469～0.711)
GFR	-0.408	5.62	0.018	0.665(0.475～0.932)
triglycerides	-0.37	5.062	0.024	0.691(0.5～0.953)
constant	1.517	3.62	0.057	4.558

body mass index, BMI; standard deviation of blood glucose, SDBG; natural logarithm of homeostasis model assessment of insulin resistance, lnHOMA-IR; estimated glomerular filtration rate, GFR

Disclosure: **X. Hu:** None.

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Is obstructive sleep apnoea and/or nocturnal hypoglycaemia associated with arrhythmia in type 1 diabetes?

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Background and aims: Sudden nocturnal death in type 1 diabetes is known as the "*dead-in bed syndrome*" and supposedly caused by malignant cardiac arrythmias. The mechanisms are unknown but are assumed to be an interplay of different factors including nocturnal hypoglycaemia. A possible contributing factor may be obstructive sleep apnoea (OSA) with recurrent hypoxia. OSA is associated with increased risk of cardiac arrythmia and is common in type 1 diabetes. Therefore, OSA may be a contributing factor in causing the "*dead-in-bed syndrome*". The aim was to assess the association between OSA, hypoxia and frequency of cardiac arrythmias during daily life in type 1 diabetes and the potential interaction of hypoglycaemia.

Materials and methods: Fifth-five individuals with type 1 diabetes underwent nocturnal simultaneous recording of breathing (including oxygenation), recording of electrocardiogram (ECG) and continuous glucose monitoring (CGM). Half of the recruited participants had OSA and/or a history of cardiovascular disease. Frequency of cardiac arrythmias (ventricular premature beats (VPBs), atrial ectopic beats (AEBs) and bradycardia (< 45 bpm) (BRA)) and relation to presence of OSA (apnoea-hypopnoea index (AHI) ≥ 5/h), occurrence of hypoxia and hypoglycaemia (< 4.0 mmol/l) was explored.

Results: A total of 2,188 hours of simultaneous recorded breathing during sleep, ECG and CGM was obtained. Presence of OSA was not associated with frequency of nocturnal cardiac arrythmias (VPBs: IRR 0.95 [95% CI 0.23, 3.90], $p = 0.95$; AEBs: 4.81 [0.92, 25.23], $p = 0.06$; BRA: 0.79 [0.22, 2.78], $p = 0.71$). Occurrence of hypoxia was not associated to frequency of cardiac arrythmias during sleep (VPBs: 1.06 [0.96, 1.16], $p = 0.25$; AEBs: 0.97 [0.86, 1.09], $p = 0.58$; BRA: 1.15 [0.96, 1.39], $p = 0.14$). Occurrence of nocturnal hypoglycaemia was not related to risk of nocturnal arrythmias (VPBs: 1.12 [0.78, 1.60], $p = 0.54$, AEBs: 1.34 [0.40, 4.55], $p = 0.64$, BRA: 1.26 [0.47, 3.40], $p = 0.64$). Occurrence of simultaneous nocturnal hypoglycaemia and hypoxia was not associated with increased risk of nocturnal arrythmias (VPBs: 1.23 [0.82, 1.83], $p = 0.31$, AEBs: 0.88 [0.29, 2.67], $p = 0.82$, BRA: 1.56 [0.62, 3.94], $p = 0.35$).

Conclusion: Neither OSA nor hypoxia nor hypoglycaemia were associated with increased risk of nocturnal cardiac arrythmias in type 1 diabetes.

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Physical activity and type 1 diabetes - habits, management and obstacles

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Background and aims: Physical activity reduces the risk of diabetic complications and increases the psychological well-being of persons with type 1 diabetes. However, exercise induced hypo- and hyperglycaemia remains a challenge despite better insulins and new technology and may reduce participation in physical activity. Therefore, we measured self-reported exercise related problems and exercise habits in people with T1DM.

Materials and methods: This is a questionnaire-based study. We approached adults (age>18 years) with T1DM from the diabetes

outpatient clinics at Northern General Hospital in Sheffield (UK), Nordsjællands Hospital (DK) and Steno Diabetes Center Aarhus (DK) at their routine clinic visits. The questionnaire focused on exercise habits, diabetes management in relation to exercise, hypoglycaemia awareness status, and comorbidity. Clinical variables were extracted from patient records. Respondents were divided in two groups based on activity level assessed by the Saltin-Grimby scale: High activity and low activity.

Results: Questionnaires were obtained from 195 persons (54% from UK and 46% from DK, 49% females, duration of diabetes 26 (SD 16) years, 27% were insulin pump users).44% were high/vigorous physically active (high activity group) and 54% were inactive/moderate active (low activity group) (2% did not list activity level). 45% in the high and 74% in the low activity group wanted to be more active ($p<0.001$). 66% in the high and 55% in the low activity group stated that exercise made it easier to have glucose levels in the glycaemic target ($p=0.16$). 93% in the high and 84% in the low activity group had experienced hypoglycaemia which they considered to be caused by exercise ($p=0.08$). 51% in the high and 38% in low activity group experienced hyperglycaemia which they considered to be caused by exercise ($p=0.08$). The top three reasons for not performing more exercise were busyness at work (32%) and in private life (21%) and health related issues (20%). The top three reasons for performing exercise were physical and mental well-being (69%), to improve health (61%) and to improve glucose control (48%). 50% of participants reported that hypoglycaemia had stopped them from doing exercise and 85% reported that they take measures before or after exercise to prevent hypoglycaemia. 3% had experienced accidents (e.g. falls) during exercise related to hypoglycaemia. 54% in the high and 46% in the low activity group experienced that they had been guided about exercise and T1DM issues in the outpatient clinic ($p=0.11$).

Conclusion: People with T1DM participate in exercise to improve health, mental wellbeing and to maintain glucose targets. Most had experienced hypoglycaemia during or after exercise, which for around half had necessitated cessation of exercise in the situation. Disappointingly, only half reported receiving professional guidance regarding exercise. Systematic guidance in education programmes supplemented by written and online information may reduce hypoglycaemic risk and enable more individuals to increase their levels of physical activity.

Clinical Trial Registration Number: IRAS: 257851

Disclosure: **R.F. Johansen:** None.

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Hypoglycaemia increases the left ventricular ejection fraction in people with diabetes and healthy controls

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Background and aims: Insulin-induced hypoglycaemia, the most common adverse event in people with diabetes treated with insulin, is associated with increased risk of cardiovascular events. The effect of hypoglycaemia on cardiac function has not yet been fully clarified. Our aim was to investigate the effect of hypoglycaemia on left ventricular ejection fraction (LVEF) and global longitudinal strain (GLS) in people with type 1 diabetes (T1DM) and in healthy controls (HC).

Materials and methods: We enrolled 15 adults with T1DM (M/F 9/6, age 47±19 years, HbA1c 7.9±2.9%, diabetes duration 22.5±12.6 years) and 14 HCs (M/F 7/7, age 39±17 years). All participants underwent a hyperinsulinaemic normoglycaemic (5.3±0.4 mmol/L, 30 min) hypoglycaemic (2.8±0.5 mmol/L, 60 min) glucose clamp. At baseline and approximately 30 min into the hypoglycaemic phase (steady-state), a cardiac ultrasound was performed (by the same person) for later analysis.

Results: All participants had sinus rhythm at baseline and none developed arrhythmias during hypoglycaemia. We found no difference between T1DM and HC for LVEF measured at baseline. In response to hypoglycaemia, LVEF increased from 58.1±2.6% at baseline to 63.7 ±4.0% in the T1DM group ($p<0.0005$) and from 58.0±3.8% to 64.7 ±2.4%, ($p<0.005$) in the HC group. GLS was unchanged (-20.9±1.5% to -21.3±3.5% ($p=0.800$)) in the T1DM group, but a numerical decrease from -19.6±3.0% to -22.0±2.7% ($p=0.084$) was seen in the HC. Age did not modulate the effect of hypoglycemia on LVEF or GLS.

Conclusion: An event of hypoglycaemia increases the LVEF significantly in people with diabetes. Presumably due to the catecholamines chronotropic, inotropic and peripheral contracting effect. The result of this would be increased cardiac output, increased oxygen consumption and metabolism and thereby increased load on the heart. This may contribute to explain the link between hypoglycaemia and cardiovascular disease.

Clinical Trial Registration Number: NCT03976271

Supported by: IMI2 JU No. 777460

Disclosure: **T. Wilbek Fabricius:** Grants; Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 777460.

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Momentary assessment of type 1 diabetes patient's experiences in glucose variability and mood in real life (MERITS): first findings

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Background and aims: Mood fluctuations resulting from blood glucose excursions are commonly reported sources of diabetes distress. Research to date found small if any association between standard deviation (SD) or covariance (CV) measures of glucose variability and mood states. Time in Range (TIR) might be associated with mood. We aimed to assess the relationship between real-time glucose variability (GV) and glycemic control with current mood in adults with type 1 diabetes (T1D), taking into account inter-individual differences in psychological traits.

Materials and methods: Participants (type 1 diabetes > 1 year on MDI) wore a blinded diagnostic glucose sensor (Medtronic iPro) and received prompts on their smartphones 6 times a day to answer short questions about their current mood, measured with the Profile Of Mood States (POMS)-SF (dimensions: Anxiety, Dejection, Anger, Fatigue, Vigor) for a minimum of 1 week to a maximum of 4 weeks. At baseline, participants completed questionnaires to assess trait anxiety (GAD-7), emotional well-being (WHO-5) and fatigue (CIS-8). Covariance (CV), TIR (≥3.9 and ≤10 mmol/l), Time Above Range (TAR, >10 mmol/l) and Time Below Range (TBR, < 3.9 mmol/l) were calculated to represent GV and glycemic control within one day. Mixed model analyses examined the association between GV measures and POMS dimensions (mean within one day) with a random intercept to account for clustering within patients. Fixed effects included were: GV, time, age, sex, HbA1c, diabetes duration. For the POMS subscales Anxiety, Dejection and Fatigue, we added baseline GAD-7, WHO-5 and CIS-8 respectively, to the models in interaction terms with GV.

Results: N=22 (12 female) with T1D, mean age 44.1 ± 13.6, mean diabetes duration 19.7 ± 12.3 years, mean TIR 58.6 ± 24.1% and mean HbA1c 58.8 ± 11.2 mmol/mol participated. Days of observations ranged from 8 to 30 days (median 16 days). Mixed methods analyses showed that GV (CV, TAR and TBR) was significantly associated with the mean POMS Anxiety score with an interaction between GV and the GAD-7 score (CV: Estimate = 0.49, SE = 0.30, GAD-7 Estimate = 0.06, SE = 0.20, Interaction Estimate = -0.11, SE = 0.17,p = 0.001; TAR: Estimate = -.07, SE = 0.16, GAD-7 Estimate = 0.005, SE = 0.02, Interaction Estimate = 0.04, SE = 0.02 , p<0.05; TBR: Estimate = 0.41, SE = 0.36, GAD-7 Estimate = 0.03, SE = 0.02, Interaction Estimate = -0.11, SE = 0.05, p = 0.03): Only for those with higher trait anxiety at baseline, lower CV and less TBR were associated with higher state anxiety. Increased TAR was associated with more state anxiety, especially in those with higher trait anxiety at baseline.Further, we found trends for higher GV (CV) to be associated with more Fatigue (CV Estimate=0.44, SE=0.24, p=0.06) and increased TIR with more Vigor (TIR Estimate = 0.3, SE=0.1, p=0.07).

Conclusion: Our findings suggest that higher glucose variability and decreased glycemic control within one day are associated with more fatigue and less vigor, while the association with anxiety seems to be moderated by trait anxiety. The direction of the association between real-time mood and glucose variability and glycemic control warrants further investigation.

Supported by: Sanofi

Disclosure: **M. de Wit:** Grants; Sanofi.

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The glycaemic status determines the direction of the relationship between the red cell distribution width and HbA_{1c}

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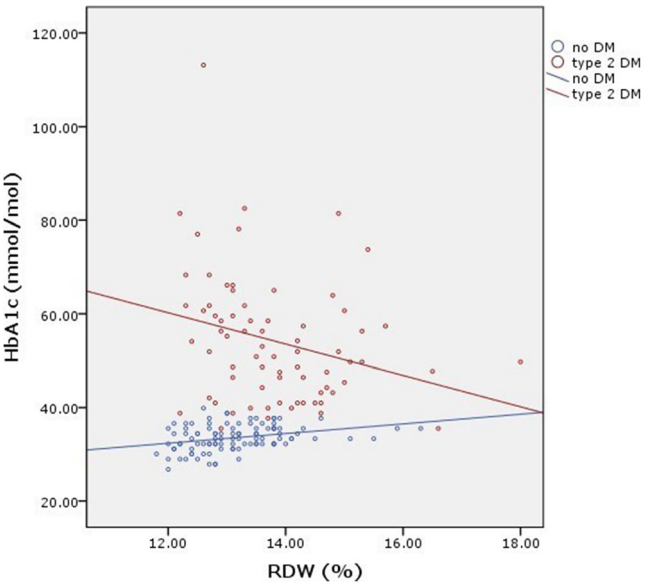
Background and aims: The erythrocyte distribution width (RDW) is a sensitive marker not specifically affected in the early stages of hematologic and other morbid conditions. Available data on the relationship between RDW and glycated hemoglobin (HbA1c) are equivocal and potentially confounded by overlooked hematologic abnormalities. The aim of the present study is to investigate the relationship between RDW and HbA1c within two groups of selected hematologically healthy individuals with and without T2DM.

Materials and methods: Paired measurements of RDW and HbA1c were obtained within two groups comprised of hematologically healthy participants (group 1: n=100 without DM, mean HbA1c 5.22±0.25%, mean RDW 13.2±0.8% and group 2: n=87 with T2DM, mean HbA1c 7.03 ±1.21% mean RDW 13.8±1.1%). All participants had hemoglobin concentrations and erythrocyte indices within the normal range. The presence of hemoglobin variants or thalassemic syndromes was excluded through hemoglobin electrophoresis. The association of HbA1c with hematologic parameters (hemoglobin, log[ferritin], RDW) and factors related to glycemia (BMI, fructosamine, FPG) was examined in the two groups separately and within the sum of the study sample.

Results: There was a significant positive correlation of RDW with HbA1c among those without DM while the opposite was true among individuals with T2DM (r=0.315, p=0.001 and r=-0.258, p=0.021). In the T2DM group a significant negative correlation between RDW and fructosamine was noted (r=-0.374, p=0.001) which was absent among normoglycemic individuals (r=-0.144, p=0.154). Among those without DM the association between HbA1c and RDW remained significant after adjustment for all tested parameters. In the population with T2DM the significance was attenuated after including glycemia-related factors. Across quartiles of ascending RDW values, there was a gradual increase of HbA1c (p= 0.013) and decreases of Fructosamine/HbA1c and FPG/HbA1c ratios (p=0.018 and <0.001, respectively) among those without DM and decreases of HbA1c and fructosamine among those with T2DM (p=0.009 and 0.004, respectively). In multivariable regression in the sum of the study sample, the interaction between diabetes status and RDW as regards HbA1c was significant (p=0.002) and remained significant (p<0.001) after adjustment for multiple potential confounders which differed between the two groups (age, BMI, hemoglobin concentration, FPG, fructosamine).

Conclusion: The relationship between RDW and HbA1c may reflect distinct phenomena among those with and without T2DM. Among normoglycemic individuals, the RDW likely reflects the non-glycemic interference on HbA1c values, while in T2DM RDW may serve as an indirect index of glycemia and dysmetabolism.



Disclosure: **D. Tsilingiris:** None.

SO 37 New approaches to health care delivery

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Impact of diabetes group visits on patient clinical outcomes: results from a cluster randomised intervention trial among U.S. Midwestern health centres

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Background and aims: U.S. health centers (HCs), community-based clinics that provide primary care in underserved areas, care for 2.5 million American adults with diabetes, of whom 30% have suboptimal glycemic control. Diabetes group visits (GVs) have been shown to improve clinical outcomes but few have reported results from multi-center trials or in the HC setting.

Materials and methods: In a cluster randomized trial, we assigned 14 HCs to a GV intervention arm or usual care. Intervention sites conducted six monthly GVs with up to 15 adult patients with uncontrolled type 2 diabetes (A1C≥8%). 75 adult patients were enrolled in GVs. In the usual care arm, chart abstraction was conducted on 120 patients. Primary outcome was change in A1C from baseline to 12 months. Secondary outcomes were changes in blood pressure and low density lipoproteins (LDL) and processes of care. GV patients completed surveys at baseline, 6 and 12 months. Generalized linear mixed models and linear mixed models were used to test the effects of GV, timepoint and their interaction. Models were adjusted for age, gender, baseline insurance, number of complications, depression and anxiety status.

Results: 195 patients were enrolled (mean age 53 ± 12 years, 61% female, 27% African American, 40% white, 25% Latino, 6% American Indian/Native American, mean baseline A1C 9.5% ± 1.8%). At baseline, the intervention group had higher rates of diabetes-related comorbidities (65% vs. 49%, p=0.04), anxiety (33% vs. 15%, p=0.005) and depression (48% vs. 22%, p=0.0002). Intervention patients attended an average of 3.5 ± 1.9 GVs. At 12 months, A1C was not significantly different in the intervention (8.91% ± 1.90%) compared to usual care (9.18% ± 1.68%, p=0.57). However, attending 4-6 group visits was associated with significant reduction in A1C compared to no visits (-0.48% vs. 0.6%, p=0.02) at 12 months. There was no significant difference in blood pressure or LDL. GV patients were more likely to have BMI, A1C and annual lipids drawn compared to usual care (p<0.05). GV patients had more visits with a certified diabetes educator (21% vs. 2%, p<0.001) in the 12-month post-intervention period. Patient satisfaction with current diabetes treatment improved (p=0.02). At 6 months, diabetes social support (p=0.02), diabetes self-empowerment (p=0.05) and diabetes distress improved (p=0.03). Among those who reported a mental health problem, there was an increase in the percentage of patients who reported being prescribed a medication (50% vs. 94%, p=0.01) and seen by a mental health provider (44% to 68%, p=0.01) from baseline to 6 months.

Conclusion: We did not see improved glycemic control in GV patients compared to control. However, GV attendance was associated with improved A1C. GV patients were more likely to have improved processes of care and more engagement with diabetes education post-intervention. GV patients, who had high rates of comorbidities, anxiety and depression, noted improvements in social support, distress and treatment for mental health concerns.

Clinical Trial Registration Number: NCT03487692

Supported by: US Dept of HHS, OMH and NIH

Disclosure: **A.A. Baig:** Grants; U.S. Department of Health and Human Services, Office of Minority Health, U.S. National Institutes of Health.

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Person-centered approach for elderly in chronic disease management

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Background and aims: Individuals with type 2 diabetes, COPD, increased cardiovascular risk and cardiovascular disease are, in the Netherlands, treated in single-disease oriented chronic disease management programs. Although, in recent years a shift has taken place from focusing on single-disease management to a more holistic person-centered care approach, this is not daily yet practice. The latter stresses the need to individualize treatment targets and considering preferences, needs and values of the patients. In alignment with this shift, the Dutch Diabetes Federation has developed a toolkit for patients and general practitioners to encourage a person-centered consultation method. A recent adaptation of the tool makes it applicable for people with any chronic condition. This adapted tool gives us the opportunity for a multimorbidity-oriented integrated person-centered consultation approach. Therefore, the aim of this study was to evaluate treatment satisfaction of patients in chronic disease management and of their general practitioners (GPs) who participated in the multimorbidity-oriented integrated, person-centered annual consultation. Moreover this study gives more insight in patient activation and illness perception of elderly patients with chronic diseases like diabetes.

Materials and methods: An observational, cross-sectional study at five healthcare centers in the center of the Netherlands, between April and December 2019. Individuals aged ≥65 years old enrolled in chronic disease management programs were approached to participate in an multimorbidity-oriented integrated annual consultation with their GP in which all conditions over all domains were discussed. Patient characteristics were extracted from the electronic medical records. Profiles of participants and non-participants were compared. Outcomes of process evaluation questionnaires were evaluated among GPs and patients. Patient activation and Illness Perception were measured with validated questionnaires (PAM and BIPQ).

Results: Of 508 patients eligible to participate, 254 (50%) agreed, 48.5% men, and mean age 73.0 (SD 5). Participants and non-participants did not substantially differ in various patient-specific and disease-specific characteristics. Participants indicated 'Physical health' most often (64.1%) as topic to discuss during the consultation, followed by topics regarding 'Wellbeing' (48.5%), 'Selfcare' (41.9%) and 'Lifestyle' (40.9%). GPs reported that the consultation was partly or completely appropriate for the patient in 76%. Participants rated their consultation with a median score of 9.0 (IQR 1.00) on a scale from 1 to 10, and 93% reported they would like to continue having annual multimorbidity-oriented integrated consultations annually. No differences were found within the participants between chronic disease groups on patient activation and illness perceptions.

Conclusion: Both patients and GPs were highly satisfied with this new person-centered way of consultation and preferred to continue this multimorbidity-oriented consultation annually.

Disclosure: **H.E. Hart:** None.

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The pattern of testing for glycosylated haemoglobin (HbA_{1c}) in people with diabetes is linked to the long term trajectory of blood glucose control

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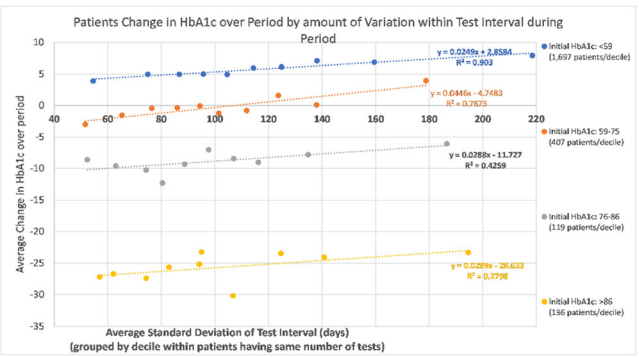
Background and aims: American Diabetes Association Guidelines (ADA) advocate testing "at least two times a year in patients who are meeting treatment goals" and "quarterly in patients who are not meeting glycaemic goals". We previously described links between HbA1c testing frequency and outcome to show that many people with DM are not tested at the recommended frequency. Here we describe how variability in test interval over time relates to the deterioration of HbA1c control.

Materials and methods: Laboratory HbA1c level data, number of tests, interval between tests, variability in this interval and demographic data from a large UK hospital between June 2012-July 2019 was analysed. The outcome measure was the change in HbA1c over a 5-year period, focusing on those patients with a HbA1c within the first two years of the study period who also had a HbA1c 5 years±3 months after the starting HbA1c. To examine the link between variability in HbA1c testing interval, we focused on those with at least 6 tests during this period (n=23,582 patients).To minimise the impact of test numbers on interval SD, we separated the individuals into groups based on the number of tests between t0 and t0+5yrs and calculated the SD decile for each group. We then examined the effect of the combined SD deciles, together with other variables, on the change in HbA1c level between t0 and t0+5yrs, stratifying by starting HbA1c category.

Results: We show greater variability in testing frequency is associated with deterioration in HbA1c control over the 5-year period. This effect was most pronounced in those with lower starting HbA1c levels. In those with a starting HbA1c of <59mmol/mol, the lowest SD decile was associated with an increase in mean HbA1c of 3.9mmol/mol while those with the highest decile was more than double this 7.9mmol/mol. Similarly, in those with a starting HbA1c of 59-75mmol/mol, the lowest SD decile was associated with a mean reduction of 3mmol/mol, while those in the highest decile demonstrated a 4mmol/mol rise in HbA1c. In those with starting HbA1c values of 76-86mmol/mol and >86mmol/mol, the same trends were observed but were less marked. These effects were independent of the interval between tests. Mean HbA1c level also increased with increasing SD decile, an effect that was apparent irrespective of starting HbA1c.

Conclusion: We show that increased variability in the interval between HbA1c monitoring tests is linked to increasing HbA1c over time, irrespective of the interval between tests. These findings indicated that regular monitoring, not just HbA1c level or the number of tests/year, is important in order to maintain diabetes control, especially in those people with relatively well-maintained diabetes control. This has implications for the ongoing management of those patients who attend sporadically for testing and suggests that general practitioners may need to develop



Disclosure: **A.A. Fryer:** None.

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Effectiveness of diabetes education tailored to psychiatric nurses on quality of diabetes care and psycho-social outcomes in people with diabetes and severe mental illness

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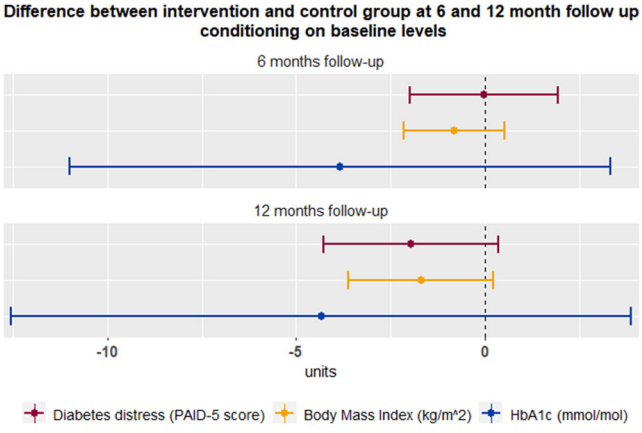
Background and aims: Co-existence of diabetes and severe mental illness (SMI) is associated with barriers in diabetes management, higher risk of diabetes complications and death. Psychiatric nurses may have a key role in improving diabetes outcomes in this population but need more diabetes knowledge and skills. This study aims to examine the effectiveness of diabetes education tailored to psychiatric nurses on quality of diabetes care and psycho-social outcomes in persons with diabetes and SMI.

Materials and methods: This pragmatic non-randomized cluster trial was conducted in 8 psychiatric out-patient clinics in Denmark. In the 4 clinics in the intervention group, all psychiatric nurses completed a 3-days education on diabetes symptoms, screening, complications and treatment to increase knowledge and skills to support persons with SMI and diabetes in better diabetes care. The control clinics continued usual clinical practice. We recruited persons with any type of diabetes treated in the 8 clinics. Data from medical records (HbA_{1c}, BMI) and questionnaires (diabetes distress (PAID-5), diabetes support) was collected at baseline, 6- and 12-months (mo) post intervention. Data was analyzed in linear regression models conditioning on values at baseline.

Results: 108 participants were included with baseline mean (SD) age of 52 (12) years, 47 (44%) were women, 84 (78%) had type 2 diabetes and 63 (58%) had schizophrenia. There were no significant statistical differences at baseline in age, sex or diagnoses between the intervention (n=51, 47%) and control group. The proportion of participants reporting 'some to high diabetes support' from the psychiatric nurses was higher at follow-up in the intervention than in the control group; baseline (23 (74%) vs. 31 (67%)), at 6 mo (23 (82%) vs. 29 (66%)) and at 12 mo follow-up (16 (80%) vs. 23 (74%)). As shown in the Figure we saw a statistically insignificant difference in the levels of HbA1c, BMI, diabetes distress (PAID-5) in the intervention group compared to the control group at 6- and 12-mo follow-up. There was a higher decrease in proportion of participants with high diabetes distress (PAID-5≥8) in the intervention group compared to the control group at follow-up; baseline (19 (51%) vs. 24 (50%)), at 6 mo (12 (41%) vs. 17 (39%) and at 12 mo (8 (36%) vs. 14 (42%)).

Conclusion: This trial suggests a tendency to improvements on indicators of quality of diabetes care and lowering of diabetes distress in the intervention compared to the control group. The statistical insignificant results may be due to the small sample size but could be meta-analyzed with similar data. Increasing diabetes knowledge and skills among psychiatric nurses may improve diabetes outcomes, however, there is a need for more and larger studies.



Clinical Trial Registration Number: ISRCTN15523920

Supported by: This study has been funded by Steno Diabetes Center Copenhagen through an unrestricted grant from Novo Nordisk Foundation and Jascha Foundation; case number 6994

Disclosure: **L. Knudsen:** None.

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A core outcome set for the treatment of pregnant women with pregestational diabetes

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Background and aims: Meaningful comparisons between studies evaluating interventions of pregnant women with pregestational diabetes mellitus (PGDM) are limited due to the heterogeneity in outcome selection and reporting. This study developed a core outcome set (COS) for randomised controlled trials (RCTs) evaluating the effectiveness of interventions for the treatment of pregnant women with PGDM.

Materials and methods: The study consisted of three components. 1) A systematic review of the literature to produce a list of outcomes reported in RCTs assessing the effectiveness of interventions for the treatment of pregnant women with PGDM. 2) A three-round, online eDelphi survey to prioritise these outcomes by international stakeholders (including women with PGDM, healthcare professionals and researchers). 3) A consensus meeting where stakeholders from each group decided on the final COS.

Results: We extracted 131 unique outcomes from 67 records meeting the full inclusion criteria. A total of 205 stakeholders completed round 1. Of these, 174/205 (85%) and 165/174 (95%) completed round 2 and 3, respectively. Participants at the subsequent consensus meeting chose 19 outcomes for inclusion into the COS: trimester specific HbA1c, maternal weight gain during pregnancy, severe maternal hypoglycaemia, diabetic ketoacidosis, miscarriage, pregnancy induced hypertension, pre-eclampsia, maternal death, birth weight, large for gestational age, small for gestational age, gestational age at birth, preterm birth, mode of birth, shoulder dystocia, neonatal hypoglycaemia, congenital malformations, stillbirth and neonatal death.

Conclusion: This COS will enable better comparison between RCTs to produce robust evidence synthesis, improve trial reporting and optimise research efficiency in studies assessing treatment of pregnant women with PGDM.

Disclosure: **O. Kgosidialwa:** None.

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Effect of traditional versus communication technology-based health educational intervention focusing on diabetes in Bangladesh: a randomised controlled trial

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Background and aims: Communication Technology based Health Education (CTHE) and Traditional Health Education (THE) both type of intervention played an effective role to foster long term improvements in adherence to diabetes self-management. But it is not yet identified that which one is more effective for peripheral patients of Bangladesh. This study was designed to compare the effectiveness of these two types of intervention.

Materials and methods: This was a randomized controlled trial conducted in Thakurgaon district of Bangladesh with two types of intervention groups (CTHE and THE) and one control. CTHE received educational session with reminder and monitoring through mobile phone voice calling; THE group received educational session with home visit-based reminder and monitoring; while the control received only educational session. Each group had 330 adult diabetics. Entire the 12 months intervention, educational session was conducted in the 1st month by using a pictorial educational material including logbook; 11 reminders and monitoring were conducted on the rest 11 months. Data were collected by face to face interview using semi-structured questionnaire. Analysis of Covariance and regression were used in the analysis.

Results: CTHE and THE groups showed significant ($p<0.01$) improvement in knowledge, adherence to self-management and health outcome compared to control. Bonferroni post hoc comparison between groups showed that in most components of knowledge (diet, mean difference: 6.04; physical exercise/ activities, 3.48; follow-up visit/ blood glucose test, 4.88; avoid tobacco intake, 3.09; basic knowledge, 1.49 and technical knowledge of diabetes, 2.65) and waist circumference (mean difference: 5.12) of CTHE group was significantly improved than the THE group. Likewise, adherence (percentage) to drug (CTHE vs. THE, 57% vs. 53%); physical exercise/ activities (55% vs. 42%); follow-up visit/ blood glucose test (69% vs. 50%); and avoid tobacco intake (26% vs. 25%) improved in the CTHE group compared to THE group. Furthermore, CTHE (mean±SD, 21.96±7.94) was revealed as the cost effective techniques in diabetes self-management among peripheral and disadvantaged patients compared to THE (mean±SD, 30.21±16.00).

Conclusion: CTHE based intervention with reminder and monitoring seems to more effective compared to the THE based intervention in improving knowledge, management adherence and health outcomes of peripheral diabetic patients of Bangladesh.

Supported by: Heidelberg University Germany

Disclosure: **B. Banu:** None.

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Virtual versus in-person pump and CGM training experiences among adults with diabetes in Europe

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Background and aims: While the COVID-19 pandemic has not stopped the influx of new pump and CGM users, it has altered the means through which new users receive training. Proper training and guidance are essential to ensuring people with diabetes can use their devices comfortably and confidently. Given the shift to online platforms, the present study

aims to investigate differences in experiences among those receiving virtual and in-person pump and CGM training.

Materials and methods: From October-November 2020, adults with diabetes in Europe from an opted-in panel were surveyed. Respondents who started using a new pump or CGM model in the last year and who received training from a professional either in person or virtually (pump users: n=178, 81% in person, 99% type 1, 38±13 years, 35% new to pump therapy; CGM users: n=261, 85% in person, 91% type 1, 41±14 years, 41% new to continuous glucose monitoring) were asked about the training methods they engaged in and confidence in using their new device immediately after training. Statistical analysis was conducted using two-proportion z-tests.

Results: Confidence ratings (percentage of those rating their confidence as 9 or 10 on a 1-10 scale) did not differ significantly across in-person and virtual training methods for both pump users (53% vs. 59%, p=0.58) and CGM users (59% vs. 64%, p=0.58). Those trained virtually were significantly more likely than those trained in person to report engaging in additional training methods for both pump (62% vs. 22%, p<0.001) and CGM use (41% vs. 24%, p=0.03). New pump users trained virtually were more likely than those trained in person to report watching online training videos (41% vs. 9%, p<0.001), completing a self-guided online tutorial (21% vs. 3%, p<0.001), and reading pamphlets and training materials (38% vs. 20%, p=0.03). New CGM users trained virtually were significantly more likely than those trained in person to report completing a self-guided online tutorial (31% vs. 6%, p<0.001) and watching online training videos (28% vs. 15%, p=0.04).

Conclusion: The majority of new pump and CGM users were highly confident in using their devices regardless of whether they were trained in person or virtually, suggesting that virtual training can be a strong alternative to in-person guidance. However, those trained virtually are more likely to engage in additional self-guided training methods compared to those trained in person, suggesting that current virtual training methods may not be comprehensive enough for new device users. Future research should aim to identify the unmet needs and areas for improvement of virtual device training programs.

Disclosure: **E.R. Ye:** Other; I am an employee of dQ&A, a company that provides research services for a fee to several clients (>10) in the diabetes field.

SO 38 Psychological aspects of diabetes care

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A qualitative evidence synthesis exploring the determinants of self-management in adults with severe mental illness

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Background and aims: People living with severe mental illness (SMI) have a reduced life expectancy of around 15-20 years, principally due to the high prevalence of long-term conditions (LTCs) such as diabetes. Management of these LTCs relies predominately on self-management. People living with SMI experience numerous challenges when trying to self-manage their physical health. Little is known about the determinants of self-management of LTCs in this population. This review aims to synthesise qualitative research exploring determinants of self-management in adults with SMI, to inform the development of programmes to support self-management for people with both SMI and diabetes.

Materials and methods: Six databases, including CINAHL and Medline, were searched to identify qualitative studies that explored people's perceptions about determinants of self-management in adults with SMI (with or without co-morbid LTCs). Studies were eligible if they included participants with SMI and/ or those who provide care or support for people with SMI. Self-management behaviours were identified using the American Association of Diabetes Educator's self-care behaviours (AADE7), while determinants were identified using the Capabilities, Opportunity, Motivations and Behaviours (COM-B) framework. Purposive sampling was used to identify eligible studies for thematic synthesis, according to data richness (assessed using Ames et al (2017)'s data richness scale).

Results: Twenty-five articles were included in the synthesis. Six studies focused on self-management of diabetes alongside SMI, with the remaining articles exploring different aspects of self-management in people with SMI. Six analytic themes and 28 sub-themes were identified from the synthesis. The themes included: the additional burden of SMI; living with co-morbidities; beliefs and attitudes about self-management; support from others for self-management; social and environmental factors; routine, structure and planning.

Conclusion: For people living with SMI, self-management behaviours are directly influenced by their experience of SMI. The burden and symptoms of SMI can act as barriers to self-management of LTCs, such as diabetes. Social and professional support, improved access to community and financial resources, and increased patient involvement in their own care, could promote self-management. Support programmes for people living with SMI and diabetes should address these experiences and the unique needs of this population.

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Prevalence of needle phobia and anxiety among younger subjects with diabetes who are uncontrolled on multiple oral glucose lowering drugs and influence counselling

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Background and aims: Many patients express reluctance to begin injectable therapy, leading to delayed initiation and low persistence with treatment. Multiple studies have identified both provider-related and patient-related barriers to the initiation of insulin. The main objective of the study was to evaluate prevalence of needle phobia and anxiety among younger subjects with diabetes who are uncontrolled on multiple oral glucose lowering drugs and influence of experience and psychological counselling.

Materials and methods: This is an observational trial. The Brief Illness Perception Questionnaire was used to assess the cognitive and emotional aspects of illness. Hamilton Anxiety Rating Scale for assessing severity of anxiety. statistical software like IBM Statistical Package for Social Sciences (SPSS Ver. 25) were used to calculate the statistical analysis. mean ± standard deviation were used to express the mean data value of continuous variables.

Results: Total 450 adult T2DM patients were included in this study who denied to take insulin. The mean age of the subjects was 42.81 (± 10.31) years, duration of diabetes was 5.87 (± 2.57) years, and A1C during the prior year was 8.5 (± 1.7)%, and 62% were female. On counselling it was revealed that a high percentage (68%) of patients reported high fear and distress with needles at diagnosis. Anxiety for injection or needles is associated with higher HbA1c levels and greater avoidance behaviour of diabetes management, such as fewer insulin injections. Needle phobia was more prevalent in females. Out of 450 patients, 262 (58.2%) patients had psychiatric comorbidity. Main factors associated with the insulin rejection were timing of insulin injection, thicker needles, pain sensation, apprehension of nodules formation and higher cost. Majority of patients express anxiety as insulin is difficult to take, insulin means treatment failure, fear of weight gain and fear of hypoglycaemia. Comprehensive assessment of patient's fear, appropriate needle selection, patient education, the use of behavioural interventions such as breathing exercises, and follow-up monitoring has drastically reduced the fear by 29.7% at day 1 and 23% at subsequent follow up visit within 3 months. Although most improved, 13.6% of patients continued to report high fear and distress 6 to 9 months later.

Conclusion: Fear of needles is common in younger diabetic subjects requiring insulin therapy. Comprehensive assessment of patient's fear, appropriate needle selection, patient education, the use of behavioural interventions and follow-up monitoring has drastically reduced the fear.

Disclosure: **A. Baidya:** None.

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Cognitive decrements and ventricular volume increase are related to white matter lesion presence in type 1 diabetes without peripheral microangiopathy

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Background and aims: Cognitive impairments in type 1 diabetes mellitus (T1DM) are mainly found in processing speed and executive functions. In T1DM, cognition seems most affected in those with peripheral microangiopathy, which could be a marker of cerebral microvascular disease. We aimed to explore the relationship between white matter lesions (WML), a common marker of cerebral microvascular disease, brain structure and cognition in T1DM patients.

Materials and methods: Presence and severity (Fazekas) of WML as well as brain volume (FSL-SIENAX) were quantified with 1.5T MR-imaging in T1DM patients with (n = 51) and without (n = 53) peripheral microangiopathy and in 48 healthy control subjects. In addition, participants underwent a cognitive assessment. Effects of WML in T1DM and the additional effect of peripheral microangiopathy were determined using linear regression, unadjusted and adjusted for age, sex, systolic blood pressure and depressive symptoms.

Results: WML were present in 43 (41.3%) of all T1DM patients versus 22 (45.8%) of controls ($p = 0.73$). Patients with WML (age: 43.3±8.0 years; females: 36 (59%); systolic blood pressure: 133.3±13.2; depressive symptoms: 8.8±7.1) were significantly older ($p = 0.03$) than those without WML (age: 39.5±9.1 years; females: 27 (62.8%); systolic blood pressure: 129.9±17.8; depressive symptoms: 9.3±9.5). All, but 1, patients with WML had Fazekas 1. In all T1DM, WML presence did not influence brain volume or cognition (all $p > 0.05$). Assessing the influence of peripheral microangiopathy, no effects of WML were found in those with peripheral microangiopathy (all $p > 0.05$). In those without clinically manifest peripheral microangiopathy, WML presence was related to lower memory ($\beta = -0.296$; $p = 0.03$) and executive functions ($\beta = -0.282$; $p = 0.04$) performance, as well as increased ventricular volume ($\beta = 0.325$; $p = 0.02$). All effects remained significant in the fully adjusted models.

Conclusion: The presence of WML moderately affected cognition and ventricular volume in this group of middle-aged T1DM patients, but only in those without clinically manifest peripheral microangiopathy. These findings may support an early moderate effect of cerebral microangiopathy on cognition and brain volume, which might be overshadowed when peripheral microangiopathy advances.

Supported by: Dutch Diabetes Research Foundation

Disclosure: **C.G. Pariz:** None.

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Effects of bariatric surgery on quality of life, body image and sex life in obese women

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Background and aims: Bariatric surgery and consequent weight loss improve metabolic and anthropometric parameters in obese subjects. However, a detailed assessment about the impact of bariatric surgery on quality of life (QoL), body image perception, and sex life is lacking. The aim of this study was to assess the QoL, body image satisfaction and sexual function in 389 female patients with obesity (age 40±10 years, pre-surgery weight 120.4±17.7 kg) before and at least 6 months after bariatric surgery (60% sleeve gastrectomy, 40% gastric bypass).

Materials and methods: All participants were asked to complete the following online self-report questionnaires seven days before and six

months after bariatric surgery: the Body Uneasiness Test (BUT) to assess self-perception of body image, the Short Form-12 (SF-12) to QoL as per the physical (PCS) and mental (MCS) domains, and the Female Sexual Function Index (FSFI) to screen for sexual dysfunctions in women.

Results: After bariatric surgery, patients lost 44.5±16.5 kg ($p<0.001$), with an increase of the PCS (53.3±7.2 vs 31.9±21.4, $p<0.001$) and MCS (50.5±8.8 vs 39.6±12.5, $p<0.001$) scores, that fell within the normal range of healthy individuals. All patients showed a significant improvement of the global score index domain of the BUT (1.9±1.1 vs 3.7±0.9, $p<0.001$). The youngest patients (18-39 years old) who lost at least 40% of the baseline weight obtained a normalization of their body image concern domain results, reaching the reference range for healthy non-obese subjects. After bariatric surgery, patients also had a significant improvement of the FSFI (26.8±9.7 vs 19.6±8.8, $p<0.001$), with an increase of non-dysfunctional, sexually active women from 26.2% to 78.2%. Significant correlations were found between the extent of weight loss and the improvement of all the psychometric parameters.

Conclusion: Bariatric surgery is an efficient and safe treatment for obesity, and the physical gains related to weight loss are also mirrored by significant improvements in mental and physical health and sexual function.

Disclosure: **L. Di Gioia:** None.

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Motivational interviewing and self-care in type 1 diabetes: a randomised controlled clinical trial

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Background and aims: Motivational interviewing is a communication tool that seeks a change of habits motivated by the patient him/herself. It has proved efficacy in addictions and obesity. It is associated with an improvement in glycemic control in type 2 diabetes, but the results are contradictory in adolescents with type 1 diabetes, and there are no previous studies in adults with this disease. The aim of the study was to evaluate the impact of motivational interviewing, applied in endocrinology visits, on self-care in adults with type 1 diabetes.

Materials and methods: Three endocrinologists were trained in motivational interviewing and performed the interventions. A randomized controlled (1:1 ratio) single-blind parallel-group clinical trial was performed. Adults with type 1 diabetes of at least 1 year duration and an Hb1Ac ≥8% and/or an episode of severe hypoglycemia in the past 6 months were included. Both groups were followed for 16 months in parallel, with 4 visits (intervention with motivational interviewing or usual practice) in each group, and a 5th evaluation visit. Fidelity to the intervention was assessed by recording the visits and review by two psychologists, blinded to the intervention, following the EVEM scale. The primary outcome variable was self-care, assessed using the Spanish version of the validated Diabetes Self-Care Inventory-Revised version. The secondary outcomes were glycosylated hemoglobin (Hb1Ac), self-efficacy, motivation and satisfaction with self-care, health-related quality of life (ViDa1), satisfaction with the doctor-patient relationship and self-defined goals. The results will be analysed by intention-to-treat. For most variables, a general lineal model will be used, including the treatment arm and the baseline value of the variable of interest and other unbalanced variables between groups at baseline as covariates.

Results: Between March and August 2019, 66 patients (38 women), with a mean age of 39.8 ±11.4 years, 21.7 ±10.1 years since diagnosis, initial HbA1c of 9.1 ±0.95 %, agreed to participate and were randomized: 33 to the intervention group and 33 to the control group. One participant was lost to follow-up due to pregnancy, three of them were lost to follow-up during medical visits, and four did not submit the final questionnaire. A total of 57 participants completed the study (29 in one group and 28 in the other). Preliminary analyses show that there was no significant change in self-care behaviours, HbA1c or quality of life (except for self-care block of ViDa1, reduction in one of the groups) during the study, in any of the treatment groups (Wilcoxon's test). More detailed statistical analyses will be performed following the study protocol

Conclusion: Preliminary analyses do not show a significant effect of motivational interviewing, applied by trained endocrinologists, on self-care and glycaemic control in adults with type 1 diabetes. Detailed analysis will allow us to confirm or refute and interpret the results

Clinical Trial Registration Number: NCT03906786

Disclosure: **J.C. Betancort Acosta:** None.

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High diabetes-related distress is associated with cerebral functional and structural alterations in middle-aged patients with type 1 diabetes

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Background and aims: Diabetes-related distress (diabetes distress) affects 20-40% of patients with type 1 diabetes (T1D). Although there is some overlap, diabetes distress has been shown to be a different construct from depressive symptoms, and its neuronal correlates are unknown. Therefore, the aim of this study was to investigate neural correlates of diabetes distress in T1D patients.

Materials and methods: All participants with T1D (104; 51 with and 53 without proliferative retinopathy) filled out the Problem Areas in Diabetes (PAID) for diabetes distress and Center for Epidemiological Studies - Depression scale (CESD), and underwent MR-imaging for structural and functional neuronal correlates. A PAID score of 40 and above was used as cut-off for high diabetes distress. Group analyses in cerebral volume (FreeSurfer) and functional connectivity (FSL-PALM) were adjusted for age, sex, retinopathy, and additionally for depressive symptoms. Family Wise Error (FWE) correction was used for multiple voxel testing.

Results: Ninety participants (age: 41.19 ± 0.94 years, 51 [56.7%] women, 43 [47.8%] with proliferative retinopathy, CESD: 7.45 ± 0.67) had low versus 14 participants (age: 40.57 ± 2.43 years, 12 [85.7%] women, 8 [57.1%] with proliferative retinopathy, CESD: 20.0 ± 3.36) with high diabetes distress, the latter having higher depressive symptoms compared to the former ($p<0.001$). Those with high, compared to low diabetes distress showed increased bilateral occipital cortical volume and higher functional connectivity in the default mode, prefrontal, frontoparietal, and visual networks, after adjusting for age, sex, and retinopathy (all $p_{FWE}<0.05$). Despite the low number of patients with high PAID scores, after correction for CESD score, occipital volume remained significantly

higher. A significant correlation was found between higher occipital volume and higher prefrontal connectivity in the fully adjusted model (β=0.266, p=0.007).

Conclusion: In this relatively small group of T1D patients with high diabetes distress, occipital volume and visual, prefrontal, and default mode network connectivity were elevated, of which volume survived additional correction for depressive symptoms. The correlation between occipital volume and prefrontal connectivity suggests cross-talk between visual and attentional areas in T1D with high diabetes distress, cross-talk that has also been observed in other neuroimaging studies of stress-related events, such as occupational and life stress. These results shed light onto the neural correlates of diabetes distress and may help in directing further studies.

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Are the benefits of FreeStyle Libre evident across a range of indications for its use? A UK Diabetes centre experience

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Background and aims: Recent studies have shown the benefits of Freestyle Libre (FSL) on multiple aspects of diabetes care. In the UK, FSL is available on the National Health Service (NHS) for select indications only. We evaluated whether the benefits of Freestyle Libre (FSL) are evident across the range of such indications permitted on the NHS

Materials and methods: We conducted a retrospective study of 210 consecutive participants commenced on FSL from August-2019 to March-2020 at a subcentre of a UK tertiary diabetes centre. Routine care data were collected from the LibreView database and clinical records. The statistical analysis was restricted to those with 6-month follow-up data. The impact of FSL use on glycaemic control was examined in the whole cohort, and further evaluated across the subgroups created based on the indications of its use. Additionally benefits of FSL on reducing hospital admissions for diabetic ketoacidosis (DKA) and hypoglycaemia were assessed. Paired two-tailed t-test was used to compare continuous variables before and after the use of FSL. Wilcoxon Signed Ranks test was used to assess the impact of FSL on DKA admissions.

Results: Data were available for 192 patients (type 1 diabetes 98.9%). Baseline characteristics revealed: White Europeans (87%), male (57%), age 42.7 (±14.5) years, diabetes duration 19.35 (±13.10) years and microvascular complications (23%). The indications for FSL use included: Frequent hypoglycaemia (28.3%); occupational reasons (25.3%); high Haemoglobin A1c (HbA1c) (15.9%); psychosocial conditions (12.3%); hypoglycaemia unawareness (11.6%), and self-monitoring of blood glucose (SMBG) undertaken more than eight times per day (6.5%). Improvement in HbA1c was noted in the whole cohort and across all subgroups and was statistically significant in patients using the FSL due to frequent hypoglycaemia, occupational reasons and high HbA1c (Table 1). At 6-months follow-up, HbA1c improved by -4.5 mmol/mol (pre-FSL and post-FSL HbA1c 69.28 (±18.18) mmol/mol and 63.57 (±13.23) mmol/mol respectively, p = 0.001). HbA1c reduction was greatest in those with baseline HbA1c >100mmol/mol (pre-and post-FSL HbA1c 114.33 (±14.92) and 81.89 (±17.98) respectively, $p<0.05$, Pearson correlation coefficient 19%) (Table 1). Total number of hospital admissions for DKA reduced from 52 to 2 episodes and for hypoglycaemia from 5 to 0 episodes, pre- and post-FSL, respectively. The Wilcoxon Signed Ranks test was indicative of a positive impact of FSL on DKA admissions

Conclusion: In our centre, FSL use for 6 months significantly improved HbA1c across all indications, particularly for frequent hypoglycaemia, occupational reasons and high HbA1c. Significant reduction in hospital admissions for DKA and hypoglycaemia were observed. These results have significant implications for resource utilization and justify unit cost of FSL across a broad range of indications.

Table 1. Impact of FSL on HbA1c reduction at six months across the subgroups of indications of FSL use and additionally categories of baseline HbA1c

Parameters	Pre-FSL HbA1c (Mean±SD)	Post-FSL HbA1c (Mean±SD)	P* value
Indications for FSL (n=132):			
Frequent SMBG (>8times/day) (n=9)	56.56 ± 13.58	54.57 ± 9.09	0.398
High HbA1c (n=22)	77.91 ± 20.19	68.77 ± 13.22	0.033**
Frequent Hypoglycaemia (<4.0 mmol/L) (n=34)	64.74 ± 19.93	58.03 ± 12.92	0.004**
Occupation (n=35)	69.00 ± 17.07	64.49 ± 12.37	0.028**
Hypoglycaemia unawareness (n=16)	65.25 ± 11.76	62.75 ± 12.03	0.276
Psychosocial problems (n=16)	78.35 ± 15.41	73.88 ± 13.99	0.204
Baseline HbA1c (mmol/mol) (n=132)			
< 70 (n= 79)	57.68 ± 7.87	56.95 ± 8.95	0.383
70 – 100 (n= 44)	81.07 ± 7.40	72.75 ± 11.14	<0.05**
> 100 (n= 9)	114.33 ± 14.92	81.89 ± 17.98	<0.05**

FSL – Freestyle Libre, HbA1c – Glycosylated Haemoglobin, SD – Standard Deviation, SMBG – Self monitoring of blood glucose
** Statistically significant

Disclosure: **P. Thadani:** None.

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Assessing the SPUR adherence diagnostic framework

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Background and aims: The SPUR framework has been designed to determine the risk of non-adherence to medication for patients with chronic diseases, as well as providing insights into the patient behavioral drivers. An interactive questionnaire, the SPUR tool, has been developed to assess the risk of non-adherence for patients[1]. This study is part of an international initiative to assess this tool. The study is designed to test the correlation between answers on the SPUR questionnaire with two other established Patient Reported Outcome Measures (PROMs): The Medical Adherence Rating Scale (MARS) and the Beliefs About Medicines (BMQ) questionnaire. It also examined the relationships between the results of these questionnaires and Medication Possession Ratio (MPR) over six months prior to the administration of the PROMs, in order to assess the value of the tools in assessing adherence. Further analysis aimed at streamlining the SPUR tool.

Materials and methods: This cross-sectional study surveyed randomly selected adult participants in France with a confirmed diagnosis of T2D who were prescribed a minimum of one anti-hyperglycaemic medicine for the previous 6 months. The survey consisted of questions relating to socio-demographic and clinical data, the SPUR tool and MARS and BMQ. MPR was calculated using 6 months of patient medication history with respect to anti-hyperglycaemic medicines only. In the case of polymedicated patients, the lowest MPR was taken into account. 221 patients participated in the study.

Results: SPUR was correlated to MARS, (r = 0.57) and to both measures of the BMQ; necessity and concerns, with r of .47 and .60 respectively. In all three cases $p < .001$. MPR for the analysed patients ranged from 0 to 100 with a mean of 75 and a median of 93.4, indicating a level of adherence considerably higher than has otherwise been determined among French T2 diabetes patients[2]. This can be explained both by the means of recruitment, through healthcare professionals, and by the short period over which MPR was calculated. Of the 221 patients analysed, 88 had an MPR of 100. It can be safely surmised that a longer study period would reduce average MPR. Given the relatively limited variability of the sample population's MPR, none of the PROMs were correlated significantly to MPR. A further analysis was investigated the ability of each PROM to explain variability among the 133 patients with MPR < 100. In this case, while MARS and the two BMQ measures remain statistically insignificant, SPUR was correlated to MPR ($p = .049$). It should also be noted that the responses allowed investigation of a more streamlined SPUR tool, consisting of 15 out of the total 43 questions tested. This refined tool did prove to be correlated to MPR for the population as a whole (r=0.19, p=.004) and even more highly correlated to the 133 patients with MPR < 100 (r=.26, $p = .003$).

Conclusion: The study showed SPUR to be a valid measure of the risk of non-adherence as compared to existing measures. SPUR's ability both to predict the risk of non-adherence and provide an understanding of the drivers of that non-adherence represent a powerful tool to help coach patients towards better adherence.

Disclosure: **K. Dolgin:** None.

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Initiating the digital diabetes questionnaire as a clinical tool in routine diabetes care: patients' and professionals' perspectives captured in focus group discussions

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Background and aims: The Diabetes Questionnaire is a digital patient-reported outcome and experience measure for people with diabetes intended to be used in clinical visits and as an integrated part of the Swedish National Diabetes Register. The Diabetes Questionnaire has been developed based on patients' perspectives and evaluated for its measurement qualities. The results from the questionnaire are presented instantly enabling the patient and the healthcare professional to talk about the findings at the visit. The questionnaire has the potential to facilitate patient participation and to support steps toward a more person-centred care. The aim of this study was to describe patients' and healthcare professionals' experiences from initiating the use of the digital Diabetes Questionnaire as a clinical tool in routine diabetes care supported by a structured implementation strategy involving initial education, local facilitators, and regular follow-ups.

Materials and methods: In this qualitative study semi-structured focus group discussions were held with diabetes specialist nurses and medical doctors at out-patient hospital clinics or in primary health care (4 groups) and with adults with type 1 diabetes or type 2 diabetes (4 groups). The audio-taped transcripts were analysed with inductive qualitative content analysis.

Results: The central findings were the two main categories that together formed the overarching theme 'While implementation demands new ways of working, the Diabetes Questionnaire adds a broader picture'. The theme and its main categories and categories are presented in Table 1.

Conclusion: The Diabetes Questionnaire can broaden the horizons of health data in routine diabetes care. Patients and healthcare professionals saw potential positive impacts of using the questionnaire on both the individual and group levels. These results inform further development of implementation strategies to support clinical use. The long-term goal is for the digital Diabetes Questionnaire to be used to strengthen patient perspectives in diabetes care and to be considered together with medical variables in the Swedish National

Diabetes Register.

Table 1. Theme, main categories and categories

Theme	Main categories	Categories
While implementation demands new ways of working, the Diabetes Questionnaire adds a broader picture	The Diabetes Questionnaire, a tool for more person-centred clinical visits	Preparations for clinical visits given another dimension
		Can bring the important aspects to light
		Can broaden the horizons
	The processes of initiating the implementation of the Diabetes Questionnaire	Differences in engagement among healthcare management and co-workers
		To start and to establish new routines
		Healthcare professionals' experiences of the support during implementation
		Pros and cons regarding the questionnaire, its items, and dimensions
		Manners of administration and completion of the Diabetes Questionnaire
		Thoughts ahead – opportunities and concerns

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Effect of WhatsApp messaging based intervention on insulin adherence and treatment effectiveness in diabetic patients in central India

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Background and aims: Low adherence to pharmacological therapy and monitoring by patients with diabetes mellitus is very common and represents a challenge. Technological Help has came to a rescue in connecting with patients during this covid-19 period by enhancing communication and the reach for the healthcare. WhatsApp is a very commonly used social media app and its available with most of the patients. This study examined the effect of an intervention using WhatsApp® messaging on insulin adherence and treatment effectiveness in patients with diabetes

Materials and methods: A randomized clinical trial was performed with 573 patients who had diabetes and/or hypertension and who had enrolled in a diabetes clinic. The patients were randomly assigned to either the intervention group ($n = 296$), which received usual care plus WhatsApp messages-based dose titration for insulin with an emphasis on medication adherence, or the control group. The control group ($n = 277$) only received usual care. Medication adherence, as measured by the Morisky-Green Test, was compared after 32 weeks

Results: After the follow-up period (8 months), 78.5% of the patients in the intervention group were adherent versus 57.2% in the control group. Also, the number of patients achieving target hba1c <7% were more in intervention group 73 % as compared to control group 52 %.

Conclusion: Diabetes is a progressive disease, and the treatment should be continuous. Technological aids using WhatsApp could be useful as a reinforcement to increase adherence to medication and achieving target levels of optimum health. This approach is well accepted by patients and easily accessible to them and it maintains connection between patient and caregivers during and after covid-19 period. If used judiciously it can reduce the burden on our healthcare system and yield better outcomes in terms of better treatment adherence and treatment outcomes.

Disclosure: **S. Saboo:** None.

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Long-term HbA_{1c} outcomes with and without intermittent CGM use in adults with type 2 diabetes participating in the Onduo Program

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Background and aims: The Onduo Virtual Diabetes Clinic (VDC) for people with type 2 diabetes (T2D) combines a mobile app, remote lifestyle coaching and video consultations with board-certified endocrinologists for medication management and prescription of real-time (rt)CGM devices for intermittent use in high risk participants. This analysis examined change in HbA1c at 6 months and 1 year in VDC participants with and without intermittent rtCGM use.

Materials and methods: Adults ≥18 years of age with T2D who enrolled in the VDC program from February 2018 through April 2019 with baseline and follow-up HbA1c values at 1 year were included. The CGM group was required to have used CGM ≥30 days prior to the follow-up HbA1c measurement. Outcomes included within group change in mean HbA1c with and without CGM use. Subgroup analysis was performed for participants with 6-month and 1-year data. Between group comparisons for change in HbA1c stratified by baseline categories of >9.0%, 8.0 to 9.0%, 7.0 to <8.0%, <7.0% and by <8.0% and ≥8.0% were evaluated by a two-sample t-test for equivalence of mean change in HbA1c between groups.

Results: Of the cohort (n=772), 45.9% (n=354) used CGM and 54.1% (n=418) did not use CGM. At baseline the CGM group was significantly younger (mean±SD): 53.3±8.6 vs 55.1±9.4 years, had higher HbA1c: 7.9%±1.8 vs 7.6%±1.7, and greater insulin use: 36.2% vs 28.5%. Change in HbA1c at 1 year is presented in the Table. The increase in participants meeting the Health Effectiveness Data and Information Set (HEDIS) target of HbA1c <8.0% was greater in the CGM group, 55.1% to 78.3%, vs no CGM, 60.8% to 74.0% (p=0.002). Subgroup analysis (n=468, 60.6%) revealed significant improvement in HbA1c at 6 months and 1 year for both the CGM and no CGM groups with a baseline HbA1c >8.0% (p<0.001). On average participants meeting the American Diabetes Association (ADA) treatment target of HbA1c <7.0% at baseline remained at target at 6 months and 1 year.

Conclusion: Participation in the Onduo VDC was associated with a significant reduction in HbA1c at 1 year in those not meeting treatment targets, with approximately 2-fold greater improvement with CGM use. These results suggest that improvement in HbA1c observed at 6 months was maintained at 1 year.

Table. Change in HbA1c at 1-year Stratified by Baseline HbA1c Category and CGM Use

	CGM (n=354)			No CGM (n=418)			
Baseline HbA1c	Baseline HbA1c	1-yr HbA1c	Change	Baseline HbA1c	1-yr HbA1c	Change	p-value
Overall	7.9±1.8	7.2±1.3	-0.7±1.7	7.6±1.7	7.4±1.4	-0.2±1.3	<0.001
>9.0%	10.8±1.5	8.0±1.9	-2.8±2.2	10.7±1.8	9.0±1.8	-1.8±2.0	0.006
8.0% to 9.0%	8.4±0.3	7.4±1.1	-1.1±1.2	8.4±0.3	7.9±1.1	-0.6±1.2	0.02
7.0% to 7.9%	7.4±0.3	7.3±1.0	-0.2±0.9	7.4±0.3	7.4±1.0	-0.1±1.0	0.50
<7.0%	6.4±0.4	6.5±0.8	0.1±0.7	6.3±0.4	6.6±0.8	0.3±0.7	0.02

*Equivalence of mean change in HbA1c between groups; data are mean±SD

Disclosure: **J.E. Layne:** Employment/Consultancy; Employee of Onduo, LLC, the study Sponsor.

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Development of a new device for screening for peripheral diabetic neuropathy

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Background and aims: We developed a new tool called 'Vibrascan' that utilises a vibratory plate for the screening of peripheral diabetic neuropathy. Its design was based on the mechanics of the Neurothesiometer, but is intended as a more intuitive, operator- independent testing instrument.

Materials and methods: Twenty healthy subjects were tested using both Neurothesiometer and VibraScan. For using the Neurothesiometer, a

single operator measured VPT (vibration perception thresholds in Volts (V). VibraScan can be operated independently by the subject in the sitting position and placing both feet on the vibrating plate. The range of vibration used is the same for both devices (i.e. from 0V to 50V). The frequency and amplitude of VibraScan is completely programmable such that severity level can be interpreted automatically by the device. VPT measurements by both devices were correlated using the Bland - Altman method in order to measure the agreement between both devices.

Results: Mean VPT measured for left and right foot using Neurothesiometer was 4.53 ± 0.65 V and 4.97 ± 0.57 V, and 4.76 ± 0.60 V and 5.22 ± 0.67 V for left and right foot using VibraScan. There was very good correlation between individual values for each device ($r = 0.893$, $p < 0.01$ for right foot and $r = 0.816$, $p < 0.01$ for left foot). Despite the differences in operation technique, there was very little difference in VPT measurements between devices. when using the using Bland-Altman method.

Conclusion: The intuitive, quick and essentially observer independent nature of VibraScan enables an important new normal method of screening for the at risk foot in diabetic individuals. Further studies on diabetic subjects with varying severity of neuropathy are planned to help strengthen its possible role for screening patients in tandem with their annual eye screening visit, a much awaited unified approach in the modern management of diabetes.

Disclosure: **D. Coppini:** None.

SO 40 CGM

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High treatment satisfaction and less severe hypoglycaemia after 24-month use of intermittently scanned continuous glucose monitoring

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Background and aims: Nationwide reimbursement of intermittently scanned continuous glucose monitoring (isCGM) was introduced in Belgium in 2016. This prospective observational multicentre real-world trial (FUTURE) studies the impact of isCGM on quality of life (QOL) and glycaemic control of people with type 1 diabetes (T1D) in 3 diabetes centres. Here, we report the 24-month data of the FUTURE trial.

Materials and methods: Between July 2016 and July 2018, 1906 T1D adults were consecutively recruited. Demographic, metabolic, glucose, and QOL (SF-36, Problem Areas In Diabetes-Short Form, Hypoglycaemia Fear Survey, Diabetes Treatment Satisfaction Questionnaire [DTSQ]) data were collected at start, 6, 12, and 24 months of standard follow-up. Primary endpoint was evolution of QOL. Secondary endpoints were change in HbA_{1c}, self-reported severe hypoglycaemia and diabetes-related work absence, and number of people who reach clinical CGM consensus targets. Data are mean±SD, least-square mean (95% CI), or n (%).

Results: Of 1906 people who started isCGM, 1577 (83%) used isCGM for at least 24 months. Twelve percent (n=237) discontinued isCGM due to switching to real-time CGM (n=75; 32%), skin irritation or allergy (n=39; 17%), and frequent sensor loss (n=26; 11%). People were 46±15 years old, had T1D for 23±14 years, 22% were using an insulin pump, 16% (n=299) were hypoglycaemia unaware, with HbA_{1c} of 7.8±1.2%. QOL scores were high at baseline and remained stable. DTSQ satisfaction improved from 28.0 (26.1-29.8) to 30.4 (28.6-32.2) ($p<0.0001$), with high self-reported treatment satisfaction (8.5±1.4 on a scale of 10). HbA_{1c} did not change over 24 months. Compared to 6 months before isCGM initiation, fewer people experienced severe hypoglycaemic events needing help from others (17.7% [n=314] vs 9.5% [n=150]; p<0.0001) and hypoglycaemic comas (3.8% [n=67] vs 1.1% [n=18]; p<0.0001) in the 6 months prior to the 24-month time point. The same evolution was observed in number of severe hypoglycaemic events needing help from others (83.1 vs 44.5 events/100 patient-years; p=0.003) and hypoglycaemic comas (9.5 vs 3.0 events/100 patient-years; p=0.009). Additionally, fewer people were absent from work (7.7% [n=134] vs 2.5% [n=39]; p<0.0001) and missed fewer days of work (80.8 vs 35.1 days/100 patient-years; p=0.009). From the first two weeks of isCGM use up to 24 months, more people reached the targets of <4% of time <70 mg/dL (24.0% [n=331] vs 29.5% [n=367]; p<0.0001), >70% of time 70-180 mg/dL (10.4% [n=143] vs 12.0% [n=148]; p=0.046), <25% of time >180 mg/dL (18.5% [n=256] vs 20.1% [n=249]; p=0.025), and <5% of time >250 mg/dL (15.4% [n=212] vs 20.6% [n=254]; p<0.0001). Results are comparable when omitting drop-outs from analyses.

Conclusion: isCGM use over 24 months in a large T1D population increases treatment satisfaction while maintaining other aspects of QOL and HbA_{1c}. The lower prevalence of people who experience severe hypoglycaemia and who miss work, together with more people who achieve the consensus targets for hypoglycaemia and hyperglycaemia indicate that isCGM can be beneficial in long-term diabetes care.

Clinical Trial Registration Number: NCT02898714

Disclosure: **S. Charleer:** None.

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Safety and performance evaluation of a novel continuous glucose monitoring system: Lumee Glucose

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Background and aims: Profusa is developing a novel Continuous Glucose Monitoring (CGM) System which tracks glucose concentrations in interstitial fluid for several months. It consists of a small hydrogel sensor (2.5 x 0.4 x 0.3 mm) that is permanently inserted by subcutaneous injection, and a wireless optical reader that is placed over the skin to measure the sensor's fluorescent signal which changes proportionally with glucose. The aim of this ongoing study is to evaluate the safety and performance of the Lumee Glucose System in a clinical setting.

Materials and methods: Seven (7) subjects with type 2 diabetes were enrolled. Each subject received two subcutaneous sensor injections in the upper arm during the first visit. Subjects returned to the clinic for multiple ambulatory monitoring visits (6-hour sessions) over several months. During each visit, blood glucose (BG) fluctuations of study subjects were captured using a commercial capillary glucose meter every 15 minutes and compared to the Lumee sensor signal. Strong tracking correlation between reference BG values and Lumee sensor signal is defined by r2 > 0.5. Furthermore, reference capillary BG data is plotted against sensor signal values on the Parkes Error Grid.

Results: All sensors injected (n=14) show tracking of glucose excursions during every monitoring visit which was continued for up to day 95. At this time of reporting, retrospective calibration analysis was applied on 104 sensor traces, showing strong correlation with reference BG values in 78% of sensor traces. Parkes Error Grid Analysis shows that 99.8% of the paired sensor-reference values fell within zones A and B (91.2% in zone A and 8.6% in zone B), and only 0.2% fell within zone C. (Figure 1). According to up-to-date reporting there are no safety concerns related to the investigational Lumee Glucose System.

Conclusion: The Lumee Glucose System demonstrates a good safety profile and glucose tracking functionality for a minimum of 3 months in subjects with type 2 diabetes. Studies are currently underway to assess Lumee Glucose System's performance and safety at timepoints beyond three months, and to support the development of a prospective calibration algorithm.

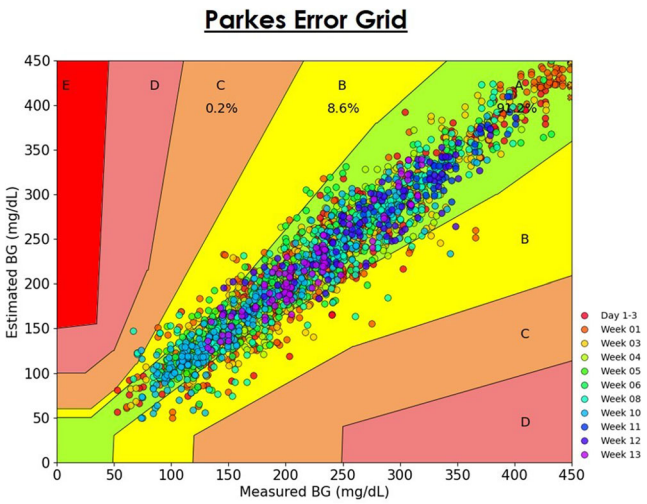

Figure 1: Parkes Error Grid analysis of 2492 paired sensor - reference values in 7 subjects (14 sensors), up to 95 days

Disclosure: **C. Nguyen:** Employment/Consultancy; Profusa.

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Performance evaluation of the Glunovo® continuous blood glucose monitoring system in Chinese participants with diabetes

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Background and aims: Continuous Glucose Monitoring System (CGMS) measures and monitor 24-hour continuous interstitial glucose through sensors, provides accurate data to individuals and physicians about blood glucose variability during the measurement period. Glunovo® , a CGMS with 14 days sensor life, when inserted subcutaneously in abdomen, generates electrical signals in response to contact with the interstitial fluid. These signals are transmitted to application via Bluetooth to provide blood glucose readings in real time (every 3 minutes). The accuracy of the CGMS needs to be validated for clinical decision making. The present study evaluated efficacy and safety of Glunovo® S1 glucose sensor in combination with the real-time CGMS in monitoring interstitial fluid glucose in adult diabetic subjects using venous blood glucose as control.

Materials and methods: This multicenter, self-controlled study included 78 Chinese adults aged 18-70 years old, previously diagnosed with type 1 (n = 25) or type 2 (n = 53) diabetes. Subjects used CGMS for 14 days, wherein each subject was inserted with 2 sensors subcutaneously on either side of the abdomen for better performance evaluation and matched with their corresponding venous blood glucose levels measured by EKF blood glucose detector (EKF diagnostics, Cardiff, United Kingdom). After 14 days of wear-in period, paired continuous blood glucose values and venous blood glucose values at different time periods were collected randomly (day 1 or 2, day 7±1 and day 14). The study outcomes were analysed as per the standards of Chinese guidelines for technical review of CGMS registration.

Results: A total of 12,688 pairs of CGMS and EKF venous blood glucose values were available for performance evaluation. Compared with EKF venous blood glucose, 90.05% of the sensor values were within 20% of the deviation, meeting the expected guideline standards. It was observed that 99.08% (95% CI, 98.91% - 99.24%) of the measuring points fell within A+B zones of Clarke error grid analysis meeting clinical accuracy. A total of 99.82% (95% CI, 99.74% - 99.89%) of the measuring points fell in A+B zone of Parkes error grid analysis. The mean absolute relative error (MAR%) was 10.00% ± 3.34% (Table 1). Survival analysis revealed that when sensors were worn over 14 days, the survival probability of sensors was 96.76%. All the subjects were completely satisfied with the performance and wearing comfort of the CGMS.

Conclusion: The Glunovo® CGMS showed high accuracy in both monitoring real-time continuous changes and predicting varying trends in blood glucose level. Glunovo® CGMS had excellent accuracy and limited clinical risk compared with venous blood glucose in range of 2.2-22.2 mmol/L over 14 days.

Table 1- Study outcomes corresponding to main evaluation indexes in Chinese guidelines for technical review of CGMS registration

Evaluation Indexes	Chinese guidelines criteria		Study results	
	Point Estimation	95% confidence interval	Point Estimation	95% confidence interval
Agreement rate with 20/20% reference value	>65%	>60%	90.05%	89.51% - 90.59%
Proportion of points falling in A+B area of Clarke error grid	>95%	>90%	99.08%	98.91% - 99.24%
Proportion of points falling in A+B area of Parkes error grid	>95%	>90%	99.82%	99.74% - 99.89%
Mean absolute relative error (MAR%)	<18%	<20%*	10.00%	9.45%-10.56%

- *Evaluate the upper limit of 95% confidence interval and the lower limit of other indexes.
- For the evaluation of CGM system, the above four main evaluation indexes should meet the standards listed in the above table at the same time, before the product performance can be considered to meet the needs of clinical applications, that is, set according to the common primary endpoint.
- Abbreviations: CGMS, continuous glucose monitoring system

Supported by: Infinovo

Disclosure: **R. Meng:** None.

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Accuracy comparison of the WaveForm cascade CGM system at different body sites over 15 days

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Background and aims: To assess the efficacy of the WaveForm Diabetes Cascade CGM system when applied to the arm and thigh, respectively. A 15-day clinical pilot study was performed to compare the performance of sensors when applied to the upper arm and thigh areas versus sensors applied to the abdomen.

Materials and methods: Each diabetic subject (n=10) wore three Cascade CGM devices simultaneously. The devices were each applied to the abdomen, arm and thigh. There were four in-clinic days (1, 5, 10, and 15-day intervals) during which the accuracy/performance of the CGM system was assessed. The YSI glucose measurements were performed on plasma obtained from venous blood sampled every 15 minutes during the 12-hour sessions on in-clinic days. The overall MARD and MAD calculations for the Cascade CGM devices were based on a comparison of time-paired YSI values and Cascade CGM values. The Cascade CGM values were obtained by prospectively applying an advanced algorithm to data collected during the study.

Results: MARD and MAD of the Cascade CGM system were as follows: • 9.8% and 12.0 mg/dL in the abdomen; • 11.0% and 17.2mg/dL in the arm; and • 13.4% and 27.7 mg/dL in the thigh.

Conclusion: In this pilot study, the performance of the Cascade CGM device applied to the arm was comparable to the performance of the device when applied to the abdomen over a 15-day period, while placement in the thigh area was found to be less suitable, mostly as a result of more frequent sensor dislodgement. The potential applicability of the arm site for the insertion of the Cascade CGM system is encouraging and would improve convenience for users of the system living with Type 1 and Type 2 diabetes. We anticipate making some adjustments to limit the thigh applications to provide comparable utility in the future.

Disclosure: **M. Rebec:** None.

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Understanding why we do not have consensus for coefficient of variation with CGM

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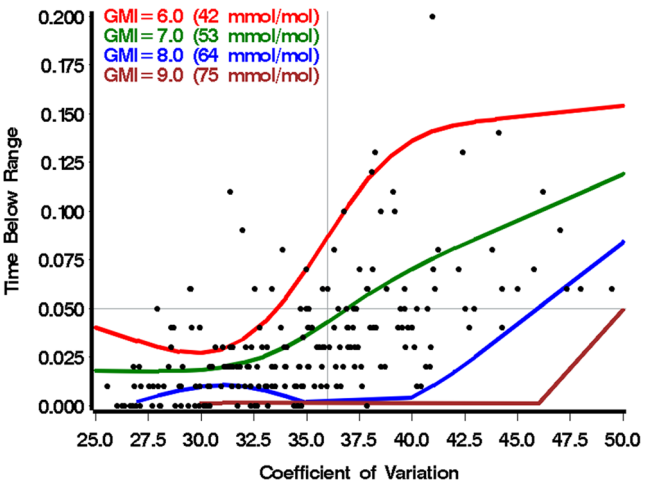
Background and aims: Continuous glucose monitoring (CGM) provides more complete information about glycemic control than HbA1c. In addition to average glucose, standard CGM parameters include: 1) time-in-range (TIR), 2) time-above-range (TAR), 3) time-below-range (TBR) and, 4) standard deviation (SD) from which a coefficient of variation (CV) can be calculated. Recent guidelines from the International Consensus on TIR define clinical targets for these CGM metrics. Their statement includes a global recommendation to restrict glucose variation. Previous studies have shown a positive linear association between CV and TBR. Their results demonstrate considerable reduction in hypoglycemia for CV values < 34-36%. Newer data, however, suggest these findings may be less applicable at the extremes of glycemic control.

Materials and methods: We performed a retrospective review of personal CGM downloads from 219 subjects with type 1 diabetes mellitus to assess the association between CV and TBR and to ascertain whether this association differs based on the glucose management indicator (GMI). We required at least 14 days of sensor wear for analysis. We included patients using Dexcom G4-G6 and Medtronic Guardian 3 devices. There were otherwise no exclusion criteria.

Results: Our cohort was comprised of subjects aged 19-84 years. There was no gender predominance. The most common CGM used was the Dexcom G5 (n = 78 [36%]). The majority of patients were prescribed a Dexcom brand sensor (n = 148 [68%]). The mean duration of CGM wear was 24.7 days (SD ± 5.5). The mean GMI was 7.2% (range 5.3-10.4%; SD ± 0.7%). Thirty-six percent of patients had TBR > 4%. Our best-fit regression model suggested a non-linear association between CV and TBR (Figure 1; linear vs. non-linear, $p < 0.0001$). This non-linear association appeared to differ based on GMI value (interaction $p < 0.0001$). A linear regression model using TBR as the dependent variable, CV (modeled as a restricted cubic spline), GMI as a linear variable and the interaction terms for CV and GMI as independent variables yielded an R^2 of 0.7.

Conclusion: Our results indicate that GMI must be considered in order to determine a target CV for each patient that achieves sufficiently low TBR. The same CV can predict different TBR at various levels of glycemic control. Some patients who have more hyperglycemia should be allowed a looser CV restriction. As expected, as average blood glucose improves, it takes less variation to increase TBR. Based on these data, we recommend an individualized approach when selecting a target CV for personal CGM patients.



Disclosure: **J.E. Perlman:** None.

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GMI might over estimate quality of glycaemic control in diabetes patients

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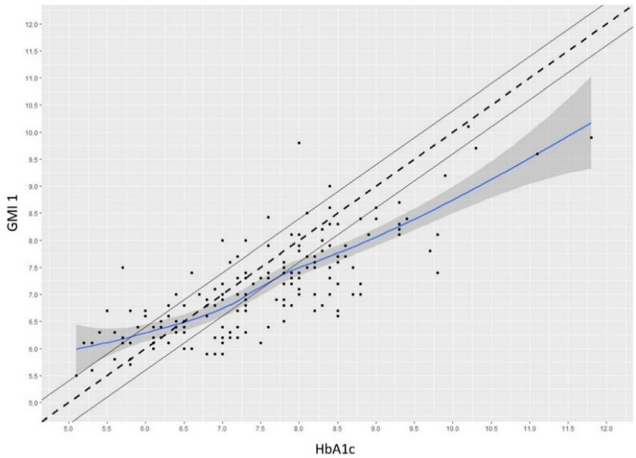
Background and aims: The introduction of continuous glucose monitoring (CGM) in the last years improved management of diabetes. By

using CGM a variety of new parameters were established to define glycemic control of patients. To estimate HbA1c, the current gold standard for glycemic control, the parameter glucose management index (GMI), based on average glucose measurements, was introduced. Through its easy accessibility, patients often rely on this parameter, especially during the last year when treatment via telemedicine played a major role. The aim of this study is to investigate whether GMI is a robust parameter for predicting HbA1c and glycemic control in diabetes patients.

Materials and methods: A retrospective data analysis of 170 patients, treated in the outpatient department of the General Hospital Vienna was performed. The parameters of the different patients were collected from clinical records and diabetes data management systems. GMI of two different time spans, namely 14 days (GMI1) and 30 days (GMI2) prior to the HbA1c measurement (T0) were compared. To detect deviations of GMI from HbA1c, we plotted GMI versus HbA1c and performed a loess curve fitting. We defined a clinically significant deviation from HbA1c as more than 0.4% points in either direction.

Results: Of the 170 patients, 77 were female, 113 patients had T1DM/LADA, 41 T2DM, with 16 patients having an unspecified type of diabetes. Most patients (166) used FreeStyle libre, with 3 using Dexcom G6 and 1 patient the CGM device by Eversense. Mean HbA1c was 7.53 +- 1.25%, whilst GMI over the 2 weeks prior to HbA1c measurement was 7.19 +- 0.95%. GMI representing 30 days prior HbA1c measurement was 7.21+- 0.93%. Correlations between HbA1c and GMI1 (R^2=.82; p<0.001) as well as GMI2 (R^2=.845; p<0.001) were statistically significant. When plotting HbA1c versus GMI (see graph 1) it is obvious that GMI deviates from HbA1c at an HbA1c level of around 8% and GMI therefore overestimate quality of glycemic control in patients with poor glycemic control.

Conclusion: Within the last years CGM had a big impact in diabetes management. As expected, there was a significant correlation between GMI and HbA1c, with a slightly higher R^2 for GMI representing CGM measurements 30 days prior to HbA1c measurement compared to 2 weeks prior. However, relying on GMI as a parameter for glycemic control could lead to inadequate treatment, especially in patients with poor glycemic control.



Graph 1: HbA1c vs GMI1 (2 weeks prior HbA1c measurement): blue line: loess curve with +/- 95% CI (grey); dotted line HbA1c=GMI with +/- 0.4% interval (continuous lines)

Disclosure: **P. Fellinger:** None.

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The impact of glycaemic variability on the relationship between hypoglycaemia and HbA_{1c}

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[1]University of Colorado Denver, Denver, [2]Eli Lilly and Company, Indianapolis, USA.

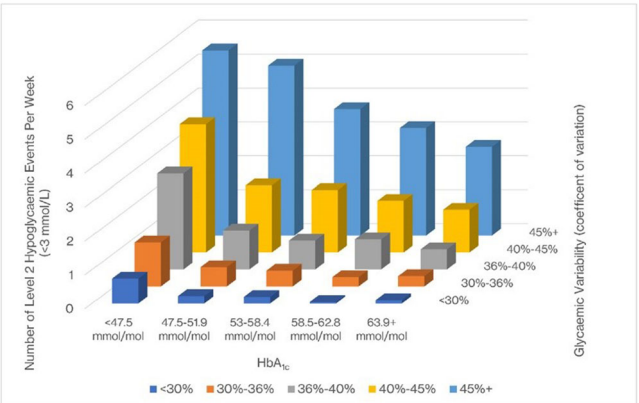
Background and aims: Continuous glucose monitoring (CGM) guidelines suggest a target glycaemic variability, as measured by coefficient of variation (CV) ≤36%, to mitigate hypoglycaemia in people with type 1 diabetes. However, they provide no context to haemoglobin A_{1c} (HbA_{1c}) values. The aim of this study was to evaluate the relationship between CV, hypoglycaemia, and HbA_{1c} among adults with type 1 diabetes.

Materials and methods: A retrospective sample of adults (≥18 years) with type 1 diabetes from the Barbara Davis Center for Diabetes who had available ambulatory glucose profiles and laboratory-measured HbA_{1c} were analysed at the patient-visit level between 2014 and 2020. Negative binomial regression of aggregated data over 7 days was used to estimate the number of level-2 (<3 mmol/L) hypoglycaemic events at cross-sections of HbA_{1c} and CV for a maximum of 2 weeks, after adjusting for time of CGM use.

Results: Among 466 adults with type 1 diabetes (mean age=37 years) at 1,648 visits, percentage of CGM use was 91%. We observed wide variation in level 2 hypoglycaemic events across CV and HbA_{1c} categories (Figure 1). The highest mean estimated number of events was 3.5 (95% CI 2.4, 4.6) per week, found in patients with CV > 36%, HbA_{1c}<47.5 mmol/mol (6.5%).

Conclusion: Glycaemic variability (CV) provides meaningful insights about level 2 hypoglycaemia that should be used in combination with HbA_{1c} and other factors to inform clinical decision making to help mitigate risk for possible hypoglycaemia.

Figure 1. Relationship between glycaemic variability, HbA_{1c}, and hypoglycaemia in patients with type 1 diabetes



Supported by: Eli Lilly and Company

Disclosure: **S.L. Ellis:** Grants; Eli Lilly.

SO 41 Closed loop systems

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Real-world data from 1,988 people with type 1 diabetes on the Omnipod DASH® Insulin Management System with continuous glucose monitoring and cloud-based data management

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[1]Schwerpunktpraxis für Diabetes und Ernährungsmedizin, Münster, Germany, [2]University of Washington, Endocrine And Diabetes Care Center, Seattle, USA, [3]SUNY Upstate Medical University, Syracuse, USA, [4]Insulet Corporation, Acton, USA.

Background and aims: Assessing real-world use of technology by people with type 1 diabetes (T1D) can provide information on clinical outcomes and treatment patterns to support advancement of care. This analysis assessed glycemic metrics and treatment patterns for a large cohort of children, adolescents and adults with type 1 diabetes using the Omnipod DASH Insulin Management System, real-time continuous glucose monitoring (CGM) and a data management system.

Materials and methods: Usage data uploaded to the data management system from July 2018 through March 2021 were matched via device serial number to a second database of self-reported demographic data and de-identified. Data from ≥3 months of system use per user were analyzed. CGM data were included when the device was used ≥15.5% of overall time (minimum of 14 days of a 3-month period).

Results: 1,988 patients using the Omnipod DASH System and CGM were identified and stratified by age (Table). Mean GMI was 7.7% and 7.6% for children younger than 6 and between 6-12 years respectively, and less than 7.5% for all other age groups. Children aged 0-12 spent an average of >52% of time in target range (70-180 mg/dL; 3.9-10 mmol/L), while all other age groups were in the target range >60% of the time. In all age groups, mean time below range (<70mg/dL; <3.9 mmol/L) was less than 3%. Mean total daily insulin dose ranged from 11.3 units/day for children less than 6 years of age, to approximately 46 units/day for adolescents and adults, and mean bolus frequency ranged from 7 times per day in young children to 5 times in adolescents and adults. The median pod change interval was 2.8 days.

Conclusion: Data from this real-world analysis provide valuable insights to treatment patterns and glycemic outcomes and support favourable glycemic control in a broad population using the Omnipod DASH System with CGM, when compared to large registries such as the T1D Exchange and SWEET.

Table. Glycemic Profiles and Insulin Use Patterns of Patients with Type 1 Diabetes Using the Omnipod DASH System and CGM.

	<6 y	6 - 12 y	13 - 17y	18 - 25y	26 - 49y	≥50 y
N	227	587	318	142	444	270
Age, yr	3.7±1.2	9.2±2.0	14.5±1.4	21.3±2.2	36.3±6.8	61.2±8.1
Female, %	43	52	48	67	64	59
GMI, %*	7.7	7.6	7.4	7.4	7.2	7.2
Time <70 mg/dL (<3.9 mmol/L), %	2.0±2.2	1.6±1.7	1.4±1.7	2.2±2.3	2.8±3.2	2.3±2.8
Time in range, %	52.7±16.9	56.8±16.6	61.4±18.4	60.5±19.6	64.3±18.6	63.9±16.8
Time >180mg/dL (>10.0mmol/L), %	45.3±17.9	41.6±17.3	37.2±18.6	37.4±20.0	32.9±19.5	33.8±17.7
TDD, U	11.3±4.2	27.1±15.5	46.0±20.6	46.1±19.4	46.3±22.3	42.9±22.6
Basal, %	40	41	45	51	53	53
Bolus size, U	1.1±0.6	2.8±1.9	5.2±2.7	5.3±3.2	4.5±2.9	4.5±3.1
Bolus, x/d	7.2±2.7	6.2±2.1	5.2±1.8	5.0±2.3	5.5±2.3	5.0±2.0

Results are mean±SD unless indicated.
*GMI: https://www.jaeb.org/gmi/

Supported by: Insulet

Disclosure: **W. Keuthage:** None.

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Daily meal size variation does not affect glycaemic control in adult type 1 diabetes patients equipped with hybrid closed loop DBLG1 system

P. Gimenez[1], S. Lachal[1], Y. Tourki[1], S. Franc[2], C. Amadou[2], A. Penfornis[2], G. Charpentier[2], P.-Y. Benhamou[3];

[1]Diabeloop SA, Grenoble, [2]Centre d'etudes et de recherches pour l'intensification du traitement du diabète, Evry, [3]Grenoble Alpes University, Grenoble, France.

Background and aims: The aim of this study is to assess the effect of meal size on glycemic control for T1D patients equipped with DBLG1 Closed Loop System, based on data from DBLG1 System Commercial Launch.

Materials and methods: The Soft Launch dataset comprises 34 adult Type-1 diabetes patients with an average duration of 206 days for a total of 7011 days of treatment. Only days with ≥70% available CGM data were included in the analysis (97.3 % of total). To assess the effect of patient meal size (the daily sum of CHO declared by each patient on his device) on glycemic control, we compute the difference in percentage point between patient daily value and patient daily mean value of both Time In Range (TIR) (Figure 1) and Time In Hyperglycemia (TIHYPER) and plot it against each daily CarboHydrates (CHO) as a percentage of the mean daily CHO for this patient. This way we can observe the effect of bigger and smaller than average meals and determine if they tend to increase or decrease TIR and TIHYPER.

Results: As presented on Figure 1, days with increased CHO consumption are not linked to a drop in TIR.We observe no correlation between the TIR and the daily amount of CHO ingested (r=0.01), nor between the TIHYPER and the daily amount of CHO ingested (r=-0.12).

Conclusion: In conclusion, DBLG1 Hybrid Closed Loop System's performance is not altered by the variations in daily amount of carbohydrates ingested by T1D patients.

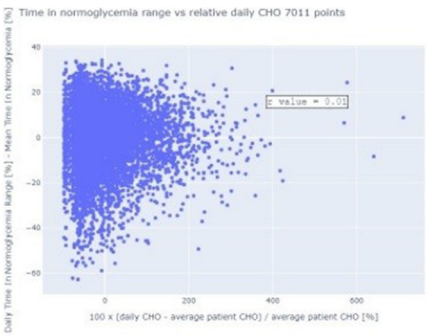

Figure 1 : Difference between daily TIR and Mean TIR plotted against the relative daily meal size.

Disclosure: **P. Gimenez:** Employment/Consultancy; Diabeloop SA.

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A novel method for unannounced meal detection and control

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Background and aims: The objective is to validate in silico an Unannounced Meal Management (UMM) feature that would detect and manage any unexpected sudden increase of Blood Glucose in a safe and efficient way.

Materials and methods: The controller was evaluated in silico with and without the activation of the UMM feature, using a simulator based on the Hovorka model involving 120 Virtual Patients. Various meal scenarios were considered with different levels of meal announcement ranging from 0% to 100% meal announcement, where an announced meal produces a bolus delivered a few minutes ahead of the meal declaration time.

Results: The simulation results showed a Time In Range 70-180 mg/dL (TIR) improvement of 5% without increasing the hypoglycemia rate (<70 mg/dL) when the UMM feature is active in a full unannounced meal (FUM) scenario. Results in a full announced meal (FAM) scenario also reveal that the UMM feature did not bring any significant hypoglycemia risk while leading to a slight TIR improvement (~0.5%) that can be imputed to unexpected glycemia excursions not related to meal intakes, yet covered by the UMM module. These figures also show that the UMM controller approached, in the FUM scenario, the performances obtained in the FAM scenario with the reference controller: the TIR is only <5% lower (56.51% with UMM in FUM VS 61.94% with reference in FAM).

Conclusion: The new UMM module was assessed in silico to be safe and effective and could be evaluated in a real-life clinical trial.

Full Unannounced Meals						
Algo	TIR [%]	Time spent > 180 mg/dL	Time spent < 70 mg/dL	Time spent < 54 mg/dL	Average glycemia	CV
Reference	51.14 (+/-1.19)	48.15 (+/-1.23)	0.71 (+/-0.22)	0.17 (+/-0.11)	194.70 (+/-1.91)	35.15 (+/-0.97)
UMM	56.51 (+/-1.02)	42.42 (+/-1.04)	1.07 (+/-0.23)	0.29 (+/-0.10)	186.68 (+/-1.65)	35.98 (+/-1.06)
Full Announced Meals						
Reference	61.94 (+/-1.28)	35.74 (+/-1.33)	2.32 (+/-0.31)	0.71 (+/-0.16)	173.48 (+/-2.02)	33.57 (+/-1.01)
UMM	62.50 (+/-1.26)	35.20 (+/-1.32)	2.30 (+/-0.31)	0.73 (+/-0.16)	172.34 (+/-1.99)	33.49 (+/-0.99)

Table: Performances of the UMM algorithm compared to the reference algorithm in the Full Unannounced Meals and Full Announced Meals scenarios obtained on a 120 virtual patients cohort

Disclosure: **S. Lachal:** None.

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Glycaemic outcomes and patient satisfaction after 3 months of use of an advanced hybrid closed-loop system use

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Background and aims: The Advanced hybrid closed-loop system (AHCL) MiniMed Medtronic 780G was launched in Europe in October 2020. This system automatically infuses insulin according to interstitial sensor glucose values, to different glucose targets (100, 110 or 120 mg/dl) and it also delivers auto-correction boluses to a glucose target of 120 mg/dl. The aim of the study was to evaluate the outcomes and patient acceptance after 3 months of use of the MiniMed Medtronic 780G AHCL in people with type 1 diabetes (T1D).

Materials and methods: Subjects with T1D were simultaneously upgraded from sensor-augmented pump with predictive low-glucose suspend (SAP-PLGS) to AHCL. Capillary HbA1c and 2-week system downloads were collected at baseline and after 3 months of use. Subjects were asked to complete the Hypoglycaemia Fear Survey (HFS), Diabetes Quality of Life (DQOL), Glucose Monitoring Experience (GME-Q), DDS (Diabetes Distress Scale) and Pittsburgh Sleep Quality Index questionnaires, both at baseline and at the end of the study.

Results: 52 T1D subjects were included (age: 43 ± 12 years, sex: 73% females, diabetes duration: 27 ± 11 years, duration of SAP-PLGS use: 5 ± 2 years, duration of pump use: 7 ±4 years, higher education: 31%). A target of 100 mg/dl and an active insulin time of 2 hours were programmed for all the patients. Outcomes at 3 months are summarised in Table 1. At the 3 months visit, time in auto-mode was 94 ± 10%, number of exits per week: 1.0 ± 0.8, autocorrection insulin: 7 ± 5 boluses per day (29 ± 12 % of bolus insulin), SMBG: 3.6 ± 1.1 per day, calibrations: 3.2 ± 1.0 per day. Bolus insulin increased from 49 ± 12% to 57 ± 8% and basal insulin decreased from 51 ± 12% to 43 ± 8% ($p < 0.001$) at 3 months compared to baseline. Sensor use increased from 85 ± 11% at baseline to 90 ± 11% at 3 months ($p = 0.001$). No significant changes were found in coefficient of variation of glucose (33 ± 4%), carbohydrate intake per day (151 ± 78 grams), system alarms per day (5 ± 3) or DDS questionnaire score (37 ± 17%) at 3 months compared to baseline. 94% of the participants maintained the glucose target of 100 mg/dl an active insulin time of 2 hours during the 3 months of study. The percentage of patients with HbA1c ≤ 7% increased from 46% at baseline to 69% at 3 months ($p = 0.001$). The percentage of patients with TIR (70-180 mg/dl) > 70% increased from 46% at baseline to 89% at 3 months ($p < 0.001$). The percentage of patients with TIR (70-180 mg/dl) > 70% and time < 70 mg/dl < 4% increased from 31% at baseline to 60% at 3 months ($p = 0.001$). No severe hypoglycaemia or DKA episodes occurred.

Conclusion: The AHCL Medtronic 780G improves glycaemic control and increases user satisfaction after 3 months of use in a real-life clinical setting.

Table 1. Outcomes at 3 months compared to baseline

	Baseline (SAP-PLGS)	3 months (AHCL)	p
HbA1c (%)	7.2 ± 0.9	6.7 ± 0.6	< 0.001
Time 70-180 mg/dl (%)	67.3 ± 13.6	80.1 ± 7.5	< 0.001
Time > 180 mg/dl (%)	29.4 ± 15.1	16.8 ± 8.4	< 0.001
Time > 250 mg/dl (%)	6.9 ± 7.8	2.7 ± 3.0	< 0.001
Time < 70 mg/dl (%)	3.4 ± 3.4	3.1 ± 2.5	0.562
Time < 54 mg/dl (%)	0.9 ± 1.2	0.7 ± 0.9	0.127
Sensor glucose (mg/dl)	155 ± 23	137 ± 13	< 0.001
Insulin dose (U/Kg/day)	0.61 ± 0.23	0.64 ± 0.26	0.002
HFS	46 ± 26	37 ± 23	0.001
DQOL	81 ± 21	76 ± 16	0.036
GME-Q	3.8 ± 0.4	4.0 ± 0.4	0.007
Pittsburgh Sleep Quality Index	6.6 ± 3.8	5.9 ± 3.5	0.047

HFS (Hypoglycaemia Fear Survey): lower scores indicating less fear of hypoglycemia, DQOL (Diabetes Quality of Life): lower scores indicating a better quality of life, GME-Q (Glucose monitoring experience questionnaire): higher scores indicating higher satisfaction with the monitoring system, Pittsburgh Sleep Quality Index: lower scores indicating better sleep Quality.

Disclosure: **P.I. Beato-Víbora:** None.

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Insulin pump interoperability of Diabeloop DBLG1 system

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Background and aims: This study aims to assess the interoperability of Diabeloop's DBLG1 Closed Loop System on glycemic

control for T1D patients, based on data from clinical trials SP7 and SP8.

Materials and methods: SP7 study was conducted on 63 out of 68 patients randomly assigned to two arms equipped with DBLG1 system using Cellnovo pump for arm 1 and Kaleido pump for arm 2. All patients attended a 12-week open loop (OL) phase (receiving their usual treatment) and a 12-week closed loop (CL) phase (using DBLG1 system).In SP8 study, 178 out of 184 patients, randomly assigned to two arms, started with a 2-week baseline phase in OL. The next 12 weeks, patients in arm 2 remained in OL while patients in arm 1 switched to CL equipped with DBLG1 System using DANA Diabecare-i insulin pump. The study was shortened due to COVID-19 epidemic.To assess the improvement reached by DBLG1 System, we compared the average time in range 70-180 mg/dL (TIR) between OL and active CL phases. For SP7 study, we analysed data from both arms. For SP8 study, to assess the TIRs for the same patients, we compared baseline and CL phases for arm 1.

Results: As shown in Figure 1, for SP7 the average TIRs increased by 8.6% for arm 1 (32 patients) and by 14.0% for arm 2 (31 patients in CL, 30 in OL). For SP8 arm 1 the average TIR increased by 12.8% (142 patients).

Conclusion: DBLG1 System improved the TIR despite the use of 3 different pumps. Therefore, improvement linked with DBLG1 System on the average TIR is independent of the pump used.

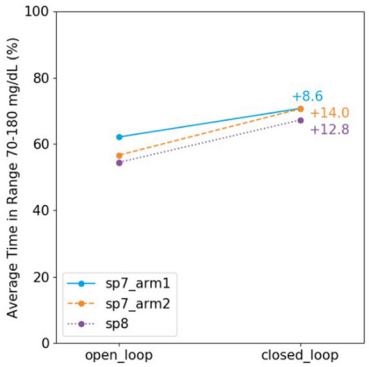

Figure 1: Average TIR (%) in OL and active CL for patients using DBLG1 System with (i) Cellnovo for SP7 arm 1, (ii) Kaleido for SP7 arm 2 and (iii) DANA Diabecare-i insulin for SP8.

Clinical Trial Registration Number: NCT02987556 and NCT04190277

Disclosure: **A. Adenis:** None.

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Improvement of outcomes in type 1 diabetic patients with HCL system compared to SAP and PLGS system

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Background and aims: Predictive low-glucose suspend (PLGS) insulin delivery system and hybrid closed loop (HCL) systems may improve glucose control and quality of life in type 1 diabetic individuals. This is a retrospective study to comparate the effect on metabolic control and glucose variability of sensor-augmented pump (SAP) therapy, PLGS and HCL systems.

Materials and methods: We retrospectively analyzed 134 adults (mean age 47±14.1 years, F/M 66/68, BMI 25.9±6.7 Kg/m^2) with T1D on insulin pump therapy, and divided into three groups, accordingly to type of insulin pump system (*Group 1*: SAP, 24 subjects; *Group 2*: PLGS, 47 subjects; *Group 3*: HCL, 63 subjects). The groups were matched for age, gender and BMI.

Results: The analysis of glucose variability parameters, in the three groups, showed a statistically significant different percentage of time within the target range, defined as 70-180 mg/dl (*gr.1*: 62.7±15.2 vs *gr.2*: 62.1±15.1 vs *gr.3*: 70.3±12.5%, p=0.012), and a significant lower time spent in hypoglycemic range (<70 mg/dl) (*gr.1*: 3.45±3.6 vs *gr.2*: 2.7±2.8 vs *gr.3*: 1.8±1.5%, p=0.04). The three groups were statistically different also for estimated-HbA1c levels and coefficient of variation percentage (HbA1c: *gr.1*: 7.3 ±0.8 vs *gr.2*: 7.2±0.7 vs *gr.3*: 6.9±0.4%, p=0.04; CV: *gr.1*: 34.7 ±6.5 vs *gr.2*: 34.5±5.7 vs *gr.3*: 31.4±4.1%, p=0.01). Also, datas show a positive correlation between percentage of TIR above 70% and the use of HCL-system (r=0.224, p=0.015). Finally, quality of life questionnaire regarding psychological acceptance, showed higher values in *group 3*, even if in a non statistically significant manner.

Conclusion: HCL systems were more effective in improving glucose control and in reducing the risk of hypoglycaemia in patients with type 1 diabetes, thereby mitigating risk for acute and chronic complications and improving quality of life.

Disclosure: **P.S. Morpurgo:** None.

SO 42 Other aspects of managing blood glucose levels

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Indirect treatment comparison of ready-to-use glucagon rescue treatments for severe hypoglycaemia: nasal glucagon versus liquid stable glucagon

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Eli Lilly and Company, Indianapolis, USA.

Background and aims: An indirect treatment comparison (ITC) evaluated the efficacy and safety differences between 2 ready-to-use severe hypoglycaemia rescue treatments, nasal glucagon (NG, Eli Lilly and Company) and liquid stable glucagon rescue pen (GRP, Xeris Pharmaceuticals), in adults with type 1 or type 2 diabetes.

Materials and methods: Systematic literature reviews identified 3 randomised cross-over clinical trials assessing the efficacy and safety of NG versus reconstituted injectable glucagon (IG), and 3 trials of GRP versus IG. No head-to-head trials of NG versus GRP were identified. The Bayesian fixed-effect network meta-analysis was used to perform the ITC. Endpoints included the proportion of participants achieving treatment success (defined as increase in blood glucose to ≥3.9 mmol/L [70 mg/dL] or an increase of ≥1.1 mmol/L [20 mg/dL] from nadir blood glucose within 30 mins), maximum blood glucose, and treatment-emergent adverse events (TEAE). In order to more closely match the study populations, participants with a nadir blood glucose value of ≤3.0 mmol/L (54 mg/dL) were analysed.

Results: A similar proportion of GRP (98.9% [279/282]) and NG participants (99.4% [155/156]) achieved treatment success (Wald method p=0.63). The mean max blood glucose values were 12.2 mmol/L (220 mg/dL) for GRP and 9.3 mmol/L (168 mg/dL) for NG, with a significant treatment difference between GRP and NG, while adjusting IG as a comparator (0.96 mmol/L [17.32 mg/dL], 95% credible interval: [3.94, 30.97]). Proportions of participants experiencing ≥1 TEAE were 48.8% for GRP and 38.5% for NG (odds ratio: 1.31 [0.67, 2.31]). Subgroup analyses showed consistent results.

Conclusion: NG and GRP had comparable efficacy in reversing insulin-induced hypoglycaemia in adults with diabetes. NG had a mean max blood glucose below 10.0 mmol/L (180 mg/dL), which may have implications on the re-establishment of euglycaemia after severe hypoglycaemia rescue.

Supported by: Eli Lilly and Company

Disclosure: **Y. Yan:** Employment/Consultancy; Eli Lilly and Company.

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Integrated safety analysis of dasiglucagon for the treatment of severe hypoglycaemia

S. Heller[1], T. Battelino[2], T. Bailey[3], R. Tehranchi[4], L. Klaff[5], T. Pieber[6], U. Hovelmann[7], L. Plum-Morschel[7], A.E. Melgaard[4], R. Aronson[8], L. DiMeglio[9], T. Danne[10];

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Background and aims: Dasiglucagon, a ready-to-use glucagon analogue in aqueous formulation, was developed to provide a fast and effective treatment for severe hypoglycemia (SH) in people with diabetes.An integrated cross-program analysis was performed to assess the safety of dasiglucagon in a representative population of people with T1DM.

Materials and methods: Three randomized, placebo-controlled phase 3 trials (2 in adults and 1 in pediatric participants) were included in the analysis (212 adults and 41 children/adolescents with T1DM).

Results: No severe or adverse events (AEs) leading to withdrawal were reported. Most AEs were mild or moderate in severity. Nausea and vomiting were the most-frequent AEs reported with active treatment both in adults and children. No differences in the frequency of nausea and vomiting were observed between adults receiving dasiglucagon versus glucagon. A higher percentage of adolescents experienced these events with dasiglucagon than with glucagon, while no treatment-related imbalance was observed in the 6- to 11-year-old age group. In the pediatric trial, no relationship between exposure ($AUC_{0\text{-}5\ hr}$ or C_{max}) to dasiglucagon, and nausea and vomiting was found. The majority of nausea and vomiting occurred within 1-3 hr and 2-3 hr of dosing, respectively.

Conclusion: In conclusion, dasiglucagon 0.6 mg for treatment of SH was found to be generally safe, and the safety profile of dasiglucagon was consistent with that observed across the class of glucagon products.

Common adverse events in ≥2% patients within 12 hr post-dose – placebo-controlled trials

Adverse reaction type, % adults	Dasiglucagon (N=116)	Glucagon (N=43)	Placebo (N=53)
Nausea	57	54	4
Vomiting	25	21	2
Headache	11	12	4
Diarrhea	5	2	0
Injection site reactions*	3	7	4
Adverse reaction type, % children	**Dasiglucagon (N=20)**	**Glucagon (N=10)**	**Placebo (N=11)**
Nausea	65	30	0
Vomiting	50	10	0
Headache	10	10	0
Injection site reactions*	5	0	0

* preferred terms included injection site pain, erythema, induration and edema

Clinical Trial Registration Number: NCT03378635; NCT03688711; NCT03667053

Supported by: Zealand Pharma A/S

Disclosure: **S. Heller:** Employment/Consultancy; Eli Lilly, Novo Nordisk, Zealand Pharma A/S, Mylan. Lecture/other fees; Novo Nordisk.

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The next-generation glucagon analogue dasiglucagon consistently achieves rapid recovery from hypoglycaemia across subgroups

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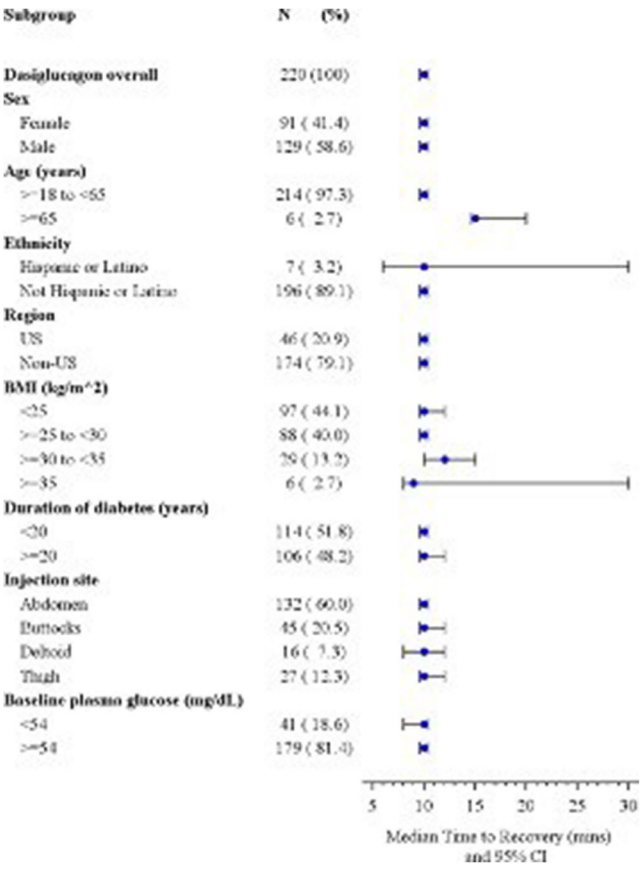
Background and aims: The efficacy of dasiglucagon 0.6 mg, a glucagon analog stable in aqueous formulation for treatment of severe hypoglycemia, has been established versus placebo in previously reported randomized, double-blind, placebo-controlled trials in adults with type 1 diabetes mellitus. An integrated analysis was conducted to investigate efficacy in demographic and other subgroups.

Materials and methods: To allow as many individuals as possible in the evaluation, the analysis comprised data from 4 trials in adults, including 2 pivotal trials, an additional phase 3 trial, and a phase 2 trial. The trials were conducted under similar conditions with respect to design characteristics, such as target population, background therapy and treatment duration. All trials included efficacy assessments following insulin-induced hypoglycemia and showed consistent efficacy results across trials. The primary endpoint was time to plasma glucose (PG) recovery, defined as first PG increase ≥ 20 mg/dL after treatment initiation without need for rescue intravenous glucose. A total of 220 participants were exposed to dasiglucagon 0.6 mg across trials.

Results: Results of the integrated analysis are shown as a Forest plot of median time to PG recovery for dasiglucagon, including 95% confidence intervals by subgroup. The results showed that the efficacy of dasiglucagon was highly consistent across subgroups, including sex, age, ethnicity, region, BMI, duration of diabetes, injection site, and baseline PG. The median time to recovery from insulin-induced hypoglycemia was 10 minutes in most groups.

Conclusion: Dasiglucagon provided rapid and effective reversal of hypoglycemia in adults with type 1 diabetes. The ready-to-use, aqueous formulation of dasiglucagon offers the potential to provide a rapid and reliable treatment for severe hypoglycemia.



Clinical Trial Registration Number: NCT02660008; NCT03378635; NCT03688711; NCT03216226

Supported by: Zealand Pharma A/S

Disclosure: **T. Danne:** Employment/Consultancy; AstraZeneca, Boehringer Ingelheim International GmbH, Eli Lilly, Medtronic, Novo Nordisk, Sanofi, Abbott Diabetes Care, Zealand Pharma A/S. Stock/Shareholding; DreaMed Diabetes.

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Glycaemic tracking of HbA_{1c} in patients with type 1 diabetes has a strong modifiable component reliant on intensive diabetes support

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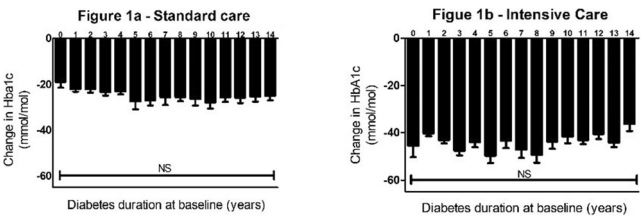
Background and aims: We have recently shown that HbA1c in people with newly diagnosed type 1 diabetes gradually rises from diagnosis for the first five years, before settling into a remarkably stable plateau. This phenomenon of HbA1c stability has been referred to as glycaemic tracking. We aimed to explore whether the course of glycaemic tracking is set irrecoverably early after diagnosis with type 1 diabetes or whether it can be modified.

Materials and methods: HbA1c obtained from the Diabetes Control and Complications Trial (DCCT) and Epidemiology of Diabetes Interventions and Complications (EDIC) trials was compared between individuals randomised to intensive treatment and standard treatment using Mann Whitney and Kruskal Wallis statistical analyses. Significance level was set at $p<0.05$.

Results: A cohort of 1441 individuals with type 1 diabetes were included, of which 711 were randomised to intensive treatment; median age was 27 years (interquartile range: 22-32 years) and 53% were male. Participant HbA1c at baseline was 71mmol/mol (8.68%) (62-85mmol/mol (7.80 - 9.90%) and median duration of diabetes was 49 months (26-108 months). HbA1c improved significantly with treatment intensification ($p<0.001$) and this improvement was sustained for the duration of the DCCT study, regardless of diabetes duration at study onset. In the standard treatment group, there was no change in hba1c across the DCCT study. This improvement in hba1c in the intensive treatment group was not sustained outside of the trial setting and HbA1c returned to baseline track one year after close of DCCT and remained here in the longer term.

Conclusion: We demonstrate that HbA1c track in people with type 1 diabetes can be altered with intensive diabetes support and this support is effective regardless of diabetes duration. However, this improvement persists only as long as the support is made available. We need further research to deliver long-term intensive support to people with type 1 diabetes. Figure 1Figure legend: Figures 1a and 1b: Change in Hba1c from baseline to one year of DCCT in the standard treatment arm (Figure 1a, n=726) and the intensive treatment arm (Figure 1b, n=710), according to duration of diabetes at DCCT study onset. *Hba1c presented as Mean +/- Standard Error of the Mean (SEM). P>0.05. Number of participants in each group is described in Supplementary table 1.*



Disclosure: **L.M. Quinn:** None.

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Variability of carbohydrate estimation skills in adults with type 1 diabetes

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Background and aims: Carbohydrate (CHO) counting is an essential component of glucose control in type 1 diabetes (T1D), even with the increasing use of hybrid closed-loop systems. Previous works investigated possible relationship between the distribution of CHO-counting error (CC_{err}) and the actual CHO content and meal type. However, information about individual CC_{err} distribution may be useful for therapy optimization, since systematic under-/over-estimation of meal CHO may be more amenable to therapeutic optimization than random estimation errors. Here we aim to assess the distribution of CC_{err} variability among adults with T1D.

Materials and methods: An anonymized online survey containing images of known common non-packaged foods of varying serving sizes was used to assess the CC_{err} of 61 T1D adults (43 female, BMI = 24 ± 3 kg/m^2, A1c = 6.9 ± 1.0%, insulin pump to multiple injections ratio 2.5:1). Survey participation resulted in a random selection of 8 out of 70 possible food variants requiring entry of estimated CHO content. CC_{err} was defined as the difference between participants' estimate and true CHO amount expressed as signed (sCC_{err}) and relative signed errors ($rsCC_{err}$). Analysis of variance with significant p-value set at 0.05 was performed to determine possible relationship between sCC_{err} (or $rsCC_{err}$) and true CHO amount, meal type, and subjects. A linear regression model was used to describe the relationship between sCC_{err} and true CHO amount. In order to quantify within-subject variability of CC_{err}, the interquartile (IQR) range was determined for each subject's $rsCC_{err}$, and classified as small (IQR ≤ 30%), mid (30% < IQR ≤ 60% g), or large (IQR > 60%).

Results: Overall, meal CHO was underestimated (median (IQR) sCC_{err} = -9 (31) g, $rsCC_{err}$ = -25% (67%)). Analysis of variance indicated that both sCC_{err} and $rsCC_{err}$ are predicted by true CHO amount and subject factors (p<0.001), $rsCC_{err}$ also by meal type (p=0.006). In particular, sCC_{err} was inversely correlated with true CHO amount (Pearson coefficient ρ=-0.64, p=<0.001), indicating a tendency to overestimate small CHO amounts and progressively underestimate large ones (Figure 1A). The coefficient of determination R^2 was 0.421, meaning that true CHO amount explained about 40% of sCC_{err} variance. With respect to subjects' error variability (Figure 1B), 13% of subjects showed a small $rsCC_{err}$ variability (systematic underestimation in 7/8), 41% a medium variability (systematic underestimation in 13/35) and 46% a large variability (systematic underestimation in 1/28, overestimation in 4/28).

Conclusion: Findings suggest that CC_{err} is influenced by CHO amount with high variability in the population, in agreement with the literature. In addition, results highlight that CC_{err} distributes differently among T1D subjects, showing that most of those with mid-low error variability tend to a systematic underestimation. This information may be exploited by clinicians to adjust patient's insulin therapy.

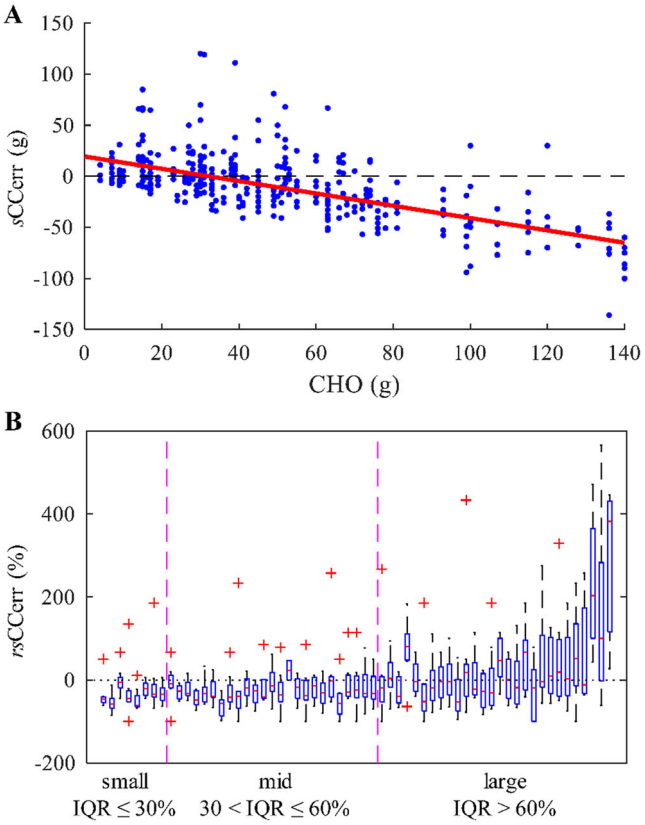

Figure 1. A: distribution of signed carb-counting errors (sCC_{err}) vs. true CHO amount, with linear regression slope overlapped. B: boxplots of relative signed carb-counting errors ($rsCC_{err}$) distributed among subjects, grouped by interquartile (IQR) range of variability: small (IQR ≤ 30%), mid (30% < IQR ≤ 60% g) and large (IQR > 60%).

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Glycaemic management over 6 months with the Omnipod® 5 automated insulin delivery system

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Background and aims: Advances in diabetes technology have transformed the treatment paradigm for type 1 diabetes (T1D), yet the burden of disease remains significant. The Omnipod 5 Automated Insulin Delivery System is a novel hybrid closed-loop (HCL) system with fully on-body operation. Safe and effective use of the Omnipod 5 System was demonstrated in adults and children with type 1 diabetes (T1D) during a 3-month pivotal study. Longer studies will evaluate durability of glycemic benefit. We present results from the first 3-month follow-up of a 12-month extension study.

Materials and methods: Pivotal study participants were invited to continue system use for an additional 12 months. Following the pivotal study, A1C was measured again after 3 months (6 months of total use). Participants aged 6-70y with T1D≥6 months and A1C<10% (86 mmol/mol) used the system in HCL mode for a total of 6 months at home, after 14d of their standard therapy (pump therapy or multiple daily injections). Participants selected daily profile glucose targets from 110-150mg/dL (6.1-8.3mmol/L) in 10mg/dL (0.6mmol/L) increments. Safety and efficacy endpoints were occurrence of severe hypoglycemia (SH) and diabetic ketoacidosis (DKA), and change in A1C compared with baseline, respectively.

Results: Most participants (95%) continued into the extension phase. Children (n=108) and adults (n=109) were aged (mean±SD) 10±2y and 37±14y with T1D duration 5±3 and 17±12y, respectively. For children, mean A1C was reduced but not significantly different between end of pivotal and the 3-month extension, while adults had an additional -0.1% (-1.1 mmol/mol) decrease (p<0.01). A1C was lower after a total of 6 months of system use than at baseline for children and adults (p<0.001, Table). There was 1 episode of DKA and no episodes of SH during the 3-month extension phase. Nearly all (99%) opted to continue into the next 3-month extension.

Conclusion: The safety and improved glycemic outcomes from the pivotal study persisted for an additional 3 months of system use in this cohort of 217 participants, with almost all continuing into the next 3 month-extension. Sustained reduction of A1C indicates the potential long-term benefit of the Omnipod 5 System. The extension phase will continue for an additional 9 months with A1C recorded every 3 months, providing data for 15 total months of system use.

Table. A1C (% [mmol/mol]) and participants with A1C <7.0% (53 mmol/mol) (n (%)) at baseline and after 3 and 6 months of Omnipod® 5 Automated Insulin Delivery System use[€]

Measurement	Timepoint	Children[+]	Adults and Adolescents[+]
A1C	Baseline (Pivotal start)	7.7 ± 0.9 [61 ± 9.8]	7.2 ± 0.9 [55 ± 9.8]
	3 months (Pivotal end)[†]	7.0 ± 0.6 [53 ± 6.6]*	6.8 ± 0.7 [51 ± 7.7] *
	6 months (3-month extension)	6.9 ± 0.6 [52 ± 6.6]*	6.7 ± 0.6 [50 ± 6.6]*[¥]
Change in A1C	Baseline to 3 months[†]	-0.7 ± 0.6 [-7.7 ± 6.6]*	-0.4 ± 0.6 [-4.4± 6.6]*
	Baseline to 6 months	-0.8 ± 0.7 [-8.7 ± 7.7]*	-0.5 ± 0.6 [-5.5 ± 6.6]*
	3 months[†] to 6 months	-0.1 ± 0.4 [-1.1 ± 4.4]	-0.1 ± 0.3 [-1.1 ± 3.3][¥]
A1C <7.0% (<53 mmol/mol)	Baseline	25 (23%)	46 (42%)
	3 months[†]	56 (53%)	71 (65%)
	6 months	58 (54%)	80 (73%)

Data are mean ± SD or n (%)

[+] Children are ages 6.0-13.9 y and adults are ages 14.0-70.0 y

[€] All results are shown for 108 children and 109 adults with A1C available at first 3-month extension phase collection point (6 months from baseline). An additional 2 children and 4 adults participated but did not have a 3-month extension A1C available

[†] 3-month follow-up corresponds to the end of the pivotal study and the start of the extension phase. A1C was missing at end of pivotal study for 2 of the 108 children

* Significant change from baseline A1C based on a linear mixed effects model, p<0.001

[¥] Significant change between pivotal study end and 3-month extension phase A1C based on a linear mixed effects model, p<0.01

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Disclosure: **A.L. Carlson:** Employment/Consultancy; International Diabetes Center at Park Nicollet. Grants; Insulet, Dexcom, Medtronic, Abbott Diabetes, Sanofi, Eli Lilly, Novo Nordisk. Other; Medtronic, Sanofi, Eli Lilly, Bigfoot.

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Biomedical and patient reported outcomes from the PRO solo multinational, multi-centre clinical trial

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Background and aims: Patch pumps provide increasing choice in insulin delivery therapy as people with diabetes look to technologies to help them achieve their diabetes goals. Patient Reported Outcomes (PROs) help to understand device burden alongside benefit, as well as impact on everyday living, acceptance of therapy regimens and drivers of self-management behaviours. This study aimed to investigate biomedical outcomes as well as the effect on PROs of the newly introduced Accu-Chek Solo micropump system compared to treatment by multiple daily injections (MDI) and an established patch pump.

Materials and methods: 181 participants were enrolled in a three-armed, randomized, controlled multinational, multi-centre trial. Individuals with type 1 diabetes naïve to insulin pump therapy (39.0 ±11.9 years old, 44% females, 15.0 ±10.8 years since diagnosis of diabetes, HbA1c **63.9±5mmol/mol (**8.0 ± 0.6%) used either the Accu-Chek Solo micropump system (**ACS**), MDI or Insulet Omnipod (IO) for 6 months, followed by 3 months where all used ACS. The Diabetes Technology Questionnaire (DTQ, at baseline, 3, 6 and 9 months) was the primary endpoint. Time in target range, glycaemic variability and number of insulin boluses as well as indications for commencement of insulin pump therapy were assessed according to a pre-defined analyses plan.

Results: At six months, DTQ change score was significantly higher in ACS group (105.9 ± 2.66 SD) compared to MDI (94.8±2.63) (p=0.001) but no significant differences were identified between ACS and IO participants. ACS participants achieved a greater improvement in time in range at 26 weeks (from baseline) than either MDI or IO participants (ACS: mean BG data 54.2% ±12.9 improved to 58.2% ±19.9 compared to MDI 48.1% ±11.6 to 49.6 ±11.5 or IO 52.8% ±22.7 to 48.9% ±20.6, p=0.001. Glycaemic variability was similarly reduced from 77.0 ±15; to 75.0 ±17.0mg/dL; however it increased in the IO group slightly from 68.1 ±10.2 to 68.9 ±10.0mg/dL. A slight reduction was also observed in the MDI group (71.7 ±15.0 to 70.2 ±13.1mg/dL). Participants in the ACS and IO groups experienced a greater bolus frequency increase than the MDI groups (baseline ACS mean boluses = 4.0 ±1.0 per day rising to 4.5 ±1.4 at 26 weeks; IO 4.0 ±1.1 rising to 4.8 ±2.9) compared to MDI (baseline mean = 3.9 ±1.2, unchanged at 26 weeks). The primary indication for commencement of pump therapy (stated by *n*=124, 69%) was because HbA1c goals were not being met, followed by participants' desire for the therapy (n=31, 17%).

Conclusion: PROs are increasingly relevant as drivers of self-management behaviours and subsequent clinical outcomes, including HbA1c. Participants switching from MDI therapy to the ACS micropump system experienced a significant and meaningful positive impact regarding sustained improvement in reported outcomes, with no differences

between the ACS and IO users. Results indicate improved biomedical outcomes for participants in pump user groups compared to MDI and non-inferiority of the novel ACS patch pump to the well-established IO pump. Patient preferences are important parameters when deciding the best therapy for each individual user.

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Supported by: Roche Diabetes Care

Disclosure: **K. Barnard-Kelly:** Honorarium; Roche Diabetes Care.

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Glycaemic profiles and treatment patterns: real-world data of 2,536 people with type 2 diabetes using the Omnipod® Insulin Management Systems and cloud-based data management

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Background and aims: Diabetes technology (Continuous Subcutaneous Insulin Infusion, CSII, and Continuous Glucose Monitoring, CGM) may help improve outcomes in people with type 2 diabetes (T2D), and its usage is increasing in this population. This analysis assessed glycemic metrics and treatment patterns for a large cohort of people with type 2 diabetes using data from the Omnipod and Omnipod DASH® Insulin Management Systems and a data management system. A subgroup of these patients also had CGM data available.

Materials and methods: From January 2015 through March 2021, usage data from the Omnipod Systems were uploaded to the data management system and matched via device serial number to a second database of self-reported demographic data and de-identified. Only users that had ≥3 months of systems use were included. CGM data were included if CGM was used for ≥15.5% of overall time of product usage available (minimum of 14 days of a 3-month period).

Results: Patients with T2D (n=2,536) were age (mean±SD) 57±12y, 26% aged ≥65 yr, 53% female, and 61% used the Omnipod or Omnipod DASH Systems for ≥1 y. Mean percentage of total daily insulin from basal delivery was 61%. Users bolused 3.2±1.6 times/day, with 7.9±4.3 units/bolus. Twenty-two percent (n=569) used the Omnipod DASH system. Omnipod System users with CGM data (n=363, 14%) had a mean glucose of 172±33 mg/dL (9.6±1.8mmol/L), mean estimated HbA_{1c} of 7.4%, and time in range 70-180, <70 and >180 mg/dL (3.9-10.0, <3.9 and >10.0mmol/L): 61.7%±20.0, 1.0%±1.8, and 37.3%±20.4, respectively. Median pod change interval was 2.8 days.

Conclusion: These real-world data from a large cohort of people with type 2 diabetes provide insights to management patterns and glycemic profiles. Use of the tubeless pump can be a viable option for those with T2D requiring insulin therapy.

Table. Characteristics and Insulin Use Patterns of People with Type 2 Diabetes Using the Omnipod and Omnipod DASH System and CGM

	All Users	CGM Cohort
N	2,536	363
Age, yr	57.0 ± 11.7	56.3 ± 11.4
Female, %	53	48
System use for ≥1 y, %	61	48
TDD, U	60.0 ± 28.3	64.7 ± 30.1
Basal, %	61	57
Bolus, %	39	43
Bolus size, U	7.9 ± 4.3	7.9 ± 4.2
Bolus, x/d	3.2 ± 1.6	3.9 ± 1.8
Omnipod DASH user, %	22	40

Results are mean ± SD unless indicated.

Supported by: Insulet

Disclosure: **T. Kader:** None.

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Safety evaluation of the Omnipod® 5 automated insulin delivery system over three months of use in adults and children with type 1 diabetes

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Background and aims: Advances in diabetes technology have transformed the treatment paradigm for type 1 diabetes (T1D), yet the burden of disease remains significant. The Omnipod 5 Automated Insulin Delivery (AID) System consists of a tubeless insulin pump (pod) containing a personalized Model Predictive Control algorithm which communicates directly with a Dexcom G6 continuous glucose monitor (CGM, or sensor) to automate insulin delivery. Therapy customization is enabled through glucose targets from 110-150mg/dL, adjustable by time of day. We report on the first, pivotal outpatient safety evaluation of the system in a large cohort of adults (ages 14-70 y) and children (ages 6-13.9 y) with T1D.

Materials and methods: Participants aged 6-70y with T1D≥6 months and A1C<10% used the AID system for 3 months at home after 14 days of data collection with their standard therapy (ST) (pump therapy or multiple daily injections). The primary safety endpoints were occurrence of severe hypoglycemia (SH) and diabetic ketoacidosis (DKA). The primary effectiveness endpoints were change in A1C and sensor glucose percent time in target range (TIR) (70-180mg/dL, 3.9-10.0mmol/L) during AID compared with ST.

Results: The adult (N=128) and child (N=112) cohorts were aged 37 ±14y and 10.3±2.2y with T1D duration 18±12y and 4.7±2.6y and baseline A1C 7.16±0.86% (55±9 mmol/mol) (range 5.20-9.80%, 33-84 mmol/mol) and 7.67±0.95% (60±10 mmol/mol) (range 5.80-10.30%, 40-89 mmol/mol), respectively. In adults, TIR increased from 64.7 ±16.6% to 73.9±11.0% and A1C was reduced by 0.38% (4.2 mmol/mol) to 6.78±0.68% (51±7mmol/mol) (both p<0.0001). In children TIR increased from 52.5±15.6% to 68.0±8.1% and A1C was reduced by 0.71% (7.8 mmol/mol) to 6.99±0.63% (53±7 mmol/mol) (both p<0.0001). Additional outcomes are shown in the Table. During the 3-month AID phase there were 3 episodes of SH unrelated to the study device and 1 episode of DKA (suspected infusion site failure).

Conclusion: In this outpatient, multi-center pivotal study in a large cohort of adults and children with T1D, the Omnipod 5 System was safe and effective when used for 3 months at home. The results highlight the successful use of the AID system to increase TIR and reduce A1C with very low rates of acute complications.

Table. Primary and secondary glycemic outcomes during Standard Therapy (ST) compared with Automated Insulin Delivery (AID) using the Omnipod 5 System in adults (ages 14-70 y) and children (ages 6-13.9 y)

Glycemic Outcomes		ST, Adults	AID, Adults	ST, Children	AID, Children
A1C, %		7.16 ± 0.86	6.78 ± 0.68*	7.67 ± 0.95	6.99 ± 0.63*
A1C, mmol/mol		55 ± 9	51 ± 7*	60 ± 10	53 ± 7*
Mean glucose, mg/dL		161 ± 28	154 ± 17*	183 ± 32	160 ± 15*
Mean glucose, mmol/L		8.9 ± 1.6	8.6 ± 0.9*	10.2 ± 1.8	8.9 ± 0.8*
Percent time in range, %					
mg/dL	mmol/L				
<54	<3.0	0.22 [0.00, 0.77]	0.17 [0.06, 0.28]*	0.10 [0.00, 0.41]	0.23 [0.08, 0.42]
<70	<3.9	2.00 [0.63, 4.06]	1.09 [0.46, 1.75]*	1.38 [0.42, 2.67]	1.48 [0.65, 2.23]
70-180	3.9-10.0	64.7 ± 16.6	73.9 ± 11.0*	52.5 ± 15.6	68.0 ± 8.1*
>180	10.0	32.4 ± 17.3	24.7 ± 11.2*	45.3 ± 16.7	30.2 ± 8.7*
≥250	≥13.9	10.1 ± 10.5	5.8 ± 5.5*	19.1 ± 13.1	9.6 ± 5.4*

Data are mean ± SD or median [IQR]
*Significant change from standard therapy to automated insulin delivery system, p < 0.05

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Six months pump treatment improves cardiovascular and endothelial function compared to MDI in patients with type 1 diabetes, independently of HbA_{1c}

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Background and aims: Patients with type 1 diabetes mellitus (T1DM) present signs of vascular and endothelial dysfunction earlier compared to healthy individuals. The evidence regarding the efficacy of pump on cardiovascular markers in T1DM is scarce. The aim of this study is to investigate the effects of insulin intensification with pump on arterial stiffness and endothelial glycocalyx in patients with T1DM compared to multiple daily insulin (MDI) injections.

Materials and methods: In the study included 20 patients with poor glycemic control who were transitioned from MDI to pump and 20 patients matched for sex, age and HbA1C who remained on intensified treatment with MDI (control group). We measured at baseline and at six months post-treatment a) Carotid-femoral pulse wave velocity (PWV) b) central systolic blood pressure (cSBP) and c) perfused boundary region (PBR) of the sublingual arterial microvessels (increased PBR indicates reduced endothelial glycocalyx thickness) and d) global LV longitudinal strain (GLS) by speckle tracking imaging.

Results: At baseline, patients among the two groups had similar age, sex, HbA1c and markers of endothelial and vascular function (p>0.05). After six months treatment, patients on pump improved HbA1c (8.5±1.5% vs 7.6±0.7%, p<0.05), PBR (2.15±0.2 vs. 2±0.2 μm, p<0.05), PWV (10.07 ±1.9 vs. 8.91±1.4m/s, p<0.05), cSBP (113.45±13.6 vs. 110±11 mmHg, p<0.05) and GLS (-21.22±1.06% vs -22.02±0.97%, p<0.05). However, we did not observe statistically significant differences in PBR (2±0.3 vs. 2.11±0.3 μm, p>0.05), PWV (9.8±3.7 vs. 10.06±3.9m/s, p>0.05), cSBP (111.73±13.4 vs. 110.04±15.2 mmHg, p>0.05) and GLS (-20.67±2.74% vs -20.93±1.97%, p>0.05)in patients who remained on MDI, despite improvement of HbA1c (8.4±1.1% vs 7.5±0.8%, p<0.05).The reduction of PBR was related with the alterations in cSBP (r=0.50,p=0.035) and PWV (r=0.48,p=0.047).

Conclusion: Insulin intensification with pump improves the thickness of endothelial glycocalyx and decreases arterial stiffness six months post-treatment in patients with T1DM, independently of glycemic control.

Disclosure: **A. Kountouri:** None.

SO 44 Glycaemic management in special settings and populations

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Continuous glucose monitoring with multiple daily insulin injections improves perinatal outcomes in women with type 1 diabetes

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Background and aims: Women with type 1 diabetes mellitus (T1DM) are at increased risk of adverse pregnancy outcomes. Compared to the general population there is a higher prevalence of congenital malformations, cesarean delivery, fetal macrosomia, large for gestational age infants, preeclampsia, preterm delivery, and neonatal mortality. Insulin regimens in pregnancy include multiple daily insulin injections (MDI) and continuous subcutaneous insulin infusion via insulin pumps (CSII). With advancing technologies, continuous glucose monitoring (CGM) is increasingly used in antenatal care at the expense of self-monitoring of blood glucose. The aim of our study was to evaluate the effectiveness of different management options on perinatal and neonatal health outcomes**.**

Materials and methods: We performed a retrospective cohort study of 232 pregnant women with type 1 diabetes from a single university-affiliated perinatal center in the Czech Republic. Women were divided into four groups according to the mode of glucose monitoring and treatment: self-monitoring of blood glucose with multiple daily insulin injections (SMBG+MDI), self-monitoring of blood glucose with continuous subcutaneous insulin infusion (SMBG+CSII), continuous glucose monitoring with multiple daily insulin injections (CGM+MDI), continuous glucose monitoring with continuous subcutaneous insulin infusion (CGM+CSII). Data were retrieved from the electronic medical records.

Results: Overall, 35.3% of women attended preconception counseling, with more women in CGM+CSII and less in SMBG+MDI group (52.7% vs. 18.2%; p=0.002). We observed lower mean HbA1c concentrations prior to conception in CGM+MDI and CGM+CSII groups (55.1 ± 15.3 and 54.3 ± 12.4; p=0.005). On univariate analysis, a higher rate of liveborn infants (97.0%; p=0.031) was observed in the CGM+MDI group. There was a higher incidence of operative delivery (cesarean section or instrumental vaginal delivery) in SMBG+CSII (81.3%; p=0.048) group and fewer cases of large for gestational age (LGA) infants among women with CGM+MDI, but more in the CGM+CSII group (18.8% vs. 48.1%; p=0.039). There were no cases of umbilical artery pH < 7.15 in the CGM+MDI group (0; p=0.006). Multivariate logistic regression showed that CGM+MDI decreases the odds of operative delivery (OR 0.29, 95% CI 0.116-0.707; p=0.007), LGA (OR 0.34, 95% CI 0.124-0.923; p=0.034) and umbilical artery pH < 7.15 (OR 0.04, 95% CI 0.002-0.790; p=0.034).

Conclusion: Our study suggests that perinatal outcomes of women with T1DM are affected by the modality of glucose monitoring and insulin regimen. Continuous glucose monitoring together with multiple daily insulin injections are associated with lower rates of operative delivery, large for gestational age infants and fetal hypoxia.

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The effect of liver transplantation on total daily doses of insulin requirement

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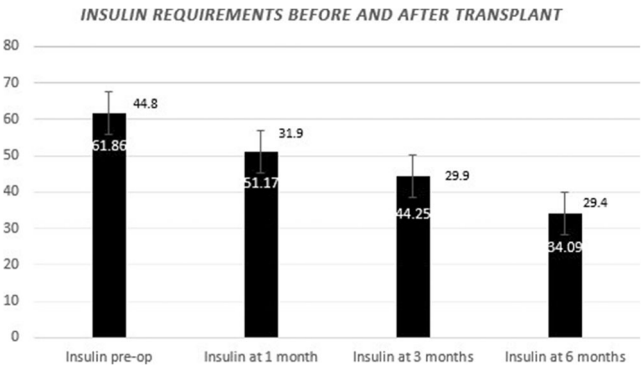
Background and aims: Liver transplantation (LT) is increasingly being offered for treatment of advanced liver disease across varied aetiologies. Anecdotally, patients with pre-existing diabetes mellitus (DM) after a liver transplant usually experience improved glycaemic control and less insulin requirement compared to other organ transplants, where glycaemic control may worsen. Our aim was to evaluate possible changes in insulin requirements post LT and to explore correlations with type of transplant, immunosuppression used and reason for transplant.

Materials and methods: An observational study of adults with pre-existing DM who underwent LT from 2009 to 2020 in a major UK liver transplantation centre. Inclusion criteria were adults (aged 18-80 years) diagnosed with DM for one year or longer before LT. Data was collected retrospectively from individual patient records and clinical databases. Patients were excluded if they died intra-operatively or within a month after LT. Parameters reviewed included demographics, DM history, indication for LT and pre- and post-transplant insulin requirements (at 1, 3 and 6 months post-operatively). Those on insulin pre-operatively until 6 months post-transplant had their daily insulin requirements analysed. A p-value of <0.05 was considered statistically significant.

Results: 359 patient cases were analysed; mean age at transplantation was 56 (±10) years, 71% were male, 327 (91%) had type 2 DM and 23 (6.4%) type 1 DM. Mean duration of DM prior to transplant was 110 (±162) months. Indication for LT included 105 (29%) Alcoholic Liver Disease (ALD); 91 (25%) Non-Alcoholic Fatty Liver Disease; 78 (22%) Hepatocellular carcinoma; 21 (6%) Autoimmune Hepatitis (AIH) and 64 (18%) other/unknown. HbA1c levels reduced from 49.96 (±18.34)mmol/mol pre-transplantation to 45.12 (±17.51)mmol/mol 3 months post-transplantation. Mean BMI was 29.36 (±5.28) pre-transplantation compared to 27.73 (±5.15) 3 months post-transplantation. 133 (37%) patients were on insulin pre-operatively, this reduced to 91 (25%) patients at 6 months, despite the use of immunosuppressants (p<0.05). Total daily insulin requirements (units/day) reduced from mean 62 units (±45) pre-transplant to mean 34 units (±29) at 6 months (44% reduction, p<0.05) as shown in Fig 1. The greatest difference on insulin reduction was observed with AIH (40.43±28.47) and ALD (36.00±10.57).

Conclusion: Our study shows significant reduction in insulin requirements post-liver transplantation, despite patients starting immunosuppressive therapy. This was most marked in patients needing transplant for ALD and AIH. To our knowledge, this is one of the first studies showing this effect. Possible mechanisms include the role of the pancreatic-liver axis in regulating glucose metabolism and insulin utilisation. We need collaborative data from major transplant centres to corroborate these findings.



Disclosure: **O. Awala:** None.

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Duodenal jejunal bypass liner (DJBL) for treatment of type 2 diabetes and obesity: risks and benefits among 926 patients in the worldwide EndoBarrier (EB) registry

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Background and aims: There is uncertainty over the balance of risks and benefits of proximal intestinal exclusion with DJBL for treatment of type 2 diabetes and obesity.

Materials and methods: During 2017, an independent, secure, online registry was established under the auspices of the Association of British Clinical Diabetologists, for the collection of DJBL safety and efficacy data from patients worldwide. To evaluate the effectiveness of DJBL, we categorised patients into groups according to their baseline HbA1c.

Results: As of March 2021, data had been submitted on 929 patients (mean ± SD age 52.2 ± 10.5 years, 52.6% male, 85.7% type 2 diabetes, BMI 41.6 ± 9.1 kg/m^2) from 31 centres in 9 countries: Australia, Austria, Brazil, Czech Republic, England, Germany, Israel, Netherlands and Scotland. In those with both baseline and time of removal data, mean ± SD weight fell by 13.8 ± 9.7 kg from 121.7 ± 25.7 to 107.9 ± 24.4 kg (n=719, p<0.001), HbA1c by 13.7 ± 16.5 from 67.6 ± 20.1 to 54.0 ± 13.7 mmol/mol (by 1.3 ± 1.5, from 8.3 ± 1.8 to 7.1 ± 1.3 %) (n=558, p<0.001), systolic BP fell from 138.3 ± 18.2 to 131.3 ± 17.3 mmHg (n=355, <0.001) and cholesterol fell from 4.9 ± 1.3 to 4.3 ± 1.0 mmol/L (n=389, p<0.001). In those with baseline HbA1c ≥ 53 mmol/mol, HbA1c fell by 17.2 ± 16.1 from 74.7 ± 17.1 to 57.6 ± 12.8 mmol/mol (n = 432, p<0.001); with baseline HbA1c ≥ 64 mmol/mol, HbA1c fell by 21.3 ± 17.6 from 81.5 ± 15.9 to 60.2 ± 13.4 mmol/mol (n=305, p<0.001); with baseline HbA1c ≥ 75 mmol/mol , HbA1c fell by 28.9 ± 18.0 from 91.4 ± 14.6 to 62.5 ± 14.6 mmol/mol (n=173, p<0.001); with baseline HbA1c ≥ 86 mmol/mol , HbA1c fell by 36.3 ± 17.9 from 100.0 ± 13.6 to 63.6 ± 15.8 mmol/mol (n=100, p<0.001). There were 39 (4.2%) serious adverse events (SAE) and118 (12.7%) less serious SAEs (table). All SAE patients made a full recovery and most derived significant benefit despite the SAE.

Conclusion: The data demonstrate that in response to DJBL treatment, the higher the initial HbA1c, the greater the fall in HbA1c, with HbA1c fall 28.9 mmol/mol when initial HbA1c ≥ 75 mmol/mol and 36.3 mmol/mol when initial HbA1c ≥ 86 mmol/mol . In view of impact on both microvascular and macrovascular risk factors, the benefits of DJBL therapy could reduce the complications of diabetes. The SAE rate was acceptable and all patients made a full recovery and many experienced significant benefit despite the SAE. This data from the EB registry in a large patient number suggests that the benefits of DJBL outweigh the risks.

Table. Serious adverse events in 926 EndoBarrier treated patients (GI = gastrointestinal).

Serious Adverse Event	n	%
Early removal because of GI bleed	22	2.4
Liver abscess (early removal = 7/10; found at time of routine explant = 3/10)	10	1.1
Liver abscess after prolonged implant (1/2 = nearly 2 years; 1/2 = 16 months)	2	0.2
Early removal because of pancreatitis	2	0.2
Early removal because of liner obstruction - surgical removal required*	1	0.1
Early removal because of cholecystitis	1	0.1
Abdominal abscess due to small perforation of bowel in relation to EndoBarrier	1	0.1
Total	**39**	**4.2**
Less Serious Adverse Event	**n**	**%**
Precautionary hospitalisation because of transient GI symptoms - removal not required	36	3.9
Early removal because of GI symptoms	35	3.8
Early removal because of GI symptoms - EndoBarrier had migrated	20	2.2
Early removal because of liner obstruction	8	0.9
Minor GI bleeding. EndoBarrier not removed	5	0.5
Precautionary hospitalisation because of transient GI problems at time of removal	4	0.4
Hospitalisation because difficult removal - needed two attempts	4	0.4
Transient obstruction of device cleared at endoscopy - device not removed	3	0.3
Transient obstruction of device cleared by gastrografin - device not removed	1	0.1
Liver abscess after 5 weeks treated successfully with antibiotics without early removal	1	0.1
Precautionary early removal because of asymptomatic EndoBarrier migration	1	0.1
Total	**118**	**12.7**

*The EndoBarrier crown became lodged in the oesophagus and then separated from the extraction hood, requiring surgical removal under general anaesthesia, through a small incision in the side of the neck.

Supported by: ABCD

Disclosure: **R.E.J. Ryder:** None.

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Exogenous insulin therapy failed to improve insulin sensitivity and beta cell function in non-diabetic cystic fibrosis assessed with the oral minimal model method

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Background and aims: Insulin deficiency in cystic fibrosis (CF) is the primary cause of impaired glucose tolerance and CF related diabetes (CFRD). Along with it, insulin sensitivity may also play a role. In early CFRD, exogenous insulin showed to be beneficial. Herein we aimed to quantify the effects of exogenous long- and fast-acting insulin on metabolic indices of glucose regulation in nondiabetic CF adults and children after an oral glucose tolerance test (OGTT).

Materials and methods: Twenty-two nondiabetic CF adults (age=21 ±3 y; BMI=22±2 kg/m^2; BW=66±9 kg) and eighteen children (age=14±2 y; BMI=21±3 kg/m^2; BW=52±13 kg) underwent a placebo-controlled, randomized, double-blind OGTT study. In the second visit, subjects were randomized to receive either placebo (PBO) or 1x/day long-acting (LA) or 3x/day fast-acting (FA) insulin analogs. Plasma glucose, insulin and C-peptide were measured for 5 h. The oral glucose and C-peptide minimal models were used to quantify insulin sensitivity, β-cell responsiveness and disposition index. Paired T-test was used to assess differences between visits.

Results: Results (mean±SE) are reported in Fig. 1. Insulin sensitivity (S_I) and disposition index (DI) are significantly reduced after LA treatment, while beta-cell responsiveness (ϕ_{tot}) is only slightly, but not significantly, reduced. Not significant differences are reported in metabolic indices after FA and placebo treatments.

Conclusion: In our cohort of CF patients, exogenous insulin administration seems not to be beneficial in postprandial glucose control with both 3x/day FA and 1x/day LA insulin treatments. More studies are needed to confirm these findings.

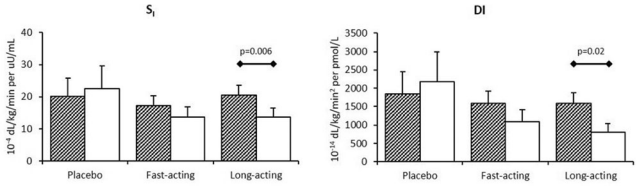

Figure 1: Insulin sensitivity (S_I, left) and disposition index (DI, right). Results are presented as mean±SE (p-value from paired T-test), with pre- vs. post-treatment (in placebo, fast-acting and long-acting insulin group) in shaded vs. blank bars, respectively.

Clinical Trial Registration Number: NCT02496780

Supported by: MIUR "Departments of Excellence" (Law 232/2016), NIH R01-DK-101402, Cystic Fibrosis Foundation, NIH UL1-TR-000114, NIH UL1-TR-000135, NIH U24-DK-100469

Disclosure: **M. Schiavon:** None.

SO 45 Seeing the full picture of diabetic retinopathy

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Impact of ethnicity and glycaemic control on developing sight threatening retinopathy in people with type 2 diabetes

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Background and aims: There is limited long term data on the interactions between traditional risk factors associated with the development of sight threatening diabetic retinopathy (STDR) and ethnicity in people with diabetes. The aim of this study was to describe the clinical, demographic and biochemical features of an ethnically diverse cohort of people with type 2 diabetes mellitus (T2DM) attending diabetes eye screening service with no retinopathy at baseline who developed STDR over a 14 year period.

Materials and methods: Data from diabetes eye screening clinics was collected alongside data from hospital records. We evaluated data from 10,782 people with T2DM (51% male) from diverse ethnicities (44% Caucasian, 40% African-Caribbean, 9% Asian, 7% other), with no retinopathy at baseline who attended the diabetic eye screening clinics between 2004-2018. Median follow up was 8.9 years. No retinopathy was defined by United Kingdom National Screening Committee (UKNSC) grades of R0 and M0. STDR was defined by the presence of any moderate to severe non proliferative or pre-proliferative retinopathy (R2) or proliferative retinopathy (R3) or maculopathy (M1) in either eye. Survival analysis using cox regression modelling was conducted with the primary end point of STDR.

Results: At baseline, the median (interquartile range-IQR) age was 55 (45-66) years, with a median (IQR) duration of diabetes of 2 (0-5) years. Of the cohort of 10,782 people with no retinopathy at baseline, 2410 (22%) developed STDR over the 14 years of the study. People who developed STDR in comparison to individuals who did not had, median (IQR), higher at baseline HbA1c 67.2 (54.1, 86.9) vs 57.4 (47.5, 77.0) mmol/mol and longer duration of diabetes 4.0 (1.0, 8.0) vs 1.0 (0.0, 4.0) years. Of the cohort who progressed to STDR 45% were of African-Caribbean ethnicity, with the remaining 55% of individuals belonging to either Caucasian, Asian or other ethnicities. We observed that 53% of people who progressed to STDR resided in areas of high levels of Index of Multiple Deprivation (IMD), in contrast to 16% who resided in areas of low IMD. In multivariable cox regression analyses, hazard ratio (95% confidence intervals), duration of diabetes 1.048 (1.042-1.054), HbA1c 1.009 (1.007-1.010) and African Caribbean ethnicity 1.093 (1.002-1.192) emerged as significant independent risk factors associated with increased risk for STDR, $p<0.05$ for all.

Conclusion: In an ethnically diverse cohort of people with T2DM, we observed that HbA1c, duration of diabetes and African Caribbean ethnicity are associated with a greater risk of STDR. Further studies are needed to better understand the underlying mechanisms that may explain our results.

Supported by: GSTT Charity

Disclosure: **A. Nirmalakumaran:** Grants; Guy's and St Thomas' Charity.

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HbA_{1c} variability is associated with microvascular complications in patients with type 1 diabetes: results of the 25-year observation programme

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Background and aims: This study aimed to evaluate associations between current HbA1c, average of longitudinal HbA1c, variability in glycated haemoglobin (HbA1c) and microvascular complications in patients with type 1 diabetes mellitus (DM1) with disease duration of 25 years.

Materials and methods: We performed a retrospective data analysis of the database of patients with DM1 from the moment of the disease manifestation until the time of the last visit. A total of 88 patients were enrolled in this study, they were divided in 3 groups depending on the registered microvascular complications (MVC): without MVC (n = 38), isolated MVC (retinopathy or nephropathy) (n = 25) and multiple MVC (retinopathy and nephropathy) (n= 25). HbA1c measurements had been ranged from one to four per year. HbA1c was characterized by current HbA1c, average level of longitudinal HbA1c (from the manifestation to the last visit - 2019), variability in HbA1c using the median and maximum of difference in changes of HbA1c (medianΔHbA1c and maxΔHbA1c). Statistical analysis was performed by IBM SPSS Statistics ver.22. A statistically significant difference is the value $p<0.05$.

Results: Clinical characteristics [median (25; 75 percentile)]: age of manifestation of DM1 is 9 years (5; 12), age of patients at the time of the last visit is 33 years (29; 35), duration of DM1 is 24 years (20; 27), body mass index 24 kg / m 2 (21; 25). Medication: basal-bolus insulin therapy (n = 82) or pump insulin therapy (n = 6). The average level of longitudinal HbA1c for the three groups was: 8% (7.6; 8.9), 8.5% (7.9; 8.9), 8.6% (7.8; 10), p = 0.2. Average of current (at the time of the last visit) HbA1c was: 8.2% (7.2; 9.0), 8.1% (7.5; 9.0), 8.4% (7.3; 9.7), p = 0.4. Statistically significant differences were determined in the group without complications and in the group with multiple complications between the levels of maxΔHbA1c 2.3% (1.8; 2.8) vs 4.7% (3.2; 5.6), $p<0.0001$ and median Δ HbA 1c 0.7% (0.6; 0.9) vs 1.4% (1; 1.7), $p<0.0001$. There were no statistically significant relationships between the maximum and median ΔHbA1c in the groups without complications and in the group with isolated complications.

Conclusion: The average level of longitudinal HbA1c and current HbA1c were above 8% and were not associated with MVC. High and non-stable phenotype of HbA1c were associated with MVC and evaluated by means of the maximum and median ΔHbA1c. Variability in HbA1c, not average levels of HbA1c, can be used for assessment of the glycemic effect on the development of MVC in longitudinal studies.

Disclosure: **L. Bolotskaya:** None.

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Urinary proteome and diabetic retinopathy in the Direct-Protect 1 and 2 trials

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Background and aims: Given the association of diabetic retinopathy (DR) to diabetic kidney disease, we investigated the urinary proteome to the presence and deterioration of DR in type 1 and type 2 diabetes in a post-hoc analysis of studies investigating the effect of candesartan on the progression of DR.

Materials and methods: Baseline urinary proteomic analysis was performed in 783 and 792 randomly chosen subjects from two RCTs: DIRECT-Protect 1 and 2. Endpoints were two-step and three-step change in DR score according to the Early Treatment of Diabetic Retinopathy Study protocol. Peptide levels were correlated to baseline DR score, using Spearman rank correlation (association presented as Rho), in a discovery set of 2/3 of participants in DIRECT-Protect 1. Identified peptide fragments were then tested cross-sectionally in a validation set of the remaining 1/3 in DIRECT-Protect 1. Thereafter, peptides identified in the discovery set were assessed in the entire DIRECT-Protect 1 and 2 cohorts in relation to baseline DR. Finally, peptides validated in the entire cohorts were tested longitudinally. Adjustment included sex, age, diabetes duration, smoking, total cholesterol, HbA_{1c}, SBP, UAER, serum creatinine, and randomization group.

Results: Follow-up ranged from 4.0-4.7 years. 24 out of 427 investigated peptide fragments were associated with baseline DR in the discovery set after adjustment for multiple testing. Two of these (COL3A1 (seq: NTG~) and COL4A1 (seq: DGA~) were also associated to baseline DR in the validation set (Rho: -0.22, $p<0.001$ and Rho: -0.14, $p=0.024$). Neither was associated with the development of endpoints. Investigating the discovered fragments in the full cohorts, several collagen fragments were associated with baseline DR and endpoints, in both type 1 and type 2 diabetes, however without overlap.

Conclusion: Several urinary peptide fragments (mainly collagen) were associated with the presence of DR, however, they were not conclusively associated with worsening of DR. Contrary to previous beliefs, our results seem to indicate low correlation between DR and diabetic kidney disease on a molecular level, but appears to support recent findings.

Clinical Trial Registration Number: NCT00252720 and NCT00252694

Disclosure: **V. Rotbain Curovic:** None.

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Study to evaluate relationship between the various components of serum lipids with retinal hard exudate formation, CSME and the occurrence and increasing severity of DR

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Background and aims: The role of dyslipidemia in the in the severity of retinopathy is still unclear. To evaluate the association of elevated serum lipids with retinal hard exudates formation, the occurrence clinically significant macular edema (CSME), occurrence and severity of diabetic retinopathy (DR) and loss of vision in type 2 diabetics.

Materials and methods: Type 2 diabetic patients seeking ocular evaluation for diabetic retinopathy were included in this cross- sectional study. They were assessed for presence and severity of diabetic retinopathy (DR), presence of hard exudates, clinically significant macular oedema (CSME) and best corrected visual acuity (BCVA). Retinal findings were correlated to serum lipids levels using univariate and multivariate analysis.

Results: Totally 350 patients were included, of which 139 had diabetic retinopathy of any grade. Retinal hard exudate formation, was found to have statistically significant correlation with the presence of dyslipidemia ($p=0.02$), increased total cholesterol ($p=0.002$) and LDL levels ($p=0.001$). On multivariate analysis, after correcting for duration, glycemic control and albuminuria, increased cholesterol remained significantly associated with increased hard exudate formation ($p=0.02$). Elevated cholesterol also showed independent association with visual loss ($p=0.04$). The occurrence CSME showed a statistically significant correlation with dyslipidemia ($p=0.04$) and increased LDL levels (0.04), which did not persist on multivariate analysis. However the there was no correlation with the occurrence and severity of diabetic retinopathy

Conclusion: Elevated serum lipids showed a significant association with retinal hard exudate formation, CSME and loss of vision in type 2 diabetics. Increased total cholesterol and LDL are significant risk factors

for the development of retinal hard exudates, CSME and decreased vision. Preserving vision may be an additional motivating factor for lowering serum lipids.

Disclosure: **A. Das:** None.

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Fenofibrate in the prevention and treatment of diabetic retinopathy: systematic review

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Background and aims: Diabetic retinopathy (DR) is the most common and severe sight-threatening microvascular complication of diabetes. This study aimed to systematically review the efficacy and safety of fenofibrate, originally indicated for mixed dyslipidemia and hypertriglyceridemia, in the prevention and treatment of DR and to supplement evidence from well-known clinical trials with data from real-world practice and non-English language studies.

Materials and methods: MEDLINE, EMBASE, Cochrane Library, Web of Science, and other medical databases were systematically searched (January 2021). Randomized controlled trials (RCT), controlled cohort studies, and observational studies were included, regardless of the language of the publication. Meta-analysis was performed, if applicable, using OpenMeta[Analyst] software. Mantel-Haenszel fixed model or DerSimonian-Laird random model (if between-study heterogeneity) were used.

Results: Sixteen experimental (7 RCT) and 14 observational studies (real-world data) met the inclusion criteria. The methodological quality of included studies was moderate to very good. Results from experimental studies indicate that the addition of fenofibrate to the current treatment compared with placebo or standard of care (SoC) significantly decreases the risk of DR progression by more than 50% (RR 0.45; 95% CI 0.30-0.65; Table 1). Experimental studies did not show any significant effect of the addition of fenofibrate to SoC in primary prophylaxis, however, real-world data indicate that this drug may be effective in this field as well (RR 0.81; 95% CI 0.69-0.95; Table 1). Additionally, in experimental studies, fenofibrate therapy reduces the general risk of laser therapy (regardless of the reason) by 31% (RR 0.69; 95% CI 0.58-0.82), but also specifically due to diabetic macular edema or DR progression. A similar trend was shown in the observational studies, however, a meta-analysis could not be performed. Data from one experimental study indicate that patients treated with fenofibrate together with SoC may experience a higher quality of life 3 months after vitrectomy compared with conventional treatment ($p = 0.007$) likely due to the possible protective effect of fenofibrate on eye tissues after invasive procedures - as demonstrated in other studies. Analysis of safety profile indicates that fenofibrate is well tolerated in patients with diabetes, however, a meta-analysis was not possible due to limited data.

Conclusion: Results of the review confirmed that fenofibrate added to SoC can be effective in secondary prevention of DR and delay the need for invasive treatment. Real-world data indicate that the therapy may also be effective in primary DR prophylaxis. Fenofibrate is well tolerated in patients with diabetes. The place of fenofibrate in the diabetic care pathway should be verified.

Table 1 Meta-analysis results

Outcome	Group	No of studies	No of patients	RR [95% CI]
DR progression	DR(+/-)	5 exp	2850	0.65 [0.52; 0.82]
	DR(+)	5 exp	1270	0.45 [0.30; 0.65]
	DR(-)	2 exp	1580	0.98 [0.71; 1.35]
		3 RWD	39127	0.81 [0.69; 0.95]
Laser therapy	DR(+/-)	4 exp	10037	0.69 [0.58; 0.82]
	DR(+)	3 exp	996	0.78 [0.64; 0.96]
	DR (-)	2 exp	9041	0.59 [0.45; 0.78]

DR(+) – DR at baseline; DR(-) – no DR at baseline; exp – experimental studies; RWD – real-world data

Supported by: Viatris Group

Disclosure: **M. Malowicka:** Honorarium; Viatris Group.

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Modified and conventional contact trans-scleral cyclophotocoagulation for diabetic neovascular glaucoma management

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Background and aims: This study aims to compare the efficacy of modified and conventional contact trans-scleral Cyclophotocoagulation laser to control intraocular pressure of diabetic Neovascular glaucoma. Also, it reports the ocular complications percentage for each laser procedure.

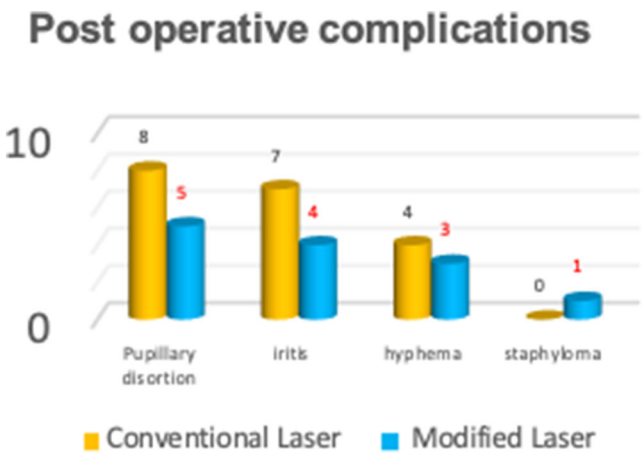
Materials and methods: The procedure was explained to each patient and signed consent form. Diode laser with wavelength of 810 nm is used to TS-CPC with G probe under L.A. *Two different laser parameters are used:* **Conventional type is** used in Group 2, the Initial power of 1250 mw and duration 2 seconds. The power is increased in 150 mw increments until hearing "Pop" which means ciliary epithelium explosion. 6 spots are used for each quadrant for total 18 spots for 270 degree. **Modified type is** used in group 1; the power was less than audible pop power by 150nm but increasing the duration to 4 seconds.12 months follow-up at intervals of one week, one month, three months, 6 months ,9 months and 12 months. Success rate is considered as IOP is less 20 mmgh and pain free without anti-glaucoma medications after 2 months of laser surgeries.

12 months follow-up of 51 eyes (5o patients) of diabetic rubeotic glaucoma post treatment by modified and Conventional contact trans-scleral cyclophotocoagulation laser procedures.Group 1 has 26 eyes (50.9%) and are treated by modified laser technique. Group 2 has 25 eyes (49%) and are treated by Conventional laser one. Each visit, pain score 1-10 were recorded, check visual acuity, IOP measure, EOM movement,

biomicroscopy examination, dilated fundoscopic exam, and record any ocular complications.

Results: 12 months follow-up of stabilization of intraocular pressure and recording any ocular complications in the two groups.Group 1: among 26 eyes (50.9%) are underwent by modified Cyclodiode laser surgery, there are 19 eyes (73%) have stable intraocular pressure after first procedure, 4 eyes (15%) are needed to repeat laser to stabilise IOP and only 3 eyes (11.5%) are repeated for third time.Group 2: among 25 eyes (49%) are treated by Conventional Cyclodiode laser surgery, there are 15 eyes (60%) have stable IOP after first procedure, 6 eyes (24%) are needed to repeat for second time and 4 eyes (16%) after third one to control IOP.Both groups have ocular complications of Cyclodiode laser. but it was less in modified than conventional technique.In modified procedure, Pupillary distortion in 5 eyes (19%), iritis in 4 eyes (15.3%) and Hyphema in 3 eyes (11.5%)However, there are pupillary distortion in 8 eyes (32 %), 7 eyes (28%) have Iritis, and hyphema in 4 eyes (16%) , Zonular dehiscence in 3 eyes (12%) and staphyloma formation in one eye (4%) in conventional one.

Conclusion: Both modified and conventional contact trans-scleral cyclodiode laser procedures are very good procedures to control IOP for management of diabetic neovascular glaucoma.But modified laser technique has higher success rate to stabilise IOP than conventional one.Also, modified technique has less complications rate.



Disclosure: **E.A. Mahmoud:** None.

SO 46 Diabetic foot: from cost to COVID

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Impact of immunosuppressive drugs on the metabolic activity and functional properties of stem cells isolated from the bone marrow of diabetic patients

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Background and aims: Autologous stem cell (SC) therapy is the last therapeutic option for patients with chronic limb-threatening ischemia (CLTI) and diabetic foot ulcers (DFU). This therapy is provided also to patients after solid organ transplantation treated by immunosuppressive drugs (ISs). However, as the impact of ISs on SCs is still not fully known, the aim of our study was to characterize and compare the effect of different ISs on the functional properties of SCs.

Materials and methods: SCs obtained from the bone marrow of 6 patients with diabetes were separated with gelofusine, centrifuged and cultured. According to phenotypic identification, the cells were divided into mesenchymal stem cells (MSCs) with clusters of differentiation $CD45^+$, $CD73^+$, endothelial colony-forming cells (ECFCs) with $CD45^-$, $CD146^+$and myeloid angiogenic cells (MACs) with $CD45^+$, $CD146^-$ markers. These SCs were cultured together with different ISs - tacrolimus (T), sirolimus (Sr) or mycophenolate mofetil (MMF) and the impact of ISs on their functional characteristics was tested.

Results: The populations of mononuclear fraction cultured 14 days contained 64% of MSCs, 24% of MACs, 7% of ECFCs, and 5% of other cell populations. The metabolic activity of SCs was significantly reduced in the presence of Sr, MMF and T at concentrations of 50 μg/ml ($p=0.002$; $p=0.003$; $p<0.001$, respectively) in comparison with SCs without ISs. The expression of genes for programmed death-ligand 1, cyclooxygenase 2 and induced nitric oxide synthase in SCs stimulated with pro-inflammatory cytokines was decreased with T and Sr at concentrations of 50 μg/ml (both $p<0.05$). Sr significantly reduced vascular endothelial growth factor production by stimulated SCs at concentrations as low as 5 μg/ml ($p<0.001$), T and MMF at 50 μg/ml ($p=0.016$; $p=0.044$). Hepatocyte growth factor production by stimulated SCs decreased with T, Sr and MMF at concentrations of 5 μg/ml ($p<0.001$; $p<0.001$; $p=0.011$, respectively). Chemokine C-C motif ligand 2 (CCL2) production by stimulated SCs was inhibited with T at a concentration of 0.05 μg/ml ($p=0.025$), MMF at 500 μg/ml ($p=0.024$) whereas Sr did not significantly alter CCL2. A reduction of interleukin 6 production by stimulated SCs was observed in the presence of T at a concentration of 50 μg/ml ($p=0.04$), with MMF and Sr at 500 μg/ml ($p<0.001$; $p=0.01$). Decreased interleukin 8 production by stimulated SCs was seen in the presence of T at a concentration of 0.5 μg/ml ($p=0.013$), MMF at 5 μg/ml ($p=0.031$) and Sr at 500 μg/ml ($p=0.021$). ISs also altered SC death rates, with apoptosis significantly decreased and necrosis increased in the presence of T and MMF at concentrations of 0.5 μg/ml ($p=0.003$, $p=0.027$, respectively) and Sr at 5 μg/ml ($p=0.006$).

Conclusion: Our study showed a significant impact of ISs on metabolic activity, cytokine production, expression of the gene encoding immunoregulatory molecules and apoptosis in SCs. Therefore, when treating transplant recipients with autologous SCs, the levels of ISs should be carefully monitored.

Supported by: Ministry of Health, Czech Republic - conceptual development of research organization ("Institute for Clinical and Experimental Medicine – IKEM, IN 00023001") and grant GAUK No. 304321/2021 provided by the Internal grant agency of Charles University.

Disclosure: **J. Husakova:** None.

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Cost-effectiveness and cost-utility of foot temperature monitoring for preventing diabetic foot ulcer recurrence: a randomised controlled trial

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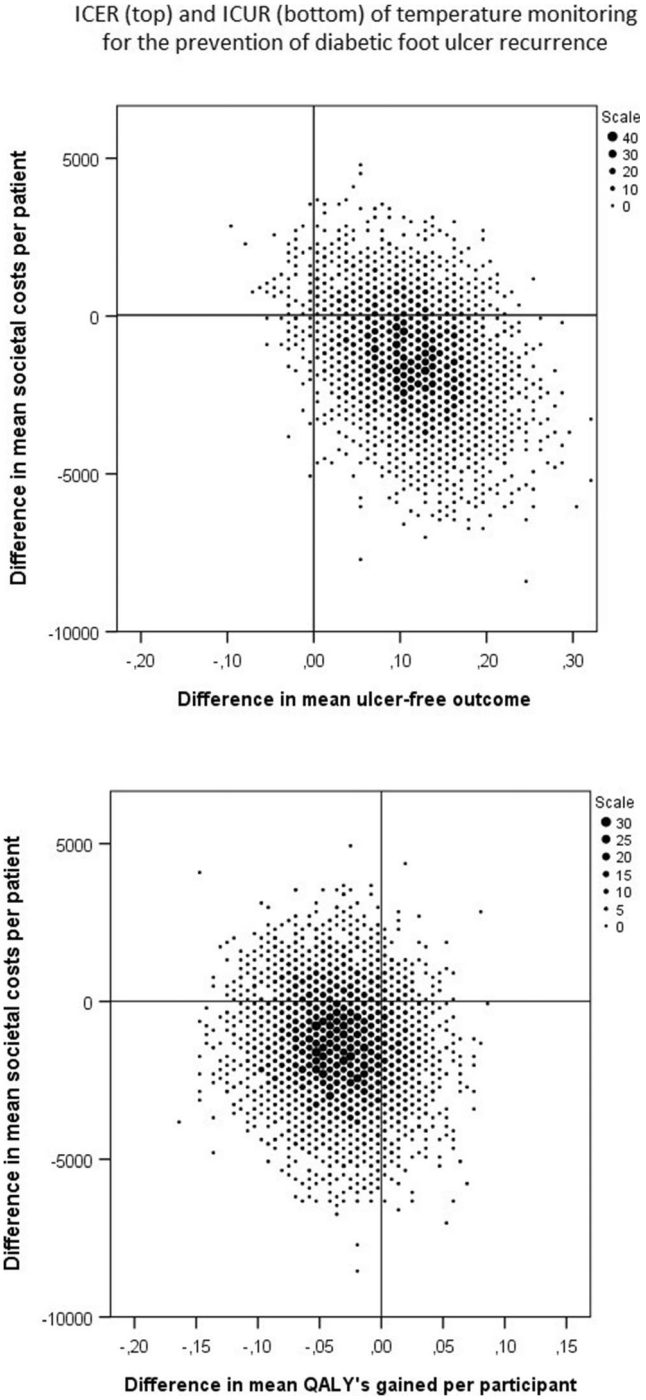
Background and aims: The skin of people with diabetic foot disease is thought to heat up from weight-bearing activity before it breaks down into ulceration. We assessed the cost-effectiveness and cost-utility of at-home foot skin temperature monitoring for prevention of diabetic foot ulcer recurrence.

Materials and methods: In this outcome-assessor-blinded multicenter RCT, we randomly assigned people with diabetes, neuropathy, foot ulcer history or Charcot's neuro-arthropathy to usual care (i.e. podiatric treatment, education, and therapeutic footwear) or usual care plus measuring skin temperatures at 6-8 plantar sites per foot each day (enhanced therapy). Foot care costs from a societal perspective were obtained via questionnaires from the institute for Medical Technology Assessment and from electronic health records. Utilities were calculated based on health-related quality of life as assessed with the EQ-5D-3L. Primary clinical outcome for effectiveness was foot ulcer recurrence in 18 months. Group differences were assessed by calculating 95% confidence intervals after correction for bias and using accelerated non-parametric bootstrapping. Incremental cost-effectiveness ratios (ICER) and incremental cost-utility ratios (ICUR) were calculated as the ratio between costs and effect/utility differences between enhanced therapy and usual care.

Results: Total foot care costs per participant during 18-months follow-up were lower in enhanced therapy (n=151; €6,067 (SD: €13,778)) compared to usual care (n=153; €7,376 (SD:15,790); p=0.450). Enhanced therapy was cheaper and more effective in 78% of the cost-effect pairs (Fig.1). Enhanced therapy had 79% probability of being cost-effective at a willingness-to-pay of €0 per participant who remains ulcer-free. Quality-adjusted life years were lower in enhanced therapy (1.085 (SD: 0.33)) than in usual care (1.119 (SD: 0.31); p=0.348). Enhanced therapy was cheaper but with lower quality of life in 68% of cost-utility pairs (Fig.1). Enhanced therapy had 45% probability of achieving cost-utility at a willingness-to-pay of €50,000 per QALY gained.

Conclusion: In this first-ever societal-perspective cost-effectiveness RCT in the field of diabetic foot disease, we found that at-home foot skin temperature monitoring is a cost-effective intervention in foot ulcer prevention, at the expense of lower quality of life.



Clinical Trial Registration Number: Netherlands Trial Registration: NTR5403

Supported by: The DIATEMP trial was funded by the Netherlands Organization for Health Research and Development (ZonMw, project nr. 837002508), with 10% matching funded by the Dutch Society for Podiatrists (NVvP) and Dutch Branch Organization for Pedicures (ProVoet). The funders had no influence on study design, data collection, management, analysis, and interpretation, the writing of the report, or the

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The impact of the COVID-19 pandemic on the presentation rate and severity of diabetic foot ulcers in Belgium

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Background and aims: A national lockdown for COVID-19 (March 14th - May 3rd 2020) placed restrictions on the free movement of persons and allowed only urgent medical consultations and interventions in hospitals. On the other hand, diabetic foot clinics (DFCs) were advised by the professional associations to consider all active diabetic foot problem as urgent. The aim of the study was to assess the impact of these restrictions on the presentation rate and severity of diabetic foot ulcers (DFU) at presentation in Belgian DFCs.

Materials and methods: A multicentre, prospective cohort study was done among 22 recognized DFCs in Belgium in the context of an ongoing biennial national initiative for care quality improvement (IQED-Foot). A survey asked which measures DFCs took during and after the lockdown to maintain their activities. Between January 1st and September 30th, patient and ulcer characteristics were recorded for 887 consecutive patients with DFUs of Wagner 2 or higher. Based on the date of presentation in the DFC, patients were assigned to the pre-lockdown group (A, first contact between January 1st and March 13th, n = 322) or the (post-)lockdown group (B, first contact between March 14th and September 30th, n = 565). Groups were compared with each other. Inclusion rate was compared to data from the IQED-Foot survey of 2018.

Results: During the lockdown, one DFC was closed, all others remained open for active foot problems, applying specific COVID-19 measures. Reported measures included selection of patients based on urgency and ulceration risk (71%), reduced frequency of consultations (54%) while switching to telemedicine by phone or mail (79%) and spreading patients in time and space to guarantee physical distancing (50%). In the lockdown period, the rate of patients presenting in the DFCs was strongly reduced (0.6 vs. 1.4 patients/week/DFC in 2018; $p < 0.001$). No differences in demographic data such as age, gender, diabetes type and duration were observed between both groups. Patients seen during and after lockdown had less frequently a history of prior DFU (B: 50% vs. A: 60%; $p = 0.0047$). No increase in median patient-reported presentation delay [P25 - P75] was observed (A: 3 [1-8] vs. B: 3 [1-7] weeks; $p = 0.8076$). Patients with a first contact during or after lockdown presented with slightly larger DFU (< 1cm^2 B: 32% vs. A: 38%; $p = 0.0003$, 1-3 cm^2 B: 45% vs. A: 40%; $p = 0.0152$). They also had less frequently critical ischemia (B: 11% vs. A: 18%; $p = 0.0103$). No significant differences in depth, infection or loss of protective sensation were detected between both groups.

Conclusion: Presentation rate was strongly reduced in Belgian DFCs during the lockdown period. Thanks to great efforts of the DFCs to remain accessible to patients with active foot problems, the presentation delay did not increase and the impact of the lockdown on the severity of the DFU at presentation was limited to slightly larger wounds. Currently, treatment and outcome data of these patients are being collected and will be available by time of the EASD meeting.

Supported by: RIZIV/INAMI

Disclosure: **A. Vanherwegen:** None.

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Diabetic foot ulcers: Should a radiological diagnosis of osteitis be treated as osteomyelitis?

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Background and aims: Effective treatment is needed at the earlier stages of diabetic foot infection to prevent progression to osteomyelitis, which results in approximately 6000 leg, foot or toe amputations each year in England alone. Osteitis is a reactive change of the bone, and may be demonstrated on Magnetic Resonance Imaging (MRI) by the finding of hyperintense bone marrow on T2-weighted images, but an absence of hypointensity on corresponding T1-weighted images. Debate exists as to whether these findings indicate early osteomyelitis, and so warrant a minimum of 6 weeks antibiotic therapy, or can be treated more conservatively. To investigate this, a retrospective analysis was conducted to assess whether MRI diagnosis of osteitis requires treatment as for osteomyelitis, and whether any clinical or microbiological factors can be used to predict outcomes.

Materials and methods: A search for the terms 'osteitis' and 'marrow oedema' for MRI reports and Diabetes Foot Multidisciplinary Team meeting records from 2016-2019 (inclusive) was conducted. Relevant biochemical, clinical and microbiological data were gathered from the clinical notes, and statistical analysis conducted, with variables compared to negative (amputation, debridement or progression to osteomyelitis) or positive (healed ulcer or persisting ulcer but no osteomyelitis) outcomes.

Results: 18 MRI reports of osteitis met the inclusion criteria, of which 8 (44%) went on to have a negative outcome within 6 months. HbA1c was significantly lower in those with a positive outcome, with a mean HbA1c of 65.7mmol/mol (8.2%) compared to 83.25mmol/mol (9.7%) for negative outcome (unpaired T-test, $p=0.045$). 15 patients (83%) were polymicrobial on their microbiology profile for the 6 months preceding their diagnosis of osteitis, with *S. aureus* (n=7) and *Corynaebacteria* (n=6) the most commonly cultured organisms. The decision to treat as osteomyelitis based on clinical picture, as decided by a consultant diabetologist, was a significant predictor of a negative outcome (Fisher's exact test, $p=0.043$).

Conclusion: Our results demonstrate the gravity of an MRI finding of osteitis in a diabetic foot ulcer, with 44% progressing to osteomyelitis, debridement or amputation within 6 months. High HbA1c and assessment by a consultant diabetologist of the ulcer as 'likely osteomyelitis' predict a negative outcome, therefore strong consideration should be given to treating these groups of patients as for a radiological diagnosis of osteomyelitis, and for optimising glycaemic control. The high prevalence of *S. aureus* colonisation, and therefore likelihood of prior antibiotic exposure, indicate the need for extended culture and sensitivities of for gram negative organisms in this population. The overall findings of this study highlight the need for clinicians treating diabetic foot ulcers to use a correlation of radiological, microbiological and clinical findings to make treatment decisions.

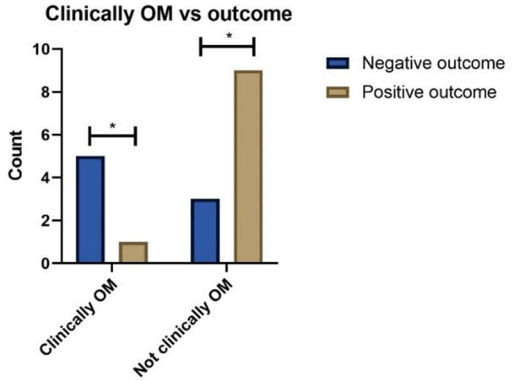

Figure 1: Decision to treat as osteomyelitis based on clinical findings compared to outcome. Fisher's exact test p= 0.043

Disclosure: **K. Bishop:** None.

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Enhancement of growth factor expression in chronic diabetic wounds by cold atmospheric plasma: data from the randomised, placebo-controlled, prospective KPWTRIAL

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Background and aims: Diabetic foot is a common late complication in diabetes mellitus. Chronic wounds are characterised by decreased levels of pivotal growth factors and a disbalance of matrix destruction and reconstruction. Cold atmospheric plasma (CAP) has been proven to induce wound healing by stimulation of granulation and reepithelialisation. However, molecular mechanisms involved are not fully evaluated. Alterations in levels of growth factors, cytokines and matrix metalloproteinases essential in wound healing mediated by CAP over the course of time compared with placebo were analysed.

Materials and methods: The prospective, randomised, patient-blinded KPWTRIAL proved beneficial effects of additional therapy with CAP in terms of wound healing efficacy in superficial, chronically infected, diabetic foot ulcers (Wagner Armstrong 1B or 2B). Within this clinical trial, wound exudate was collected from each change of dressing. Specific protein level analyses were conducted via multiplex ELISA for samples of a sub cohort of patients of the KPWTRIAL (placebo-group n=13; CAP-group n=14). Expression of FGF-2, VEGF-A, IL-1α, IL-8, and TNFα as well as various matrix metalloproteinases (MMP-1,-2,-3,-8,-9,-13) was evaluated over a treatment period of about 14 days.

Results: Analysis revealed increased levels of FGF-2 (placebo (n=13): 983±587 AU vs CAP (n=14): 1,645±579 AU, p=0.0056) and VEGF-A (placebo (n=13): 1,247±399 AU vs CAP (n=14): 1,749±694 AU, p=0.0332) throughout the treatment period and in direct head to head comparison in a daily assessment. Total protein amounts of IL-1α and IL-8 were higher, but not significantly elevated. However, wounds treated with CAP showed significantly increased levels at several points in time in the visit specific comparison. Levels of TNFα, matrix metalloproteinases 1 and 13 appeared elevated at distinct treatment days, but total levels were unaffected by CAP. Levels of other matrix metalloproteinases were not altered by therapy with CAP.

Conclusion: Treatment with CAP induces expression of growth factors essential in wound healing like FGF-2 and VEGF-A in chronically infected, superficial, diabetic foot ulcers. Through alterations in levels of primary proinflammatory proteins, such as IL-1α, IL-8 and TNFα, CAP might ensure the restart of an inflammatory response as the first phase of a well-orchestrated wound healing process. This initial inflammatory response is pivotal in disrupting the chronic inactive state of the wound and reinitiating the healing process. On account of these molecular biological changes CAP appears to generate favourable effects that promote granulation tissue formation and vascularisation, leading to the recently clinically observed improvement in wound healing in diabetic foot. These findings demonstrate for the first time that growth factor induction by cold atmospheric plasma also occurs in diabetic patients.

Clinical Trial Registration Number: NCT04205942

Disclosure: **J. Hiller:** None.

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Poor adherence to medication and high HbA_{1c} level predict risk of amputation in patients with diabetes

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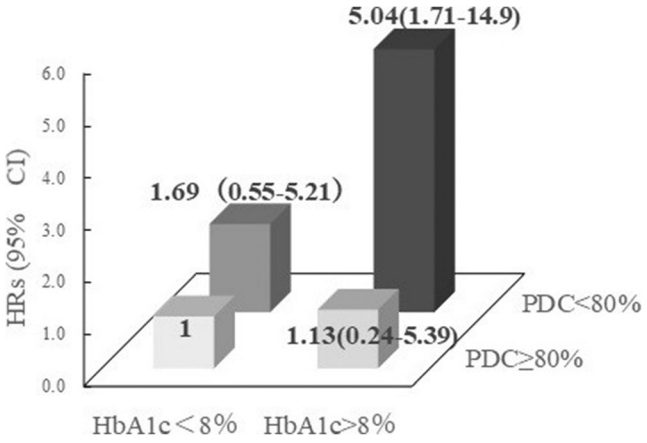
Background and aims: It is well known that poor adherence to medication in diabetic patients leads to poor glycemic control. In fact, in our previous study, HbA1c increased by 0.2% with a 25% decrease in medication adherence. It is also established that elevated level of HbA1c was a predictor for lower limb amputation. Whereas, it is still unclear whether medication adherence increases the risk of lower extremity amputation independently of or in combination with HbA1c level in diabetic patients.

Materials and methods: We analyzed data from 16,590 patients with diabetes mellitus whose medication records of oral hypoglycemic agents were available for at least 1 year. Medication adherence was evaluated by the proportion of days covered (PDC) and PDC <80% was defined as poor medication adherence. Lower limb amputation was defined as ICD-10 codes and medical procedures. Cox regression model *identified variables related to* the incidence of amputation.

Results: During a mean follow-up of 5.3 years, 22 amputation events occurred (0.25/1000 person-years). Results of Multivariate Cox regression analysis indicated that HbA1c level and PDC were independently associated with incidence of amputation (HR (95%CI) 1.19 (0.92-1.54), 0.79 (0.69-0.91). Compared with individuals with PDC ≥80% and HbA1c <8.0%, those with PDC <80% and HbA1c ≥8.0% has approximately 5 times higher risk for amputation (Figure). These findings implied necessity of increasing adherence to medication for prevention of lower limb amputation in diabetic patients. Individuals with PDC ≥80% and HbA1c <8.0% may have a lower risk of lower limb amputation with enhanced treatment. Similarly, Individuals with PDC <80% and HbA1c >8.0% may have a lower risk of lower limb amputation with enhanced medication management.

Conclusion: The combination of PDC and HbA1c may lead to a more stratified care and approach to reduce the risk of lower limb amputation.



Disclosure: **M. Kaneko:** None.

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Evaluation of mortality rate in patients amputated by diabetic foot in a third-level hospital

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Background and aims: The 5-year mortality rate after diabetic foot amputation is estimated to be 53-100%, which is even higher than reported in many types of cancer (ex. prostate, breast, and colon).The objectives of this study have been: 1) To establish mortality rate and its causes in patients who have undergone an amputation for diabetic foot in our hospital in the last 10 years. 2) To determine the clinical variables independently related to mortality.

Materials and methods: This is a retrospective, longitudinal, single-center study, which includes all amputations due to diabetic foot from 01-01-2011 to 12-31-2020. Anthropometric and clinical variables, such as arterial hypertension, dyslipidemia, smoking habit, obstructive sleep apnea syndrome (OSAS) and cardiovascular events (ex. acute myocardial infarction-AMI- and stroke) were collected. Statistical analyzes were performed with the STATA 15 package.

Results: During the period analyzed, 457 amputations were performed, and a total of 182 (39.8%) patients died 5.4±2.8 years [IQ 75-25: 4.13 years] after the surgical procedure. The mean time between amputation and death was 2.2 ± 1.8 years (CI: 2.01-2.56 years). Cardiovascular disease was the most frequent cause of death with 96 cases (53%). Mortality from postoperative complications occurred in 21 cases (11%) and in 65 cases (36%) it was due to other causes. The variables independently related to cardiovascular mortality were smoking habit, male sex, hypertension, dyslipidemia, and presence of microangiopathic complications such as diabetic retinopathy and nephropathy.Regarding the prevalence of CVRF, we observed a statistically significant reduction in the prevalence of smoking, hypertension, dyslipidemia, and OSAS in the last 5 years.

Conclusion: The mortality rate after diabetic foot amputation in our series is in the lower range of that reported in the literature. The leading cause of mortality is cardiovascular disease. Smoking, male sex, hypertension, dyslipidemia, and microangiopathic complications (diabetic retinopathy and nephropathy) are the variables independently associated with mortality of cardiovascular origin. The reduction of these CVRF in the last 5 years makes it foreseeable that the cardiovascular mortality of these patients will decline in the coming years.

Disclosure: **I. Hernández:** None.

SO 47 Autonomic neuropathy

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Does the diagnostic value of the questionnaire for autonomic symptoms COMPASS 31 differ between type 1 and type 2 diabetes?

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Background and aims: Composite Autonomic Symptom Score (COMPASS) 31 has been validated for autonomic symptoms of diabetic neuropathy and the value of 16.44 proposed as the best cut-off for abnormality with sensitivity up to 80% and specificity up to 65% for diabetic cardiovascular autonomic neuropathy (CAN). However, autonomic symptoms might be more weakly associated with autonomic deficits in type 2 than in type 1 diabetes. Thus, this study aimed to evaluate if the diagnostic performance of COMPASS 31 for CAN and diabetic polyneuropathy (DPN) differs between type 1 and type 2 diabetes.

Materials and methods: Seventy-nine patients with type 1 diabetes (age 42±13 years, duration 25±13 years) and 143 with type 2 diabetes (age 63±8 years, duration 12±9 years) completed COMPASS 31 before undergoing four cardiovascular reflex tests (CARTs) and assessment of neuropathic symptoms, signs [using Michigan Diabetic Neuro3pathy Score (MDNS)], vibration and thermal thresholds. Early and confirmed CAN were defined by 1 and 2 abnormal CARTs, and DPN by 2 abnormalities among symptoms, signs and sensory thresholds.

Results: The COMPASS 31 total weighted score (TWS) was higher in presence of CAN (early and confirmed) and DPN for both patients with type 1 (with Vs. without CAN: 30.8±22.5 Vs. 20.3 ±19.8; P=0.0132; with Vs. without DPN: 34.8±22.1 Vs. 14.3±15.1; P<0.0001) and type 2 diabetes (for CAN: 29.9±19.6 Vs. 20.0±15.6; P=0.0033; for DPN: 26.5±17.9 Vs. 6.1±13.6; P=0.0002). In patients with type 1 diabetes, COMPASS 31 TWS was related to individual CARTs and to an overall CARTs score (Spearman's rho=0.36, P=0.0016) and to DPN measures (MDNS, thermal and vibration thresholds) (P=0.01). In type 2 diabetes, COMPASS 31 TWS was related among CARTs to Valsalva ratio (rho=-0.24, P=0.0101) and among DPN measures to MDNS (rho=0.35, P<0.0001). The Table shows the diagnostic accuracy and characteristics of COMPASS 31 TWS for CAN, confirmed CAN and DPN in patients with type 1 and type 2 diabetes, i.e., area under the curve (AUC) and sensitivity and specificity (at the cut-off of 16.44) (95% confidence intervals). Among the six COMPASS 31 domains, in patients with type 1 diabetes a fair diagnostic accuracy for CAN was reached only by orthostatic intolerance (AUC: 0.690±0.072) and gastrointestinal domains (AUC: 0.713±0.081), and for DPN by orthostatic intolerance (AUC: 0.729±0.054) and pupillometer domains (AUC: 0.717±0.060), whereas in type 2 diabetes by secretomotor (AUC: 0.700±0.043) and gastrointestinal domains (AUC: 0.704±0.044) but only for DPN.

Conclusion: While confirming the diagnostic validity of COMPASS 31 for both CAN and DPN, this study documents that its diagnostic performance for CAN, in particular confirmed CAN, is better in type 1 than in type 2 diabetes. As opposed to type 1 diabetes, no single domain of COMPASS 31 reached acceptable diagnostic accuracy for CAN in type 2 diabetes. The close link between COMPASS 31 and sensorimotor neuropathy is constant for both diabetes types.

Table. Diagnostic characteristics of COMPASS 31 TWS.

	CAN (early and confirmed)		Confirmed CAN		DPN	
	AUC		AUC		AUC	
Type 1 diabetes	0.651 ± 0.066 (0.522-0.780)		0.725 ± 0.073 (0.583-0.867)		0.753 ± 0.059 (0.637-0.868)	
Type 2 diabetes	0.650 ± 0.056 (0.541-0.759)		0.606 ± 0.077 (0.456-0.756)		0.682 ± 0.045 (0.594-0.769)	
Cut-off 16.44	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Type 1 diabetes	65.5% (48.2-82.8)	62.0% (48.5-75.4)	81.2% (62.1-100)	60.3% (48.2-72.4)	76.3% (62.8-89.8)	78.0% (65.4-90.7)
Type 2 diabetes	68.7% (52.7-84.8)	52.2% (43.0-61.5)	66.7% (42.8-90.5)	49.2% (40.6-57.9)	61.9% (51.5-72.3)	61.0% (48.6-73.5)

Disclosure: **I. D'Ippolito:** None.

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Carotid baroreceptor magnetic stimulation abrupt blood pressure elevation buffering, implication to treat unstable hypertension in diabetes

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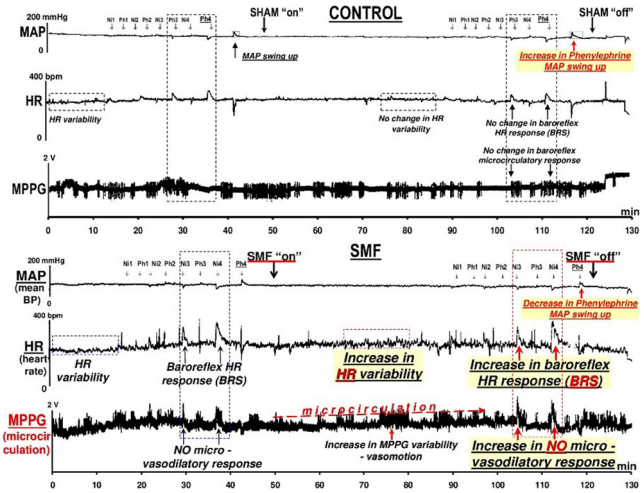
Background and aims: A mounting evidence suggests that enhanced blood pressure (BP) variability (BPV) including its most dangerous form, the catecholamine-induced abrupt BP elevation is a major, independent cardiovascular (CV) risk factor especially in the condition of distorted CV autonomic regulation in diabetes. The goal was to estimate the effect of carotid baroreceptor (CB) magnetic stimulation on sudden high elevation of BP in conjunction with arterial baroreflex sensitivity (BRS) and very short term beat-to-beat BPV, aimed to explain their relationship, CV risk and potential implementation in diabetic BP regulation dysfunction.

Materials and methods: Twenty eight experiments were performed on conscious rabbits sedated by pentobarbital i.v. infusion (5 mg kg^{-1} h^{-1}). Mean femoral artery BP (MAP), heart rate (HR), BRS and ear lobe skin microcirculatory blood flow, estimated by microphotoelectric plethysmography (MPPG), were simultaneously recorded after a 40 min CB exposure to 350 mT SMF, generated by Nd_2-Fe_{14}-B magnets (n=14), or after sham magnets exposure (n=14). BRS was assessed from HR and MAP responses to abrupt hypotension induced by i.v. bolus injections of nitroprusside and MAP abrupt elevation (MAP_{AE}) evoked by i.v. bolus of phenylephrine (Ph). The very short-term, beat-to-beat BPV was estimated by MAP standard deviation (SD), Fig. 1.

Results: CB magnetic stimulation significantly increased BRS_{Ni} (74.5 ±17.8%, p<0.001) and microcirculation (23.8%±11.0%, p=0.039); decreased MAP (-5.7±1.7%, p<0.014) and MAP_{AE} when compared with sham magnet exposure (-19.1%, p=0.043). Abrupt elevation in BP significantly positively correlated with resting MAP (MAP, r=0.342, p=0.0383) and BPV (MAP SD, r=0.383, p=0.0194) prior phenylephrine BP ramps, and negatively correlated with BRS_{Ph} (r=-0.47, p=0.0383).

Conclusion: Our results suggest arterial baroreflex involvement in catecholamine-induced abrupt BP elevation buffering mechanism. The positive correlation between beat-to-beat BPV and the magnitude of phenylephrine BP ramps suggests MAP SD to predict individual susceptibility to catecholamine-induced abrupt BP elevation, including high CV risk morning surge of BP characteristic for diabetic patients. CB magnetic stimulation seems to be an effective tool to enhance impaired arterial baroreflex BP buffering mechanism with potential implementation in diabetic autonomic CV dysfunction. **Fig. 1.** Hemodynamic recording following sham magnet, CONTROL experiments and SMF exposure to CB. In SMF run, on the background of gradual decrease of BP and microvascular dilation, the increase of the baroreflex HR (BRS) and microvasodilatory response to same dose i.v. nitroprusside took place. A notable rise of the HR variability and inhibition of the Ph-induced abrupt BP elevation (reflects CB activation) is obvious. NO = nitric oxide (generated by decomposition of nitroprusside); SMF "on" = onset, "off" = cessation of SMF exposure.



Disclosure: **J. Gmitrov:** None.

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Lowering of blood pressure and pulse rate by switching from DPP-4 inhibitor to luseogliflozin in patients with type 2 diabetes and hypertension

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Background and aims: Sodium-glucose cotransporter-2 inhibitor (SGLT2i) treatment had the effect of reducing blood pressure (BP) and cardiovascular events in recent mega-scale trials, but the effects on BP circadian rhythm remained unclear. The present study aimed to determine the nocturnal antihypertensive effect of SGLT2i compared with dipeptidyl peptidase-4 inhibitor (DPP-4i) in patients with type 2 diabetes and hypertension.

Materials and methods: In this randomized, open-label, parallel-group trial, patients treated with DPP-4i were either switched to luseogliflozin 2.5 mg/day (Luseo group; *n*=30) or continued DPP-4i (DPP-4i group; *n*=26). The patients undertook 24-h ambulatory BP monitoring before and 8 weeks after the group allocation. The primary endpoint was mean change in nighttime systolic BP (SBP).

Results: Changes in both nighttime and daytime SBP were significantly reduced in the Luseo group compared with the DPP-4i group (nighttime, −4.0±11.4 vs. 3.6±10.7 mmHg, *P*=0.01; daytime, −4.4±10.9 vs. 3.7±11.9 mmHg, *P*=0.01). Similarly, nighttime pulse rate (PR) was significantly reduced in the Luseo group compared with the DPP-4i group (−2.0±4.8 vs. 0.9±4.8 bpm, *P*=0.03). Abnormal BP circadian rhythms (non-dipper and riser types) were significantly decreased in the Luseo group (56.7% to 36.6%, *P*<0.05).

Conclusion: Switching from DPP-4i to luseogliflozin decreased not only daytime but also nighttime SBP and PR, and improved BP circadian

rhythm. These improvements could be some of the factors involved in the cardiovascular event suppression observed with SGLT2i therapy.

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Association between cardiac autonomic neuropathy, arterial stiffness and diastolic dysfunction in patients with type 2 diabetes

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Background and aims: Cardiac autonomic neuropathy (CAN), increased arterial stiffness (AS) and cardiac diastolic dysfunction are associated with increased cardiovascular mortality. This study was aimed to investigate the association between CAN, AS and left ventricular (LV) diastolic dysfunction.

Materials and methods: the study involved 101 patients with type 2 diabetes mellitus (T2DM), among them 46 with definite CAN and 55 without CAN. Patients were aged between 50-59 years, BMI 26.9 ± 0.31 kg/m^2 and HbA1c 7.4 ± 0.17 %. The mean duration of diabetes was 7.4 ± 0.6 years. CAN was confirmed by modified Ewing's traditional tests. 24-hour HRV was evaluated using ECG "EC-3H" ("Labtech"), AS monitoring - TensioMed TM Arteriograph 24 (Hungary). Diastolic dysfunction was defined according to the guideline of American Society of Echocardiography and European Association of Cardiovascular imaging. The work was done according to the principles of the Declaration of Helsinki. Statistics: ANOVA.

Results: we found out that development of CAN in patients with T2DM is associated with increased AS parameters, namely aortic augmentation index (32.9±0.97 *vs* 26.9±2.13 %, p<0.01), brachial augmentation index (-9.2±1.23 *vs* -22.3±2.11 %, p<0.001), pulse wave velocity (PWV) (10.95 ±0.23 *vs* 8.8±0.42 m/sec, p<0.001) and ambulatory arterial stiffness index (0.46±0.03 *vs* 0.35±0.04, p<0.05). PWV was considered as normal in 19.6 %, elevated in 47.6 % and pathological in 32.6 % of cases. Among T2DM patients without CAN value of PWV was normal in 87.3 % and elevated in 12.7 % of patients. Among patients with T2DM and CAN presence of LV diastolic dysfunction, namely impaired relaxation was found in 28.3 %, pseudonormal pattern in 8.7 % and restrictive mitral flow pattern in 8.7 % of cases. Diastolic dysfunction, namely impaired relaxation was presented in 12.7 % of patients with T2DM without CAN. Linear regression analysis indicated that heart rate response to deep breathing was associated with PWV and cardiac diastolic dysfunction.

Conclusion: our study suggested that CAN's development is associated with diastolic dysfunction and increased arterial stiffness parameters, that could lead to chronic heart failure.

Disclosure: **V. Serhiyenko:** None.

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Cardiac autonomic function is associated with coronary artery calcification in type 2 diabetes

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Background and aims: Cardiac autonomic neuropathy and cardiovascular disease are concomitant complications to diabetes but the link between these complications are largely unknown. Higher vascular inflammation and coronary artery calcification are related to cardiovascular disease. We examined the association between cardiac autonomic function and coronary artery calcification (CACS) in subjects with type 2 diabetes. Secondarily, we examined the association between cardiac autonomic function and vascular inflammation.

Materials and methods: Post-hoc analysis of baseline data from a randomized clinical trial including 102 persons with type 2 diabetes. Cardiac autonomic function was evaluated using heart rate variability (HRV) indices and cardiovascular autonomic reflex tests (CARTs). Lower response in CARTs and HRV measures were taken as indicators of impaired cardiac autonomic function. Vascular inflammation was assessed with ^{18}F-fluorodeoxyglucose (FDG) PET/CT and the FDG uptake was quantified as target-to-background ratio (TBR) using venous blood uptake as background. A higher TBR indicates higher vascular inflammation. CT based CACS was calculated using Agatston method.

Results: The participants had a mean age of 66.4 (SD 8.2) years, 16% were women, median diabetes duration was 10.9 [IQR 5.7 - 18.2] years, 23 % reported history of cardiovascular disease and 15% had ≥ 2 abnormal CARTs indicating cardiac autonomic neuropathy. A higher CACS was associated with a lower 30-to-15 ratio (-1.2, SE: 0.31), $p = 0.0002$), E-to-I ratio (-1.2, SE:0.3, $p = 0.0002$), standard deviation of normal-to-normal intervals (-0.66 ms, SE:0.32, $p = 0.04$), low frequency power (-0.58 ms^2, SE:0.22, $p = 0.01$), high frequency power (-0.47 ms^2, SE:0.23, $p = 0.04$) and total power (-0.79 ms^2, SE:0.32, $p = 0.02$). All these associations remained significant after adjustment for age, heart rate (only for HRV measures), sex, LDL-cholesterol, HbA_1c, systolic blood pressure, diabetes duration and weight (except for standard deviation of normal-to-normal intervals and high frequency power). Presence of cardiac autonomic neuropathy was not associated with CACS. Vascular inflammation (TBR) was not associated with any of the measures of cardiac autonomic neuropathy.

Conclusion: In persons with type 2 diabetes, we demonstrated an association between impaired cardiac autonomic function and higher coronary artery calcification. This might be a mechanism linking cardiovascular autonomic neuropathy and cardiovascular disease. We could not demonstrate an association between cardiac autonomic function and vascular inflammation.

Disclosure: **S. Sivalingam:** None.

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Low grade inflammation does not attenuate the association between glycated haemoglobin and parasympathetic tonus in people with type 2 diabetes and pre-diabetes

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Background and aims: Type 2 diabetes mellitus (T2DM) is associated with complications such as neuropathies including autonomic neuropathy. Recently, it has been shown that the parasympathetic tonus derived from 24 hours Holter monitoring is reduced in both people with prediabetes and diabetes and inversely correlated to HbA1c. We hypothesize that the association between HbA1c and parasympathetic tonus may be mediated by low-grade inflammation (LGI), a factor in the pathogenesis of T2DM.

Materials and methods: This study is based on The Copenhagen Holter Study, in which 678 community dwelling subjects aged 55 - 75 years who

were free of previous cardiovascular disease, except from well controlled hypertension, underwent a 48-hours Holter recording. Analysis of Heart Rate Variability (HRV) were available for 678 participants and this population included 141 people with well-controlled and newly recognized T2DM (mean HbA1c 55 mmol/mol (7.2%)) and 396 people with prediabetes defined as HbA1c between 39 mmol/mol (5.7%) - 47 mmol/mol (6.4%). We selected high-sensitive CRP, tumor necrosis factor alpha (TNF-α), Interleukin-18 (IL-18) and leukocyte analyses as markers of low-grade inflammation (LGI). Parasympathetic activity was assessed by measuring the root mean square of normal-to-normal beats (RMSSD), which has been acknowledged to be best linked to vagal parasympathetic tone.

Results: In the combined group of prediabetes and diabetes HbA1c was inversely correlated with 24-hour RMSSD (r=-0.16, p<0.01). The association could be detected in both pre-diabetes, r= -0.11, p=0.03), and T2DM (r= -0.21, p=0.02). In multiple linear regression HbA1c was significantly associated with 24-hour RMSSD among people with prediabetes and diabetes, adjusted for age, sex, current smoking, BMI, C-reactive protein, TNF-α, IL-18 and leukocytes (table 1). HbA1c was not associated with RMSSD among people with normal glucose metabolism.

Conclusion: We confirmed that increasing HbA1c in people with well-controlled diabetes and pre-diabetes is associated with decreasing parasympathetic tonus. Markers of LGI did not attenuate this association, challenging the hypothesis that LGI may facilitate reduced parasympathetic tonus in people with pre-diabetes and well-controlled diabetes.

Table 1: Effect of HbA1c on 24-hour RMSSD among people with normal glucose metabolism, pre-diabetes and diabetes.

Independent variables	Normal glucose metabolism	Pre-diabetes	Diabetes
HgbA1c (%)	3.77 (0.78)	-24.1 (0.02)	-8.05 (<0.01)
Sex (female)	0.09 (0.38)	0.01 (0.14)	0.12 (0.22)
Age (years)	0.02 (0.03)	0.01 (<0.01)	0.02 (0.01)
BMI (kg/m2)	0.01 (0.42)	$-4*10^{-4}$ (0.95)	$2*10^{-3}$ (0.85)
Current smoking	-0.01 (0.92)	-0.07 (0.17)	-0.12 (0.18)
hs-CRP (µg/ml)	0.03 (0.73)	-0.03 (0.54)	0.06 (0.46)
Interleukin-18 (pg/ml)	$8*10^{-5}$ (0.48)	$9*10^{-5}$ (0.28)	$6*10^{-5}$ (0.64)
TNF-α (pg/ml)	$1*10^{-3}$ (0.03)	$3*10^{-4}$ (0.52)	$4*10^{-5}$ (0.75)
WBC (10^9/L)	$2*10^{-3}$ (0.95)	0.01 (0.62)	$4*10^{-5}$ (0.53)
Constant	1.52 (0.20)	3.78 (<0.01)	2.3 (<0.01)

Results are slope (p-value)
Logarithmic values of RMSSD and CRP is used, as these are not normally distributed.
TNF-α: tumor necrosis factor alpha is dichotomized as >4 pg/ml and <4 pg/ml.
WBC (white Blood Cell Count)

Disclosure: **R. Hadad:** None.

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Five-year change in body composition is related to heart rate but not related to autonomic dysfunction in The Whitehall II study

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Background and aims: Overweight and obesity are associated with autonomic dysfunction both in non-diabetic individuals and in people with prediabetes and diabetes. Furthermore, autonomic dysfunction has been associated with changes in glucose metabolism and development of cardiovascular disease and diabetic complications. However, it is not known how temporal changes in measures of body composition assessed by e.g. fat mass (FM) and fat free mass (FFM) may affect autonomic function (AF). Exploring patterns of changes in body composition parameters may present new risk factors and pathophysiological pathways that may be modified to prevent autonomic dysfunction. We aim to investigate the effect of changes in body composition on autonomic function in patients with and without dysglycemia.

Materials and methods: Data on body composition and AF was collected twice in civil servants in 2002 and 2009. AF was assessed by measures of cardiovascular autonomic function: resting heart rate (HR) and several heart rate variability (HRV) indices. Body composition was assessed by body mass index (BMI), waist-to-hip ratio (WHR) FM and FFM. In total 3,342 participants without CVD were included. Associations between 5-year changes in body composition indices and changes in AF measures were estimated with linear regression models adjusting for baseline level of the outcome and exposure, age, sex, ethnicity, dysglycemia, metabolic covariates and medication. Analyses including HRV were adjusted for resting heart rate. The HRV indices were log transformed before analysis. A modifying effect of dysglycemia was tested. Adjustment for multiple testing was applied using the Benjamini-Hochberg method.

Results: Increase in BMI (kg/m^2), WHR, FM (kg) and FFM (kg) were associated with concurrent increases in resting HR (bpm) (BMI: 0.87 (95% CI: 0.68,1.05), WHR: 21.50 (14.9,28.2), FM: 0.44 (0.31,0.57), FFM: 0.37 (0.28,0.46)). Changes in body composition were not associated with changes in HRV indices after adjustment for multiple testing. There was no modifying effect of dysglycaemia on any association (Table 1).

Conclusion: Adverse changes in body composition assessed by BMI, WHR, FM, FFM are associated with an increase in heart rate but not autonomic dysfunction. In addition, changes in both FM and FFM seems to associate with heart rate similarly. The reason for this remains to be investigated.

		BMI (kg/m^2)		WHR		FFM (kg)		FM (kg)	
	Model	Estimate	P	Estimate	P	Estimate	P	Estimate	P
Resting Heart rate (bpm)	1	**0.9 (0.7;1)**	**<0.001**	**21.9 (15.3;28.5)**	**<0.001**	**0.4 (0.3;0.6)**	**<0.001**	**0.4 (0.3;0.5)**	**<0.001**
	2	**0.9 (0.7;1.1)**	**<0.001**	**21.5 (14.9;28.2)**	**<0.001**	**0.4 (0.3;0.6)**	**<0.001**	**0.4 (0.3;0.5)**	**<0.001**
SDNN (%-diff)	1	-0.5 (-1.6;0.6)	0.330	-24.8 (-48.3;9.4)	0.135	0.2 (-0.6;1)	0.611	-0.3 (-0.9;0.2)	0.219
	2	-0.6 (-1.7;0.5)	0.290	-20.5 (-45.6;16.3)	0.234	0.2 (-0.6;1)	0.656	-0.4 (-0.9;0.2)	0.189
RMSSD (%-diff)	1	-0.5 (-2.1;1)	0.508	-19.2 (-52.4;37)	0.427	0.5 (-0.6;1.6)	0.388	-0.4 (-1.1;0.4)	0.315
	2	-0.7 (-2.2;0.9)	0.394	-21.6 (-54.2;34.1)	0.371	0.4 (-0.7;1.5)	0.496	-0.4 (-1.2;0.3)	0.238
Low frequency power(%-diff)	1	-1.4 (-3.8;1.1)	0.262	-49.6 (-78.2;16.3)	0.107	0.1 (-1.6;1.9)	0.897	-0.7 (-1.9;0.5)	0.246
	2	-1.4 (-3.8;1.1)	0.257	-38.6 (-73.7;43.4)	0.258	0.1 (-1.6;1.8)	0.915	-0.7 (-1.9;0.5)	0.248
High frequencypower (%-diff)	1	-2 (-4.7;0.7)	0.144	-29.9 (-72.7;80.4)	0.460	0.5 (-1.4;2.5)	0.584	-1.3 (-2.6;0.1)	0.061
	2	-2.4 (-5.1;0.4)	0.092	-32.5 (-74.2;76.2)	0.419	0.4 (-1.6;2.4)	0.706	**-1.4 (-2.7;-0.1)**	**0.037**

Table 1 Effect (with 95% CI) of one population standard deviation 5-yearincrease in body composition measures on SDNN, standard deviation of normal-to-normal R–R intervals; RMSSD, the root mean square of the sum of the squares of differences between consecutive normal-to-normal R–R intervals. The associations are adjusted for age, sex, ethnicity and baseline value of the outcome studied, and BMI (model 1) and further adjusted for physical activity, smoking, systolic blood pressure, total cholesterol, triglycerides, tricyclic antidepressants, diuretics and β blockers (Model 2). Models with HRV indices as out comes were adjusted for resting heart rate. Models where BMI was the determinant was not adjusted for BMI.

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Disclosure: **C. Hansen:** None.

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Gall bladder ejection fraction as a marker of autonomic neuropathy in type 2 diabetes

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Background and aims: Diabetic neuropathy is one of the commonest complications of diabetes mellitus and associated with considerable morbidity and mortality. The influence of diabetes on gall bladder function was not demonstrated in many studies. In this study, the association of fasting gall bladder volume and gall bladder ejection fraction with

degree of cardiac autonomic neuropathy was assessed and correlated with duration of diabetes and severity of diabetes. The aim of the study was to find out the incidence of autonomic neuropathy in study group by simple bed side tests, to determine the fasting gall bladder ejection fraction in diabetics, comparison of gall bladder volume in both study and control group, correlation of gall bladder ejection fraction with autonomic neuropathy.

Materials and methods: The study was conducted as a Prospective observational study conducted among 100 patients in study group and 50 healthy subjects in control group. The fasting gall bladder volume was measured using ultrasonogram and the study and control population were grouped into three groups based on the CAN prevalence. The control group was named as Group A. The type 2 diabetes mellitus cases without CAN were group in Group B and the cases in the study group with CAN were grouped in Group C.

Results: The study population for type 2 Diabetes mellitus consisted of100 patients (46 males and 54 females) and the control group consisted of 50 subjects (23 males and 27 females). 18% belonged to age group of 41- 50 years. 34% belonged to the age group of 61-70 years. Only 3% belonged to age group of 71-80 years. All the patients in the study group had duration of the disease for more than 5 years. Among the 100 cases of Type2 Diabetes mellitus, 78 had cardiac autonomic neuropathy. Of these78 cases, 16 had Grade I cardiac autonomic neuropathy, 28 and 34 Grade II and Grade III cardiac autonomic neuropathy respectively. In the control group, 3 and 2 had Grade I and Grade II cardiac autonomic neuropathy respectively. The mean fasting gall bladder volume of group A is 19.9 ml, group B is 24.18 ml, group C is 33.55 m1. The fasting gallbladder volume was highest in group C, then in group B when compared to group A. The gall bladder ejection fraction was calculated and its mean value of group A, B and C were 60.68, 49.33 and 29.3 respectively. The gall bladder ejection fraction was decreased in group C i.e. DM patients with CAN. The incidence of CAN is found to be high with longer duration of the disease and the degree is also correlated with duration of the disease. The correlation coefficient of this association is 0.792 which indicates high correlation. The correlation of severity of DM with incidence and degree of CAN was 0.81 which indicates high correlation and also the study showed an increase in the FGBV and a decrease in the GBEF with increase in the severity of cardiac autonomic neuropathy.

Conclusion: In patients with type 2 diabetes mellitus, the gall bladder ejection fraction is significantly related to the duration of diabetes mellitus and degree of hyperglycemia in addition to cardiac autonomic neuropathy (CAN). Similarly, fasting gall bladder volume (FGBV) is significantly increased in type 2 diabetes mellitus patients with cardiac autonomic neuropathy.

Disclosure: **N. Dora:** None.

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Phase angle as indicator for sarcopenia and diabetic polyneuropathy: a cross-sectional observational study in patients with type 2 diabetes

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Background and aims: Distal sensorimotor polyneuropathy (DPN) and diabetic sarcopenia are major contributors to increased morbidity and mortality in patients with diabetes. With limited diagnostic methods in both disorders, we aimed to establish a simple and easily accessible clinical tool to improve screening. In particular, we hypothesized that parameters of the bioelectrical impedance analysis (BIA) are correlated with clinical, electrophysiological and MRI measurements in patients with DPN and sarcopenia.

Materials and methods: In this cross-sectional observational study we included 104 healthy subjects and 205 patients with type 2 diabetes (T2D). All subjects received broad serologic, clinical and electrophysiological testing. The phase angle (PA) was calculated using a multifrequency bioelectrical impedance analyzer. DPN was diagnosed with the neuropathy symptom score and neuropathy disability score (NDS). Nerve conduction studies and quantitative sensory testing (QST) were performed as well. For imaging diagnosis of sarcopenia, 37 T2D patients received MRI scans applying a Magnetization Transfer Contrast Sequence. Muscle groups of the thigh were segmented manually and the Magnetization Transfer Ratio (MTR) was calculated for quantitative analysis of each compartment. For statistical analysis we used the software IBM SPSS Statistics 27.

Results: The group analysis showed significant lower PA values in T2D patients with DPN in comparison to T2D patients without DPN (5.71+/-0.10 vs 6.07+/-0.08, $p=0.007$) and healthy controls (5.71 +/- 0.10 vs 6.18 +/- 0.08, $p<0.001$). Among T2D patients, the partial correlation analysis controlled for gender showed correlations of the PA with nerve conduction velocity (NCV) and compound motor action potentials (CMAP) of the peroneal nerve (NCV $r=0.25$, $p=0.003$; CMAP $r=0.25$, $p=0.003$), the tibial nerve (NCV $r=0.24$, $p=0.005$; CMAP $r=0.28$, $p<0.001$) and the sural nerve (NCV $r=0.31$, $p<0.001$; SNAP $r=0.22$, $p=0.008$). Clinically, there were negative correlations with the NDS ($r=-0.30$, $p<0.001$) and positive correlations with Z-scores of QST (thermal detection $r=0.34$, $p<0.001$; mechanical detection $r=0.29$, $p<0.001$). Additionally, after controlling for gender, the PA correlated with sarcopenic measurements such as hand grip strength ($r=0.40$, $p=0.002$), walking distance ($r=0.23$, $p=0.002$) and Borg scale ($r=-0.17$, $p=0.030$) in the 6-minute walking test. Also, the MTR of the anterior muscle group showed a positive correlation with the PA ($r= 0.40$, $p=0.007$ $n=37$). In the control group no correlations were found except for a positive correlation with the hand grip strength ($r=0.34$, $p=0.002$).

Conclusion: In our study we showed close associations of the PA obtained by the bioelectrical impedance analysis with neuropathic measurements and clinical/radiological assessment of sarcopenia. The PA could be a novel, easily accessible and yet powerful marker for a quick assessment of diabetic complications such as DPN and sarcopenia.

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Disclosure: **L. Schimpfle:** None.

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Histamine-induced axon flair response in people with diabetic peripheral neuropathy

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Background and aims: The axon-flair mediated response (known as neurogenic inflammation) is caused by activation of cutaneous nociceptive C-fibers inducing a spreading vasodilation, which is visible as a vasomotor response (flare) using perfusion-imaging devices. Previous studies have established a clear association between diabetes and a reduced dermal microvascular function. However, these studies have 1) failed to establish a clear association between the severity of diabetic peripheral neuropathy (DPN) and the intensity of the flair response, and 2) the influence of painful versus painless DPN likewise remains unclear.

Materials and methods: We included a total of 80 participants aged 18-70 years and divided them into four groups: 20 persons with type 1 diabetes (T1DM) and neuropathic pain (A), 20 persons with T1DM and non-painful DPN (B), 20 persons with T1DM and no DPN (C) and 20 healthy controls (D). Each participant was matched on age and gender with a participant from each of the other groups. The axon-flair mediated response was evoked by histamine injected under the skin using a needle with standardized 85 g lancet pressure. Perfusion-images of the response were captured at the start of the examination and each minute for 15 minutes using a MoorFLPI, Moor Instruments. Mean-flux was analyzed as changes from baseline. The data points were used to create a graph for each participant using time on the x-axis and mean flux on the y-axis. The curves were fitted using inverse exponential decay and the maximum flows and time-constants were estimated. Differences between the groups were calculated using students t-tests and pairwise Mann-Whitney tests with Bonferroni correction. P-values < 0.05 was considered significant.

Results: At the time of writing, the initial analysis were conducted in 62 of the 80 planned participants. Based on the current dataset, the fits showed high similarity with inverse exponential decay (R^2 = 0.95 (A), 0.88 (B), 0.91 (C) and 0.96 (D). The average maximum flows were 21.7 ± 15.9 perfusion-units (PU) (A), 23.2 ± 19.4 PU (B), 38.7 ± 18.1 PU (C) and 58.2 ± 32.5 PU (D), respectively. The average time-constants were 6.7 ± 4.3 minutes (A), 6.8 ± 3.5 minutes (B), 7.4 ± 2.9 minutes (C) and 6.7 ± 1.9 minutes (D), respectively. The maximum flow was significantly lower in people with T1DM and DPN (A, B) than in people without DPN (C, D). The maximum flow was also significantly lower in people with T1DM irrespective of degree of DPN (A, B, C) than in people without T1DM (D). We observed no difference in maximum flow between people with painful DPN (A) and people with painless DPN (B). There was no significant difference between the time-constants in any of the groups.

Conclusion: This preliminary data shows promise regarding the ability of the histamine-induced axon flair-mediated response to distinguish between people with T1DM without DPN, and people with T1DM and DPN. In addition, a reduced response in otherwise healthy people with T1DM compared to people without diabetes was found. Based on these preliminary results, the histamine-induced axon flair-mediated response seems not able to distinguish between painful and painless DPN in people with diabetes.

Clinical Trial Registration Number: NCT04078516

Disclosure: **J. Roeikjer:** None.

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Altered resting state connectivity in people with diabetic peripheral neuropathy and correlation with functional status

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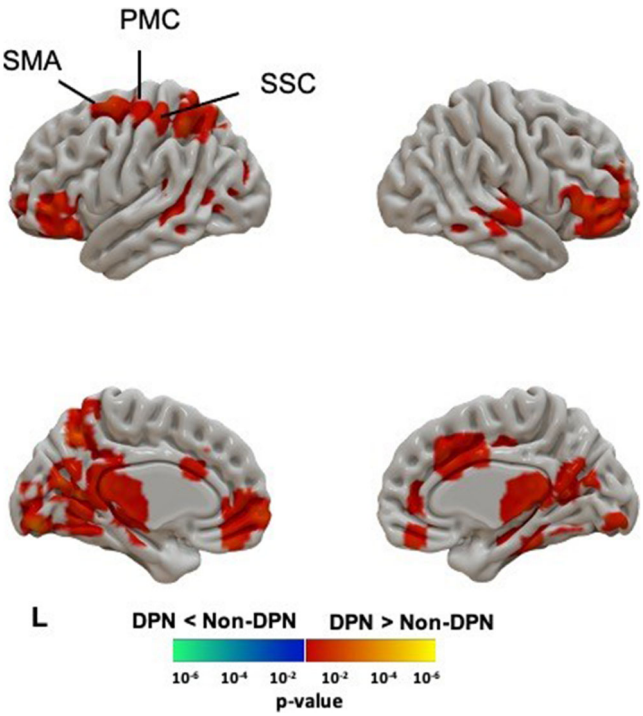
Background and aims: Chronic pain in diabetic peripheral neuropathy (DPN) has been shown to alter resting state functional connectivity within the brain. However, it is unclear how the overall decrease in afferent input from the lower limbs in DPN affects functional connectivity in the absence of pain. We examined differences in resting state functional connectivity in people having diabetes, with and without DPN.

Materials and methods: Thirty five participants with type 2 diabetes, 18 with and 17 without DPN, underwent resting state functional magnetic resonance imaging. Participants were also assessed for functional status, including muscle strength at ankle and big toe, and average body sway velocity during quiet standing with eyes closed. Group differences within each brain functional network were compared, correcting for multiple comparisons at a threshold p value <0.05. Functional connectivity maps were correlated with different functional status outcomes within the DPN group.

Results: Compared to individuals without DPN, those with DPN had significantly increased connectivity in the sensorimotor network (**Figure 1**), but reduced connectivity in the lateral visual network, right frontoparietal network and the basal ganglia network. Among individuals with DPN, sensorimotor network connectivity was negatively correlated with muscle strength at big toe and body sway velocity.

Conclusion: Our findings suggest augmented resting state connectivity within the sensorimotor network, potentially to compensate for the reduction in afferent input from the periphery. At the same time, decreased connectivity in ancillary networks important for movement may limit the compensation achieved.



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Disclosure: **K. Venkataraman:** None.

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Relationship between circulating sestrin2 level and diabetic peripheral neuropathy in type 2 diabetic patients

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Background and aims: Diabetic peripheral neuropathy (DPN) is one of the most microvascular complications of diabetes involved in redox imbalance. Sestrin2, a novel stress-inducible protein that lacks kinase activity, is involved in regulating glucose metabolism and redox homeostasis. However, the relationship between circulating sestrin2 and DPN is still elusive. Therefore, this study aimed to explore the relationship between circulating sestrin2 levels and diabetic peripheral neuropathy in type 2 diabetic patients.

Materials and methods: A total of 96 type 2 diabetic patients and 39 age- and sex-matched normal volunteers were involved in this cross-sectional study. Clinical characteristics and metabolic indices were determined. Serum sestrin2 was measured by ELISA. The relationship between serum sestrin2 and DPN was investigated. Pearson/Spearman correlation and logistic regression analysis were used to identify the associations of various metabolic indices with sestrin2 and DPN.

Results: 96 T2DM patients were divided into DPN (n=47) and diabetic patients without DPN (T2DM, n=49). There were significant differences in circulating sestrin2 between control and all diabetic patients [9.10 (5.41, 13.53) ng/ml vs. 12.75 (7.44, 23.80) ng/ml; P<0.001]. Circulating sestrin2 were significantly higher in diabetic patients than those of normal healthy volunteers [14.58 (7.93, 26.62) ng/ml vs. 9.10 (5.41, 13.53) ng/ml, P<0.01]. However, patient with DPN had a lower circulating sestrin2 compared to those without DPN [14.58(7.93,26.62) ng/mL vs. 9.86(6.72,21.71) ng/mL,P<0.01]. Bivariate correlation analysis showed that circulating sestrin2 was significantly and positively associated with HbA1c (r=0.292, P=0.000), BMI (r=0.672, P=0.000), SCr (r=0.206, P=0.016), TG (r=0.731, P=0.000), FPG (r=0.202, P=0.040) and negatively correlated with eGFR (r=-0.230, P=0.007). After adjustment for age, gender, diabetes duration and HbA1c, multiple regression analysis showed that Sestrin2 was independently associated with BMI (beta coefficient=0.422;R^2=0.608, t =5.732, P<0.0000) and TG (beta coefficient=0.443; R^2=0.608, t=6.146, P<0.0000). Logistic regression analyses showed that sestrin2, diabetes duration and HDL were strongly associated with DPN in type 2 diabetic patients (OR=0.855 CI 95% [0.750-0.975], OR=1.411 CI 95% [1.175-1.695] and OR=0.041 CI 95% [0.002-0.880], respectively).

Conclusion: Our findings strongly suggested that circulating sestrin2 level decreased gradually in diabetic patients with advanced DPN. Lower levels of sestrin2 are independently associated with DPN. Therefore, sestrin2 may be involved in the development of type 2 in DPN.

Clinical Trial Registration Number: ChiCTR1800019561

Supported by: NSFC 81870593

Disclosure: **X. Sun:** None.

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Effect of obesity on the associations of 25-hydroxyvitamin D with prevalent and incident distal sensorimotor polyneuropathy

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Background and aims: It has been hypothesized that vitamin D is associated with distal sensorimotor polyneuropathy (DSPN), but evidence from large studies is lacking. Also, it is not clear whether vitamin D and obesity have a synergistic effect on DSPN. Therefore, we examined the association of serum 25-hydroxyvitamin D (25(OH)D) with DSPN and assessed possible effect modification by obesity.

Materials and methods: The study included individuals aged 62-81 years who participated in the F4 (2006-2008) and FF4 (2013-2014) surveys of the Cooperative Health Research in the Region of Augsburg (KORA) study. DSPN was assessed using the Michigan Neuropathy Screening Instrument. Baseline 25(OH)D was modeled by a 10 nmol/L decrease and as tertiles. Cross-sectional analyses (n=1,065; 34% obese; 22% type 2 diabetes) assessed the associations of 25(OH)D with prevalent DSPN, while prospective analyses (n=422) assessed the associations of 25(OH)D with incident DSPN. In both analyses, Poisson regressions with robust error variance were used.

Results: No association was found between 25(OH)D and prevalent DSPN in the total sample after adjustment for age, sex, season of blood sampling, BMI, metabolic variables, lifestyle factors, and comorbidities. A decrease in 25(OH)D by 10 nmol/L was associated with an increased risk for prevalent DSPN (RR [95% CI] 1.08 [1.01; 1.16]) in obese individuals but not in non-obese individuals (RR 0.97 [0.92; 1.02], $P_{interaction}$=0.001). In prospective analyses, 25(OH)D levels in the first and second tertiles were associated with a higher risk of incident DSPN in the total sample (RR 1.18 [1.02; 1.38] and 1.40 [1.04; 1.90]; tertile 3 as reference) after adjustment for age, sex, season and BMI, but not in the fully adjusted model.

Conclusion: Low vitamin D and obesity may act in synergy to increase the risk of developing DSPN. Clinical trials should clarify whether obese individuals may benefit from vitamin D supplementation to treat or prevent DSPN.

Supported by: DZD

Disclosure: **H. Maalmi:** None.

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Diabetic painful neuropathy treatment response with deep learning classification

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Background and aims: A quarter of all patients with diabetic peripheral neuropathy (DPN) experiences disabling pain in the lower and upper limbs. This results in considerable disability and suffering and pharmacotherapy is often used for symptomatic relief. However, only a third of patients achieve a 50% reduction in pain intensity. We recently demonstrated how functional magnetic resonance (MR) imaging can be used to assess and stratify patients with painful DPN. This supports the idea of using neuroimaging as a mechanism-based technique to individualise

therapy for patients with painful DPN. The aim of this study was to develop and validate different machine learning algorithms to predict treatment response in patients with painful DPN.

Materials and methods: Twenty-three consecutive patients who received intravenous lidocaine treatment for painful DPN were assessed. All subjects (responders n=13 and non-responders n=10) underwent detailed clinical and neurophysiological assessments including quantitative sensory testing using the German Network on Neuropathic Pain (DFNS) protocol to phenotype their pain sensory profile. Subjects also underwent resting state brain magnetic resonance (MR) imaging. After pre-processing we performed a group concatenated ICA set to 30 components and obtained subject specific spatial maps. From these we automatically choose 7 highly correlated ($p<0.05$) ICA components from well know resting state functional connectivity networks. A 3D CNN (convolutional neural network) classification framework was trained using a VGG-Net based architecture with 100 epoch and a learning rate of 0.001. This deep learning architecture was used to compare models using (1) 7 highly correlated ICA networks 2) All 30 ICA networks generated.

Results: The mean age and duration of pain were 57.2 and 8.2 years respectively. Also mean NTSS-6 scores for all patients were 13.86. The deep learning treatment response classification model using 7 ICA spatial maps has a mean AUROC of 0.91 and an accuracy score of 0.85. However, with the extra information of all 30 ICA maps the mean AUROC increased to 0.94 with an accuracy score of 0.89.

Conclusion: Our results show that we can predict treatment response to a high AUROC and accuracy rate. We have also shown that additional information can be extracted with extra ICA spatial components as an input to our deep learning model. To our knowledge this is the first study utilising deep learning methods to classify treatment response in painful DPN. Our dataset cohort is small by machine learning standards and future works would benefit if expanded to a larger cohort.

Supported by: ESFD, NIHR

Disclosure: **K. Teh:** None.

SO 49 Neuropathy: from mechanisms to memory

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Brain network disruptions in type 1 diabetes with and without diabetic peripheral neuropathy

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Background and aims: Diabetic peripheral neuropathy and neuropathic pain involve both the peripheral- and central nervous system. The default mode network (DMN) is a brain network, which is activated at rest. The connectivity of this network is suggested to be influenced by conditions like pain and linked to cognitive function. Diabetes has also been suggested to alter DMN. However, it is uncertain whether the potential reorganization of DMN is attributed to diabetes *per se* or the complications coexisting. We aimed to investigate the DMN in type 1 diabetes (T1D) with peripheral neuropathy and neuropathic pain.

Materials and methods: This study is part of the project MEDON (Methods of Early Detection Of diabetic peripheral Neuropathy). Nineteen subjects with T1D (mean age 51.6 ± 9.8 years), 15 with T1D and neuropathy (mean age 54.1 ± 8.7 years), 19 with T1D and neuropathic pain (mean age 51.5 ± 9.8 years), and 20 healthy controls (mean age 51.5 ± 9.2 years) underwent resting-state functional MRI (3T GE scanner). The participants were age and gender matched across the groups. Seed-to-voxel analyses were performed for four DMN seeds: Medial prefrontal cortex, posterior cingulate cortex and right/left lateral parietal cortex. The strength of DMN connectivity (mean *z*-score) was calculated using the mean of seed-to-seed correlation between all four seeds.

Results: The seed-to-voxel analyses revealed increased connectivity between left lateral parietal cortex and right operculum in T1D with neuropathy compared to controls ($p = 0.001$) and decreased connectivity between left lateral parietal cortex and interior frontal cortex/precentral cortex/middle frontal cortex ($p = 0.003$). A one-way ANOVA demonstrated a significant difference in the strength of DMN between the four groups ($p = 0.04$). The mean strength of DMN was significantly higher in the T1D group (0.65 ± 0.19) as compared to healthy (0.49 ± 0.17) (Bonferroni, $p = 0.04$). T1D with neuropathy (0.59 ± 0.18) and T1D with pain (0.54 ± 0.18) showed a tendency toward higher connectivity compared to healthy, but lower than those T1D without complications. See figure 1.

Conclusion: These preliminary data confirmed the altered DMN connectivity in individuals with T1D. Increased connectivity was identified specifically for the group with T1D and neuropathy compared to healthy. DMN showed increased connectivity to operculum, which is involved in higher-order processing for somatosensory perception and decreased connectivity to frontal regions involved in cognition. The connectivity strength of DMN was highest in the T1D without complications, which could suggest DMN functioning as a cognitive compensatory system, but this is still urged to be investigated.

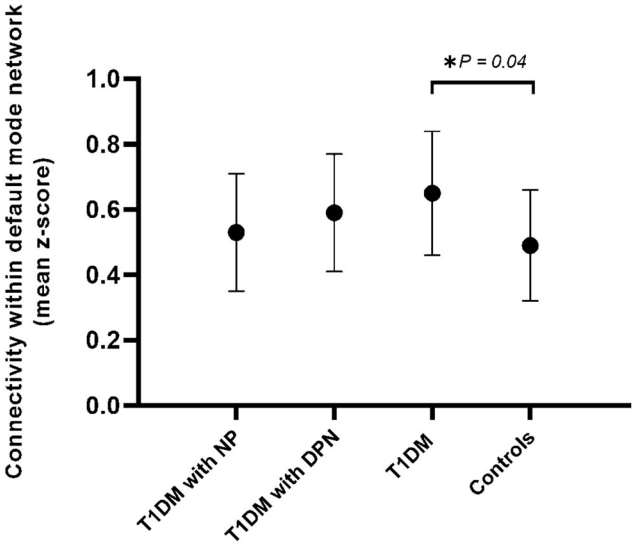

Figure 1: Strength of default mode network connectivity. NP: neuropathic pain; DPN: diabetic peripheral neuropathy

Clinical Trial Registration Number: NCT04078516

Disclosure: **S.S. Croosu:** None.

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Machine learning techniques for the analysis of tactile sensitivity in type 1 diabetes

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Background and aims: Tactile sensitivity (TS) is frequently altered in patients affected by diabetic peripheral neuropathy (DPN). Recently, we developed a novel test based on haptic technology for the evaluation of TS in type 1 diabetic patients (T1DM). We used machine learning techniques with the aims of (i) evaluating the relationship between TS as measured with haptic tests and standard tests such as biothesiometry, sensory motor nerve conduction studies (NCS) and (ii) predict the probability of DPN.

Materials and methods: 40 T1DM patients (HbA1c < 9.5%) and 18 healthy control subjects (C) were enrolled. Patients underwent a neurological assessment including vibratory perception (VP) using biothesiometry and bilateral NCS to upper and lower limbs. Patients were divided in 2 groups based on VP alterations (VP- and VP+). TS was evaluated using a haptic device that produced precise motion. The protocol was replicated with and without masking vibrations (MV). By means of Generalized Linear Mixed Models (GLMM), we tested the ability of the participants to discriminate motion speed in the two conditions. Principal Component Analysis (PCA) was performed on biothesiometer data to summarize these correlated variables with a smaller number of representative variables. The first two principal components (PCs) that explained more than 80% of the variance were retained for further analyses. Linear Discriminant Analysis (LDA) was used to predict the probability of DPN at the NCS from the following variables: disease duration, TS, biothesiometer test, Michigan Score, age and gender.

Results: T1DM group was divided into 21 VP+ and 19 VP- patients. TS in the upper limbs was significantly lower in VP+ as compared to the C without MV (estimate: -0.30 ± 0.06, $p < 0.001$) and significantly lower in VP- and in VP+ as compared to the C with MV (GLMM estimate: -0.13 ± 0.07, $p < 0.05$; estimate: -0.37± 0.03, $p < 0.001$ respectively). A positive significant linear relationship between TS with and without MV and conduction velocity (estimate = 0.009, $p = 0.017$; estimate = 0.012, $p = 0.01$, respectively) of sural and radial nerve were observed in T1DM group. The first principal component (PC1) explained more than 80% of the variance. The LDA correctly assigned the patient with and without DPN in 87% of the cases. To evaluate the predictive power of the different tests, we ran the LDA by removing either biothesiometer PCs or TS. The results were compared by means of ROC curves; the Area Under the Curve (AUC) was similar in the complete model and in the model excluding biothesiometer PC (approximately 94%), but it falls to 88% if TS is excluded from the analysis.

Conclusion: TS, measured with haptic device, was significantly lower in T1DM patients without vibration sensitivity alteration in the lower limbs. A significant relationship between sensory NCS and TS was also observed. Haptics could complement current standard quantitative sensitivity tests, and thus provides an innovative measure of outcome in DPN. Use of machine learning technique enhances DNP assessment.

Disclosure: **F. Picconi:** None.

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Mechanism of insulin-induced proliferation and myelin formation in Schwann cells

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Background and aims: Schwann cells are important for peripheral nerve function, and their dysfunction has been implicated in the pathogenesis of diabetic neuropathy and other demyelinating diseases. Here, we elucidated the insulin-induced proliferation and expression of myelinated proteins in IFRS1, and investigated the involvement of signaling pathways.

Materials and methods: Immortalized rat Schwann cells (IFRS-1), the Schwann cell line obtained from the peripheral nerve tissue of Fischer 344 rats, were stimulated by insulin for indicated periods and activation of intracellular signalings, proliferation and expression of myelin-related proteins were measured. In addition, the effects of various inhibitors on the proliferation and the expression of myelin-related proteins were examined.

Results: IFRS1 exhibited insulin receptor. Insulin phosphorylated Akt and ERK in a time-depemdent manner, while it did not phosphorylate P38MAPK. The proliferation of IFRS1 was promoted by insulin, which was significantly inhibited by the addition of LY294002, a PI3-kinase (PI3-K) inhibitor.Insulin stimulated the expressions of myelin-related proteins, such as myelin protein zero (MP0) and myelin basic protein (MBP). The insulin-increased expression of MP0 was significantly inhibited by the addition of PD98059, a MEK inhibitor and the increased

expression of MBP was significantly inhibited by the addition of LY294002.

Conclusion: These results demonstrate the physiological role of insulin and the potential involvement of the PI3-K/Akt and ERK/MEK pathways in insulin-stimulated growth and myelin-related protein synthesis in Schwann cells.

Disclosure: **N. Nakamura:** None.

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Working memory is affected in type 1 diabetes and neuropathy contributes to the cognitive deterioration

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Background and aims: Type 1 diabetes (T1D) can be accompanied by diabetic peripheral neuropathy (DPN) and neuropathic pain. T1D has been associated with cognitive impairment. While it has been observed that cognitive function is correlated with nerve conduction velocity and affected by neuropathic pain, the current evidence in T1D remains insufficient. The aim of this study was to investigate cognitive function in individuals with T1D and examine how DPN and neuropathic pain may impact cognitive function.

Materials and methods: This study is part of a larger project: MEDON (Methods of Early Detection of Diabetic Peripheral Neuropathy). The study included 20 individuals with T1D (mean age 52.9±10.0), 15 individuals with DPN (54.0±8.7), 20 individuals with neuropathic pain (51.3 ±9.5) and 20 healthy controls (51.3±9.2) matched for gender and age. The participants performed Addenbrooke's cognitive examination III (ACE-III), which assesses attention, memory, word mobilisation, language and visuospatial skills. Working memory was investigated using the N-back task, including 0-, 1- and 2-back. The discrimination index d' was calculated from the N-back results and reaction times were recorded and adjusted for accuracy. Groups were compared by performing one-way ANOVAs and Kruskal Wallis tests with Bonferroni corrections.

Results: The results are summarized in table 1. Group differences were identified in memory (p=0.02) and d' (p=0.01), whereas the total score for ACE-III was borderline significant (p=0.05). Individuals with T1D and DPN scored significantly lower compared to healthy controls on the memory domain of ACE-III (p=0.03), while the other groups did not differ significantly. Tendencies demonstrated the lowest memory scores in individuals with T1D and DPN, followed by the individuals with T1D and neuropathic pain, while the individuals with T1D scored closer to the healthy controls. No significant differences were present for the other domains (all p>0.2). Individuals with T1D and DPN exhibited lower d' scores in the 2-back task compared to healthy controls (p=0.01). No significant differences were observed in other d' scores or reaction times (all p>0.1).

Conclusion: These preliminary results suggest that working memory is affected in T1D. However, T1D does not seem to be the main driver of cognitive decline, rather it appears that DPN contributes to the T1D-related cognitive deterioration. This may reflect the potential negative effects on cognition caused by the interaction between the peripheral and central nervous system. Furthermore, it appears that neuropathic pain does not impact cognitive function in people with T1D.

Table 1: ACE-III scores and N-back results of the participants

	T1D + neuropathic pain	T1D + neuropathy	T1D	Healthy controls	p-value
ACE-III					
Total	87.6 ± 7.0	85.4 ± 7.7	89.4 ± 7.4	91.7 ± 5.4	0.053
Attention	17.4 ± 1.0	17.7 ± 0.8	17.6 ± 0.8	17.7 ± 0.6	0.742
Memory	18.5 ± 5.0	16.5 ± 4.6	20.3 ± 4.0	21.0 ± 3.1	0.022*
Word mobilisation	11.8 ± 1.8	11.5 ± 2.2	12.0 ± 1.6	12.3 ± 1.8	0.681
Language	24.5 ± 0.9	24.1 ± 1.6	24.5 ± 1.6	25.1 ± 0.9	0.208
Visuospatial skills	15.5 ± 0.9	14.7 ± 1.7	15.2 ± 1.8	15.6 ± 0.9	0.303
N-back (d')					
0-back	3.8 ± 0.2	3.9 ± 0.0	3.9 ± 0.1	3.8 ± 0.1	0.108
1-back	3.7 ± 10.3	3.6 ± 0.4	3.7 ± 0.4	3.8 ± 0.3	0.524
2-back	1.9 ± 0.8	1.6 ± 0.5	2.1 ± 0.7	2.3 ± 0.6	0.013*

[a] Data are presented as mean ± SD. T1D denotes type 1 diabetes, ACE-III Addenbrooke's Cognitive Examination III.

Clinical Trial Registration Number: NCT04078516

Disclosure: **M. Gjela:** None.

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A novel, multimodal magnetic resonance imaging and a machine learning approach to classifying sensory phenotypes in painful DPN

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Background and aims: Painful diabetic neuropathy (DN) is a common, distressing complication of diabetes that is discordant with the degree of peripheral nerve pathology. Very little is known about the cerebral processes involved in pain processing in painful DN. Here we investigated resting-state brain connectivity associated with prolonged pain in DN.

Materials and methods: 142 subjects and 36 matched controls were compared with regard to both behavioural measures of pain perception and resting-resting state functional Magnetic Resonance Imaging. The resting-state fMRI brain connectivity was investigated using 20 seed regions located in cardinal pain processing brain regions. Resting-state fMRI analysis was performed using the NITRC Functional Connectivity (CONN) Toolbox and SPM8 (welcome Trust Centre for Neuroimaging London, UK) in Matlab 2014a (the MathWorks, Natick, MA, USA). Functional connectivity matrices between the pre-specified seeds were calculated and the HV versus painful DN phenotype interaction examined.

Results: Relative to controls, painful DPN patients displayed increased brain connectivity predominately for the supplementary motor areas and the primary sensorimotor cortex (β=0.23, T(93)=3.7, p-FDR=0.004). Similar results were found when painful DPN subjects were compared with those with no DPN (β=0.23, T(96)=4.01, p-FDR=0.001). Conversely, we observed an increase in brain connectivity between the primary somatosensory cortex and cingulate cortex (β=0.13, T(101)=3.18, p-FDR=0.039), prefrontal cortex and amygdala (β=-0.14, T(101)=-3.59, p-FDR=0.01) between painful and painless DPN patients.

Conclusion: Our study provides experimental evidence of increased connectivity between frontal midline regions that are implicated in

affective pain processing and bilateral sensorimotor regions in painful DPN patients.

Supported by: ESFD, NIHR

Disclosure: **D. Selvarajah:** None.

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A 5 years follow up observational study of type 1 diabetes patients not attending secondary care clinics

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Background and aims: The standard of care is considered to be that all patients with Type 1 Diabetes (T1DM) are offered follow up in secondary care services. However, many patients do not attend their secondary care appointments but the exact percentage of patients not meeting this standard, the nature of the patients not attending and the consequences are not known. Cardiff & Vale University Health Board (CAVUHB) is one of seven health boards in Wales. Each health board oversees a number of primary care practices, as well as, secondary care centres. In this study, data from 383 patients across 18 practices was analysed. The main aim of this study is to find if clinic attendance improves glycaemic control and reduces diabetes-related complications in patients with T1DM.

Materials and methods: Between 1 June 2014 till1 June 2015, primary care practices in CAVUHB were requested to perform a search to identify records of patients with T1DM in their databases. A data table was created with defined parameters for diabetes-related complications. Attendance to secondary care was defined as recorded attendance to a diabetes clinic in hospital using the "Outpatient activity" tab from patients electronic records. Patients were divided into 3 groups; full attendance (FA) for those who attended clinics with no gaps for 2 consecutive years, intermittent attendance (IA) for those who didn't attend clinics for 2 consecutive years or more and never attended (NA) for those who never attended over 5 years. The time frame for clinic attendance was defined as 1 June 2014 till1 June 2019. Most recent HbA1c levels and Albumin/Creatinine ratio were recoded. Data on incidence of DKA, hypoglycaemia, retinopathy, nephropathy and neuropathy was collected during the study time frame.

Results: 211 patients fully attended their secondary care clinics (55,09 %), 109 attended intermittently (28.45 %) and 63 never attended (16.44 %). No significant differences were seen in the microvascular complications among the 3 study groups. Retinopathy (FA : 28%), (IA : 26%) and (NA : 29%). Nephropathy, (FA : 13%), (IA : 12%) and (NA : 14%). Neuropathy (FA : 5%), (IA : 3%) and (NA : 3%). Raised Albumin/Creatinine ratio was not significantly different among the study groups FA, IA and NA (10%, 7%, 11% respectively). Higher incidence of DKA was seen in IA group 10% in comparison with the FA and NA groups (5%, 5%). Also, 13% of patients from IA group with no significant difference from FA and NA groups (8% and 11% respectively).

Conclusion: Within CAVUHB, 16.44 % of the patients did not attend secondary care clinics. However, some variations were seen among the 18 primary care practices suggesting multifactorial determinants including practices and patients. This 5 follow up data on diabetes-related complications has shown that the prevalence of long-term complications for T1DM patients is not greater in primary care compared to that in secondary care suggesting that this group of patients whose care was kept only in primary care may be appropriately selected.

Supported by: INSPIRE Summer Studentship

Disclosure: **Q. Siah:** Grants; INSPIRE Summer Studentship.

SO 50 Nephropathy interventions: from blueberries to SGLT2

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Blueberry effects on kidney and perirenal adipose tissue in a rat model of high fat diet-induced prediabetic nephropathy

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Background and aims: Blueberries (BB) owing to their insulin sensitizing, lipid-lowering, antioxidant and anti-inflammatory effects are a promising nutraceutical for the management of Prediabetic Nephropathy (PreDN), a microvascular complication resulting from an early condition of diabetes (prediabetes). We aimed to evaluate the effects of a BB juice (BJ) on the kidney and perirenal adipose tissue (PAT) in a rat model of high fat diet-induced PreDN.

Materials and methods: Twenty-four male Wistar rats were divided in three groups (n=8) for 24 weeks: Control group (Standard chow, Sd), 45% High-Fat (HF) diet-induced PreDN group and BJ-treated group (HF+BJ) [25 g/kg BW per day from week 16 onwards]. Glycemic/insulinemic profiles were evaluated throughout the protocol. Lipid profile was examined by serum TGs, total and LDL/HDL cholesterol, hepatic/renal TG quantification and renal Oil Red O staining. Renal function was assessed by kidney weight, urine output and serum/urinary measures of creatinine, albumin, uric acid and glucose. Kidney/perirenal adipose tissue (PAT) histological characterization was performed by Periodic Acid-Shiff and Gomori's green trichrome staining. Relative gene expression (RT-PCR) was also evaluated in kidney (KIM-1, NLRP3) and PAT (PRDM16, CD36, UCP1, CPT1). Values are means ± S.E.M (ANOVA/post-hoc tests). This work was approved by the ORBEA of iCBR-FMUC (9/2018).

Results: HPLC/PDA/ESI-MSn analysis revealed that the main PP's present in BB are anthocyanins (e.g. malvidin derivative), flavonoids (e.g. quercetin-O-hexoside) and phenolic acids (e.g. caffeic acid). HF animals displayed increased BW, glucose intolerance, postprandial hyperinsulinemia, elevated circulating LDL-c, hepatic/renal lipidosis, mild impairments in renal function and histological lesions (e.g. glomerular basement membrane thickening), features resembling the stages I/II of PreDN. In addition, HF group displayed increased density of PAT browning-like fat spots and elevated UCP-1 gene expression ($p<0.05$), which could be viewed as a beneficial adaptive response. Apart from ameliorating prediabetic glucose intolerance, BJ supplementation elicited hepatic lipid accumulation, inhibition of PAT browning mechanisms and fatty acid β-oxidation, increased renal/PAT inflammasome and kidney injury markers without major improvements on renal function and/or histopathology.

Conclusion: In this animal model of PreDN, BB was able to ameliorate prediabetic glucose intolerance but promoted harmful effects in the kidney/PAT duo that may be only perceived at a sub-clinical (molecular) level.

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S_s-DS-ONJ treatment abrogates the inflammatory events underlying during diabetic nephropathy: the use of adult kidney explants from BB rat as an ex vivo model

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Background and aims: It is recognized that chronic low-grade inflammation and activation of the innate immune system are closely involved in the pathogenesis of diabetes mellitus. Diabetic Nephropathy (DN) is a multifactorial disease due to that danger signals released by injured renal cells can trigger remodeling processes by stimulating renal cells and activating immune cells. Tubular cells can secrete pro-inflammatory cytokines such as IL1b, IL-18 and TNFa, which secretion si regulated by inflammasome activation and EMT that may aggravate DN. In the early phase of renal fibrosis, cytokines play a decisive role in initiating the phenotypic change of epithelial-to-mesenchymal transition (EMT). EMT is demonstrated that it is a direct contributor to the development of renal fibrosis in DN. (S_s-DS-ONJ) belongs to a family of compounds called sulfur-linked sp2-imnosugar glycolipids (sp2-IGL). Differently from other immunoregulatory glycolipids, the sp2-IGLs are metabolically stable and can be prepared in pure anomeric form with total stereoselectivity, thereby providing a suitable platform for glycodrug design. Increasing evidence suggested that sp2-IGL may offer anti-inflammatory protection against diabetic complications as Diabetic Retinopathy. Therefore, in the present study, we examined the effects of (S_s)-DS-ONJ on an adult kidney explants from BB rat in order to test the effect of the compound in renal inflammatory changes, progress of EMT and autophagy regulation.

Materials and methods: Effect of the S_s-DS-ONJ bioactive compound on kidney tubular cells (MCTs) and kidney explants from BB rat. Proinflammatory and anti-inflammatory response were evaluated. We stimulated MCTs with a cocktel of cytokines, as an inflammatory stimulus and/or S_s-DS-ONJ. Kidney explants from BB rat at 7 weeks was cultured in the presence or absence of S_s-DS-ONJ. Proinflammatory-(M1 response) / anti-inflammatory-(M2 response), EMT proteins and cytokines, stress kinases signaling pathway were analyzed by either RT-PCR, Western blotting and siRNA procedures.

Results: Understanding all these inflammatory processes as a regulating mechanism that prevents the DN progression in the early stages of the disease, we have shown the potential effect of S_s-DS-ONJ as a possible therapeutic treatment in DN due to its potent anti-inflammatory and anti-fibrotic effect as a natural compound due to inhibition of IL18 expression. The kidney explants from BB rat reduced significantly the pro-inflammatory parameters levels in the presence of S_s-DS-ONJ, compared to kidney explant from BB rat in basal condition. As a possible participation in inflammatory resolution, the autophagy flux is increased under S_s-DS-ONJ effects in kidney explants from BB rat. Moreover, the results show that (S_s)-DS-ONJ does not reverse EMT towards a basal condition, but it does slow down EMT progression which towards a more healthy kidney situation.

Conclusion: The different events modulated by S_s-DS-ONJ trigger toward a reduction in deleterious processes associated to DN: inflammation, inflammasome complex activation and EMT progression.

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Disclosure: **A.I. Arroba:** None.

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Intermittent fasting has short-term effects on albuminuria, AGE formation and acylcarnitines in patients with type 2 diabetes

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Background and aims: This study aims to assess whether intermittent fasting (IF) can induce substantial changes in endogenous defense mechanisms in patients with type 2 diabetes (T2D) and diabetic nephropathy (DN).

Materials and methods: Patients with T2D (n=40) (HbA1c 7.8 ± 0.2 % [62.1 ± 2.3 mmol/mol], diabetes duration of 14.2 ± 1.4 years) and increased albumin-to-creatinine ratio (ACR) are randomized 1:1 to receive for 5 consecutive days once a month either a fasting-mimicking diet (intervention) or a Mediterranean diet (control) for a total of 6 diet cycles. After each diet cycle, blood-/urine- samples are collected and patients return to their normal diet till the next cycle. Primary endpoint is the difference of the change in ACR from baseline to after 3 and after 6 diet cycles. Patients are followed-up 3 months after the end of the study. Secondary endpoints are the difference of the change in MG-H1 (a dicarbonyl-derived AGE) and acylcarnitines (AC) as products of fatty acid oxidation. Statistical analysis is done by ANCOVA adjusted for respective baseline values, age, sex, and BMI.

Results: Interim analysis after 3 diet cycles shows in the intervention group a decrease of ACR in patients with microalbuminuria levels at baseline (absolute change in ACR -32.2 mg/g [95% CI -61.4, -2.9], n=18) compared to the control group (absolute change in ACR -3.1 mg/g [95% CI -26.4, 19.9], n=17) ($P < 0.05$) and a decrease of MG-H1 by 49.8% (95% CI -68.6%, -30.6%) corrected to respective baseline values and to the control group ($P < 0.01$). No change is observed in the glyoxalase detoxification system as well as in yH2Ax as an indirect marker of DNA-damage response. AC analysis shows a distinct response to fasting, with a significant increase of acetylcarnitine (end-product of fatty acid oxidation) in patients of the intervention group that respond with better ACR improvement. Interestingly, the above-mentioned changes are independent of bodyweight and ketone bodies concentration and are reversible already one week after refeeding.

Conclusion: Intermittent fasting temporarily changes ACR, MG-H1 and AC levels in T2D patients with DN. The short-lasting effects in the absence of changes in glyoxalase system and in DNA-damage response suggest that the changes observed are due more to fasting-induced altered energy consumption rather than significant changes in endogenous defense mechanisms.

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Sodium-glucose cotransporter 2 inhibitors as adjunct therapy for type 1 diabetes and the benefit on cardiovascular and renal disease evaluated by Steno risk engines

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Background and aims: Sodium-glucose cotransporter 2 inhibitors (SGLT2i) have shown effects in T1D by lowering of blood glucose, without increased risk of hypoglycemia, reduction in body weight and increased time in range. The major limitation for universal use of SGLT2i in T1D is an increase in the risk of diabetic ketoacidosis (DKA). SGLT2i have attractive beneficial cardiovascular and renal effects in persons with type 2 diabetes but there are currently no studies to show whether these effects can also be demonstrated in people with T1D. Effects that might outweigh the risk of DKA when considering SGLT2i for persons with T1D. The aim of this study is to estimate the risk of cardiovascular disease (CVD) and end-stage kidney disease (ESKD) in persons with T1D with and without treatment with SGLT2i.

Materials and methods: The study is based on 5,506 adults with T1D treated at the outpatient clinic at Steno Diabetes Center Copenhagen in the period 2001-2016. A total of 3,660 persons fulfilled the inclusion criteria of age 30-75 years and an eGFR >45 ml/min/1.73 m^2. For each patient, the 5-year cumulative risks of developing ESKD and CVD were estimated using the Steno Type 1 Risk Engines. For CVD risk estimation, only the subset of 3,284 (89.7%) without previous CVD at baseline was included, and the 10-year cumulative risk of developing CVD was also estimated. We compared the estimated risk with and without the SGLT2i-induced change in the risk variables. The effect of SGLT2i was simulated by changing the recorded HbA1c and systolic blood pressure values in accordance with results from the DEPICT studies. More specifically, individual absolute change in HbA1c and systolic blood pressure was simulated as randomly drawn numbers from a normal distribution with mean (standard deviation (SD)) of -3.6 (0.9) mmol/mmol and -1.12 (2.8) mmHg. The recorded eGFR and albuminuria were changed in accordance with results from the Tandem studies, sotagliflozin 200mg; no change in eGFR and percentage change in albuminuria with mean (SD) -23.7 (12.9).

Results: The SGLT2i induced change in the risk variables translated into an overall 5-year CVD relative risk reduction of 6.1% (95% confidence interval (95%CI) 5.9,6.3), with up to 11.1% (10.0,12.2) in the subgroup with albuminuria. Similar results were seen for the 10-year risk of CVD. For the estimated 5-year risk of ESKD, we found an overall relative risk reduction of 5.3% (5.1,5.4) with up to 7.6% (6.9,8.4) in the subgroup with albuminuria.

Conclusion: Using the Steno T1 CVD and renal risk calculator we estimated the risk of CVD and ESKD in persons with T1D with and without treatment with SGLT2i and found a substantial CVD and ESKD risk reduction, especially in the subgroup with albuminuria. Our model provides an estimate of benefit that may balance the risks associated with use of SGLT2 inhibition in T1D.

Disclosure: **E. Buur Stougaard:** None.

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Liraglutide plus SGLT2 inhibitors might be synergistically beneficial to reduce progression to end stage renal disease in rapidly progressive diabetes kidney disease

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Background and aims: Liraglutide (Lira) has been shown to reduce the development to macroalbuminuria, but not to reduce progression to end stage renal disease (ESRD), and for GLP-1 receptor agonist (GLP-1RA) and SGLT2 inhibitor (SGLT2i) combination therapy to be more effective to prevent ESRD. We evaluated add-on effects of Lira in patients with type 2 diabetes (T2D) treated by SGLT2i by assessing annual eGFR decline rate.

Materials and methods: A total of 75 patients with T2D with CKD stage 3-4, treated by SGLT2i (empagliflozin 65%) were analyzed. The duration of diabetes at the time of recruitment were as follows: stage 3a (n=29); 11.9±5.4 yrs, stage 3b (n=28); 19.9±12.1 yrs, and stage 4 (n=18); 13.8±8.5 yrs. Macroalbuminuria existed in 49% (37/75), and nephrosis in 12% (9/75). Average age was 70.3 ±12.2 yrs old. Lira was initially or additionally used in 41%, 64% and 44% respectively. Since eGFR declining rate and timing differ in each patient, most rapid eGFR slope was calculated individually from the data for 46±20 months before using SGLT2i and 33±10 months after adding it. Add-on effects of Lira were compared.

Results: 1) In total, individualized most rapid eGFR slope was improved from -6.4±9.1 to -1.5±3.2ml/min/1.73m^2/year (P<0.001) by adding SGLT2i. (n=75) 2) Proportion of responders (defined as their ratios of slopes are reduced > 1.0 after addition of SGLT2i) in each stage were as follows: stage 3a; (22/29), stage 3b; (23/28), stage 4; (16/18). Slopes in responders (61/75) were improved as follows, -7.4±9.7 to -0.9±2.9ml/min/1.73m^2/year (P<0.0001), intensified by Lira in 51%. Slopes in non-responders (14/75) were worsened as follows, -1.9±2.1 to -4.1±3.5ml/min/1.73m^2/year (P<0.001), despite intensification by Lira in 50%. Slopes in rapid decliners (defined as most rapid individual eGFR slope >-10.0ml/min/1.73m^2/y) (13/75) were remarkably improved from -18.9±16.4 to -1.4±3.5ml/min/1.73m^2/year (P<0.01), baseline using Lira in 23%, then intensified by using Lira in 85% (11/13), who highly had macro-albuminuria in 69% (9/13) and nephrosis in 54% (7/13). 3) Sub-analysis: Based on different Lira initiation regimes as follows. i) Baseline on Lira initiated -5.2±5.3 improved to -2.0 ±1.2 ml/min/1.73m^2/year (P<0.05) with SGLT2i added. (n=11) ii) Slopes -5.0±3.5 improved to -1.2±4.3ml/min/1.73m^2/year (P<0.01) with simultaneous intensification by SGLT2i and Lira. (n=13) iii) Slopes -14.6±18.0 initially improved to -5.4±5.5 (P<0.05) by adding Lira, then improved to -2.3±3.4 ml/min/1.73m^2/year (P<0.05) with SGLT2i added. (n=14) In this group, macroalbuminuria existed in 71% (10/14), and nephrosis in 36% (5/14), but nephrosis all achieved remission. One female person aged at 30s showed remarkable improvement. Initial slope declined rapidly at rate of -39.0ml/min/1.73m^2/year in 19 months. After adding Lira 0.9mg, eGFR decline rate reduced to -3.8 ml/min/1.73m^2/year for next 12 months. Then adding SGLT2i, slope was +0.68 ml/min/1.73m^2/year for next 38 months. In this person, nephrosis (urinary protein 7.7g/day, 10.4g/gCr) existed prior to adding Lira. But nephrosis came into remission by Lira (urinary protein ~3.0g/gCr), then further improved by SGLT2i (urinary protein ~0.6g/gCr).

Conclusion: Lira plus SGLT2i might be synergistically beneficial to reduce progression to ESRD in rapidly progressive DKD.

Clinical Trial Registration Number: 2-K014

Disclosure: **K. Kashima:** None.

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Study of non diabetic kidney disease in type 2 diabetic patients with renal involvement

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Background and aims: Diabetes mellitus is the most common cause of chronic kidney disease (CKD) worldwide. 20-40% patients with diabetes develop renal disease of which Diabetic Nephropathy (DN) is the most common. The prevalence of non diabetic kidney disease (NDKD) among patients with type 2 diabetes mellitus varies widely depending on the selection criteria and the populations being studied. The main objective of the study was to evaluate the renal biopsies performed on type 2 diabetic patients for suspicion of NDKD and to correlate the pathological findings with the clinical and laboratory findings.

Materials and methods: All type 2 diabetes mellitus patients aged more than 18 years were included in this study who had biopsy done for following reasons; Unexplained rapid deterioration of renal function (decrease in glomerular filtration rate (GFR) more than 1 ml/min/1.73 m2/month); proteinuria not accompanied by retinopathy; unexplained haematuria (3 or more red blood cells per high-power field in centrifuged urine sample). Basic clinical details; blood; urine investigation and USG abdomen was done. Renal biopsy was analyzed by light microscopy and immunofluorescence. Optic fundae examination was done in the Dept of Ophthalmology. Based on biopsy patients were grouped into three (i) Isolated NDKD (ii) NDKD with; underlying DN and (iii) Isolated DN.

Results: A total of 31 patients underwent kidney biopsy and were enrolled in this study. Recent onset nephrotic syndrome (13) was the most common indication of biopsy followed by active urine sediment (8). 45% patients on renal biopsy had isolated DN; while as NDKD was seen in 32% and DN plus NDKD in 26%. Focal segmental glomerulosclerosis (FSGS) and IgA nephropathy (IgAN) were the most common causes of isolated NDKD; while as chronic tubulointerstitial nephritis was common in NDKD plus DN. Female gender; short duration of diabetes (<5 yrs) and active urine sediment were independent predictors of NDKD according to multiple logistic regression analysis.

Conclusion: NDKD was seen in 55% of patients with atypical presentation. FSGS and IgAN were common in NDKD diseases. Judicious use of biopsy atypical presentation may help in diagnosis of NDKD especially in females with short duration of diabetes.

Disclosure: **P. Hans:** None.

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Age-rage and markers of bone metabolism in patients with diabetes type 1 after successful pancreas-kidney transplantation and kidney transplantation alone

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Background and aims: To compare advanced glycation end-products axis (AGE, RAGE) and 3-nitrothyrosin (3-NT) as a marker of oxidative stress in patients with DM1 after successful pancreas-kidney transplantation (PKT) with normoglycaemia and in patients with DM 1 after kidney transplantation alone (KTA). To evaluate correlation of AGE, RAGE and 3-NT with renal transplant (RT) function, glycaemic control and markers of bone metabolism.

Materials and methods: Our study included 58 patients after kidney transplantation due to ESRD, 36 of them had PKT. We evaluated glycaemic control, RT function and diabetes and CKD complications status before and after transplantation. We performed analysis for AGE, RAGE serum level, oxidative stress was evaluated using 3-NT level. As markers of bone metabolism we investigated parathyroid hormone (PTH), 25(OH)vitamin D, calcium, phosphorus, FGF23, osteoprotegerin (OPG) and fetuin A levels.

Results: All patients after PKT reached normoglycaemia (glycosylated hemoglobin (HbA1c) 5,7 [5,3; 6,1] %, C-peptide 3,24 [2,29;4,40] ng/ml), glucagone 5,23 [4,11; 8,20] pmol/l with significant difference vs patients after KTA. Arterial hypertension (AG) frequency was lower in patients with PKT after the surgery vs before transplantation (p=0,008), and comparing with patients after KTA. Patients after PKT and KTA demonstrated statistically significant difference in antihypertensive and lipid-lowering therapy (p=0,001 and p<0,001, respectively). In markers of metabolic memory statistically significant differences were shown for AGE level (p=0,0003) and RAGE level (p=0,000003). In markers of vascular calcification only OPG was significantly higher in PKT (p=0,04). In correlation analysis we revealed statistically significant correlation of 3-NT and OPG (r =0,30, p = 0,04). Also we found significant correlations (p<0,05) of RAGE level with RT function (eGFR r = -0,52), HbA1c (r = 0,48), AG duration (r = 0,34) and AGE with HbA1c (r =0,51).

Conclusion: Results of analysis of metabolic memory markers could point to their direct influence on metabolic disturbances of CKD and DM1 persistence after reaching of stable euglycaemia and kidney function restoration after PKT, and additionally their contribution to recurrent nephropathy, vascular calcification and bone disorders.

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Disclosure: **A. Severina:** None.

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The risk of hip fracture according to the diabetic kidney disease status in the Korean population

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Background and aims: Type 2 diabetes and chronic kidney disease are risk factors for hip fracture. Recently, albuminuria without reduced estimated glomerular filtration rate (eGFR) was also reported to increase the risk of incident hip fracture. In this study, we evaluated the risk of hip fracture among Korean adults with type 2 diabetes stratified by albuminuria and eGFR levels.

Materials and methods: Using the Korean National Health Insurance Service database, we included 1,545,300 patients with type 2 diabetes who underwent regular health check-ups from 2009 to 2012. The patients were classified into four groups (no-diabetic kidney disease (DKD) (A-G-), albuminuric DKD without reduced eGFR (A+G-), nonalbuminuric DKD with reduced eGFR (A-G+), and albuminuric DKD with reduced eGFR (A+G+)) and followed up until 2018. The hip fracture was defined using ICD-10 codes.

Results: The prevalence of A-G-, A+G-, A-G+, or A+G phenotype was 81.7%, 5.1%, 11.3%, and 1.8%, respectively. During the median follow-up of 6.3 years, 21,536 participants suffered a hip fracture. The hazard ratios for hip fracture were 1.45 (95% confidence interval [CI] 1.37-1.53), 1.26 (95% CI 1.22-1.30), and 2.08 (95% CI 1.94-2.22) among participants with A+G-, A-G+, and A+G+ group relative to those in A-G- group, respectively. The effects of DKD on hip fracture were exaggerated in those aged <65, with BMI <25, or with dyslipidemia.

Conclusion: Having DKD significantly increases the risk of hip fracture among type 2 diabetic patients even without a decrease in renal function.

	A-G- (n = 1,263,272)	A+G- (n = 79,113)	A-G+ (n = 174,789)	A+G+ (n = 28,126)
Hip fracture (n)	14,878	1,240	4,527	891
Hip fracture incidence rate (per 1,000 person-y)	1.76	2.43	4.13	5.45
Model 1[a] HR (95% CI)	1 (reference)	1.39 (1.31, 1.47)	2.36 (2.28, 2.44)	3.16 (2.95, 3.38)
Model 2[b] HR (95% CI)	1 (reference)	1.45 (1.37, 1.53)	1.24 (1.20, 1.29)	2.05 (1.92, 2.20)
Model 3[c] HR (95% CI)	1 (reference)	1.43 (1.35, 1.52)	1.24 (1.20, 1.28)	2.04 (1.91, 2.19)
Model 4[d] HR (95% CI)	1 (reference)	1.45 (1.37, 1.53)	1.26 (1.22, 1.30)	2.08 (1.94, 2.22)

A-G-, no-DKD; A+G-, albuminuric DKD without reduced eGFR; A-G+, nonalbuminuric DKD with reduced eGFR; A+G+, albuminuric DKD

[a]Model 1: unadjusted.

[b]Model 2: adjusted for age and sex.

[c]Model 3: adjusted for age, sex, smoking, alcohol, exercise, income

[d]Model 4: adjusted for age, sex, smoking, alcohol, exercise, income, hypertension, dyslipidemia, BMI

Disclosure: **S. Lee:** None.

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Reduced levels of the anti-ageing hormone Klotho are associated with increased aortic stiffness in people with type 2 diabetes and kidney disease

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Background and aims: Aortic pulse wave velocity (Ao-PWV), an index of aortic stiffness, predicts cardiovascular disease and renal dysfunction in type 2 diabetes (T2DM). Osteochondrogenic differentiation of human aortic smooth muscle cells (HASMCs) induced by angiotensin II (AngII) constitutes a key event driving calcification of HASMCs and resultant aortic stiffening. Soluble Klotho (sKlotho) is a circulating anti-ageing hormone that has direct cardio-renal protective effects in animal studies. sKlotho is also an endogenous inhibitor of vascular calcification. The relationship and possible associations between sKlotho and Ao-PWV in diabetic kidney disease (DKD) has not been studied.

Materials and methods: Single centre cross-sectional cohort study of 141 T2DM patients (63% male) with a clinical diagnosis of DKD. All patients had eGFR >45 ml/min and were on treatment with renin-angiotensin-aldosterone system (RAAS) inhibitors.Circulating Klotho (sKlotho) levels were measured using a validated immunoassay and Ao-PWV was determined by applanation tonometry (Sphygmocor system). Intracellular calcium ($[Ca^{2+}]_i$) was monitored in HASMC *in vitro* using the ratiometric fluorescent indicator Fura-2 AM (2μM). HASMC were pre-treated with recombinant human sKlotho (1 nM, 24h) before stimulation with AngII (100 nM) and continuous monitoring of $[Ca^{2+}]_i$ transients.Protein expression of markers of osteochondrogenic trans-differentiation; α-actin, Collagen alpha-2(I) chain protein (Col1a2) and Matrix metalloproteinase-1 (MMP-1) was also assessed by western blotting in HASMC pre-treated with sKlotho (1 nM, 24h) and exposed to AngII (200 nM) for 72h in the continuous presence of sKlotho.

Results: The mean age (±SD) of our cohort was 62.1 (±8.9) with an estimated GFR (using CKD-EPI equation) of 76.6 (±23.2) ml/min Median ((interquartile range) circulating Klotho levels and Ao-PWV were 280.6 (177.9-606.2) pg/μl and 11.8(10.2-13.5) m/s respectively. Patients with an Ao-PWV above the median as compared to those below the median had: a higher systolic blood pressure (mean ±SD) 158.7±15.3 vs 151.5±14.8 mmHg, a higher pulse pressure ((79.1±14.1 vs 70.8±11.7 mmHg) and lower Klotho levels median (interquartile range) [246.9 (159.5-530.3) vs 334.1 (189.1-615.9) pg/μl), ($p<0.05$ for all). A 10% increase in sKlotho reduced the odds of a patient having a raised Ao-PWV above the median by 8.4% in a multivariable logistic regression analysis: Odds Ratio (95% confidence intervals) 0.4 (0.19-0.83), p=0.014. *In vitro*, HASMC pre-treatment with sKlotho significantly attenuated the AngII-stimulated $[Ca^{2+}]_i$ transients (Δpeak and area under the curve) versus control pre-treatment ($p<0.05$, 2-way ANOVA, n=4). HASMC pre-treatment with sKlotho also reduced the protein levels of Col1a2 following prolonged exposure to AngII (72h) ($p<0.05$, student's *t*-test, n=3), with a similar but not significant trend achieved for MMP-1 while protein levels of a-actin remained unchanged.

Conclusion: There is an inverse association between Ao-PWV and circulating Klotho levels at the early stages of DKD. sKlotho exerts a beneficial effect on HASMCs by reducing mediators of vascular calcification and this could potentially improve arterial distensibility. Treatment strategies that increase sKlotho may attenuate aortic stiffness in DKD and protect against progression of cardio-renal disease.

Disclosure: **N. Fountoulakis:** None.

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Type 2 diabetes and congestive heart failure are mutually independent predictors of the presence of albuminuria

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Background and aims: Albuminuria is a well-known characteristic of diabetic nephropathy and it is also present in a large portion of patients with congestive heart failure (CHF). However, the single and joint effects of type 2 diabetes mellitus (T2DM) and CHF on albuminuria are unknown. This issue therefore was addressed in the present study.

Materials and methods: We investigated 180 patients with CHF, of whom 83 had T2DM (CHF+/T2DM+) and 97 did not have diabetes (CHF+/T2DM-) and 223 controls without CHF, of whom 39 had T2DM (CHF-/T2DM+) and 184 did not have diabetes (CHF-/T2DM-).

Results: The prevalence of albuminuria was lowest in CHF-/T2DM- subjects (8.7%). When compared to this group it was significantly higher in CHF-/T2DM+ (23.1%, p=0.010), CHF+/T2DM- (38.1%, $p<0.001$) and CHF+/T2DM+ patients (62.7%, $p<0.001$). It was highest in CHF+/T2DM+ patients, in whom it was higher than in CHF-/T2DM+ ($p<0.001$) and in CHF+/T2DM- (p=0.001) patients; a trend towards a higher prevalence of albuminuria in CHF-/T2DM+ patients vs. CHF+/T2DM- patients did not reach statistical significance (p=0.093). In logistic regression analysis, CHF and T2DM were mutually independent predictors of albuminuria, when adjusted for age, sex, body mass index, LDL cholesterol, history of smoking and hypertension, as well as for the use of statins and ACE inhibitors/angiotensin II receptor blockers (OR 2.57 [95% CI 1.47-4.51]; p=0.001 and OR 4.15 [2.18 - 7.88]; $p<0.001$, respectively).

Conclusion: We conclude that T2DM and CHF are mutually independent predictors of albuminuria.

Disclosure: **M. Maechler:** None.

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Long term complications in type 1 diabetes are associated with an altered inflammatory state: a proteomics approach

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Background and aims: Type 1 diabetes can lead to the development of vascular complications. Vascular complications are known to be partly driven by an altered inflammatory state. In this study, we aimed to

identify a link between disease characteristics and inflammatory state characterised by a proteomic approach in patients with type 1 diabetes.

Materials and methods: 243 type 1 diabetes patients (M:F ratio 1.17, age 51.7± 16.3 years, duration of diabetes 28.4± 15.7 years, BMI 25.8 ±4.4, mean HbA1c 66±13 mmol/mol) were recruited in this cross-sectional study. Blood samples were drawn and the levels of circulating inflammatory proteins in the serum were measured using 96-plex inflammatory panel proximity extension assay (Olink Proteomics, Uppsala, Sweden). Statistical analyses were performed using R programming language (version 4.0.3).

Results: Diabetes duration was positively correlated with circulating inflammatory proteins in our cohort. 27/96 proteins (e.g. CCL3, CCL11, and CCL19) were positively correlated with duration of diabetes. Pathway enrichment analysis showed strong correlations between the proteins and immune cell migration (adjusted p-value $1.75x10^{-11}$) and a number of signaling pathways such as ERK1 and ERK2 cascade (adjusted p-value $3.89x10^{-10}$). Glycemic control (HbA1c) positively affected the levels of 4/96 proteins in the panel (i.e. CCL28, CDCP1, HGF, and OPG). Little to no association was found between circulating inflammatory proteins and other characteristics, including insulin need and BMI.We also found strong correlations between diabetic complications and elevated inflammatory markers, independent of diabetes duration and glycemic control. Nephropathy was positively associated with 16/96 proteins such as TNF, VEGFA, and FGF21. Nephropathy was shown to be particularly enriched in proteins related to positive chemotaxis (adjusted p-value $3.60x10^{-4}$) and Ras signaling (adjusted p-value 0.019). Stroke was associated with 11/96 proteins, of which 9 of them overlapped with the ones associated with nephropathy (e.g. CD5, CD40, TGFa, and PD-L1). Other diabetic complications, i.e. peripheral neuropathy, retinopathy, PAD, and coronary AD, did not seem to associate with elevated inflammatory proteome levels.

Conclusion: Glucose control (HbA1c) and diabetes duration seem to drive an enhanced inflammatory state in T1D patients. Moreover, diabetic complications are independently associated with increased circulating levels of several inflammatory proteins. Future studies are needed to determine the mechanisms of action underlying the association between the identified pathways, such as Ras cascade and chemokine signaling and diabetic complications.

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Disclosure: **M. Ajie:** None.

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Inside CKD: modelling the future global burden of chronic kidney disease in patients with type 2 diabetes

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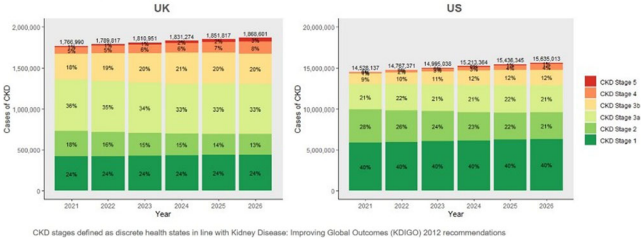
Background and aims: Type 2 diabetes (T2D) is a leading cause of chronic kidney disease (CKD) worldwide. Future trajectories of CKD prevalence, progression and outcomes in patients with T2D are key considerations for policy planning. Using country-specific patient-level microsimulation, *Inside CKD* models the global clinical and economic burden of CKD from 2021 to 2026.

Materials and methods: We used the *Inside CKD* microsimulation to estimate the clinical burden of CKD in patients with concomitant T2D worldwide. We constructed virtual populations using published country-specific data, including demographics and prevalence of concomitant CKD (by stage, according to KDIGO guidelines) and T2D from multiple sources.

Results: Preliminary data from three Western countries demonstrate that from 2021 to 2026 the number of patients with T2D and CKD is expected to rise by 6% in the UK, 8% in the US (Figure), and 16% in Canada. The largest increase is expected in the 35 to 64-years age group in the UK (18%) and in the ≥ 65-years age group in the US and Canada (12% and 21%, respectively).

Conclusion: This patient-level microsimulation models a large linear increase in the number of patients with T2D and CKD over 5 years, including consistent increases in the number of patients with T2D and CKD stages 3b to 5. Reliable epidemiological models can help inform country-specific healthcare policy and intervention strategies.

Figure: Projected growth in annual disease burden of CKD in patients with T2D from 2021 to 2026



CKD stages defined as discrete health states in line with Kidney Disease: Improving Global Outcomes (KDIGO) 2012 recommendations

Disclosure: **N. Tangri:** Employment/Consultancy; Dr Tangri received consulting fees from AstraZeneca to provide advice on this work. Grants; Dr Tangri reports grants from AstraZeneca and Janssen. Honorarium; Dr Tangri reports personal fees from Roche, AstraZeneca, Otsuka, Janssen, Boehringer Ingelheim/Eli Lilly and Triceda. Other; Dr Tangri has received fees from ClinPredict, Tricida, Pulse Data and Mesentech.

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Inside CKD: modelling the direct economic burden of concomitant chronic kidney disease and type 2 diabetes

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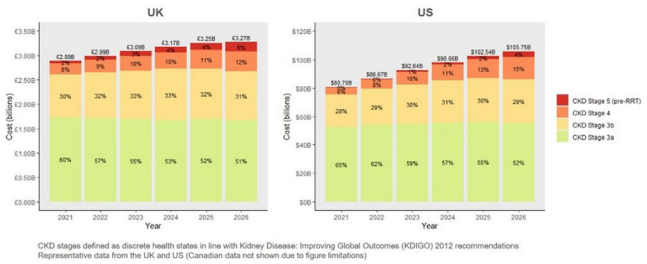
Background and aims: *Inside CKD* aims to model the future global clinical and economic burden of chronic kidney disease (CKD). Type 2 diabetes (T2D) is a leading cause of CKD, and concomitant CKD and T2D place a significant burden on healthcare systems worldwide.

Materials and methods: *Inside CKD* is an international study across the Americas, Asia-Pacific and European regions. We used the *Inside CKD* country-specific, patient-level microsimulation to model healthcare costs for patients with concomitant CKD and T2D from 2021 to 2026. We constructed virtual populations using country-specific data including demographics, prevalence of CKD (by stage), T2D and complications, as well as direct costs, from multiple published sources (e.g. NHANES for US and HSE for UK).

Results: Preliminary results from three countries show that annual healthcare costs for patients with both CKD and T2D are expected to increase from 2021 to 2026 (UK, from £2.89B to £3.27B; US, from $80.70B to $105.75B; [**Figure**]; Canada, from C$7.68B to C$12.81B). Costs attributable to CKD stage 4 are expected to increase the most (UK, from 8% to 12% of overall costs; US, from 6 to 15%; Canada, from 10 to 15%).

Conclusion: Healthcare costs for patients with CKD and T2D are projected to increase in the UK, US and Canada from 2021 to 2026. Later CKD stages are associated with more pronounced cost increases, likely due to both increased prevalence and greater treatment complexity. Early diagnosis and interventions to slow CKD progression are needed to reduce the economic burden of concomitant stage 3 to 5 CKD and T2D.

Figure: Projected annual healthcare costs for patients with CKD and T2D from 2021 to 2026



CKD stages defined as discrete health states in line with Kidney Disease: Improving Global Outcomes (KDIGO) 2012 recommendations
Representative data from the UK and US (Canadian data not shown due to figure limitations)

Disclosure: **A. Power:** Employment/Consultancy; Dr Power received consulting fees from AstraZeneca to provide advice on this work. Honorarium; Dr Power has received honorarium from AstraZeneca, Vifor Fresenius Renal Pharma, Napp Pharmaceuticals, Bayer and Alexion. Lecture/other fees; Dr Power has received speaker and advisory board fees from AstraZeneca, Vifor Fresenius Renal Pharma, Napp Pharmaceutics, Bayer and Alexion.

SO 52 Predictors of diabetic kidney disease

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Central obesity is associated with the onset of albuminuria in type 1 diabetes

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Background and aims: As type 1 diabetes (T1D) usually manifests early in life, the affected individuals are at high life-time risk of diabetic kidney disease (DKD). Obesity defined by BMI is a causal, non-linear risk factor for DKD where both a low and high BMI increases the risk. However, it is not known how central obesity relates to DKD. Thus, we investigated how central obesity defined as waist-height ratio (WHtR) associates with new-onset albuminuria.

Materials and methods: This longitudinal study, part of the FinnDiane Study, included 2,260 individuals with type 1 diabetes. Albuminuria was defined as AER > 30 mg/24h in two out of three measurements. Individuals were followed for 10.7 (± 5.2) years until onset of albuminuria (245 events) or last known date without albuminuria (24,286 person-years). Individuals with albuminuria or on renal replacement therapy at baseline and those with an albuminuria onset in less than 6 months were excluded. Baseline BMI and WHtR (scaled by 10) were each tested separately in multiple Cox regression models; a minimal model adjusted for sex, age of diabetes onset and duration of diabetes, a clinical model additionally adjusted for smoking, triglycerides, total cholesterol, systolic BP, HbA_{1c} and eGFR, and a medication model additionally adjusted for baseline use of inhibitors of the renin-angiotensin-aldosterone system (RAAS). Linearity of association was tested and in its absence the variable was cut into quintiles and the second lowest quintile was used as a reference group.

Results: Individuals who developed albuminuria were more likely to be men, had a higher HbA_{1c}, systolic BP and were more likely prescribed a RAAS inhibitor at baseline. However, there was no difference in duration of diabetes, age of diabetes onset and eGFR. Importantly, individuals with new-onset albuminuria had higher WHtR at baseline (0.50 vs. 0.49, p = 0.004) but no difference in BMI (25.3 kg/m^2 vs. 24.8 kg/m^2, p = 0.08). In the minimally adjusted model, WHtR was associated with new-onset albuminuria (HR = 1.49 [1.20, 1.84], p = 0.0002). The nature of this association was linear (p = 0.25 for non-linearity). The association with BMI was non-linear and we only found an association in the highest quintile (>27.3 kg/m^2) (HR = 2.01 [1.35, 2.98], p = 0.0006). Even when adjusted for clinical risk factors, the WHtR was still associated with new-onset albuminuria with a HR of 1.32 ([1.04, 1.67], p = 0.02). The association remained significant when adjusting for RAAS inhibitors (HR = 1.28 [1.01, 1.63], p = 0.04).

Conclusion: Central obesity is a significant marker of new-onset albuminuria in individuals with type 1 diabetes on top of established, clinical risk factors and RAAS inhibition. The risk increases linearly with the WHtR. Therefore, the WHtR shows potential to identify individuals at risk of albuminuria.

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Circulating sphingomyelin: A causal risk-factor of chronic kidney disease?

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Background and aims: Abnormal levels of circulating sphingomyelin (SM) have been shown to associate with albuminuria and kidney function in type 1 diabetes. However, the sign of association has varied from cohort to another, suggesting potential influence of confounding factors. In one of these efforts, we previously demonstrated in a cohort at Steno Diabetes Center Copenhagen that three individual SM species measured at baseline were inversely associated with albuminuria at baseline as well as with all-cause mortality during a four-year follow-up in. In this study, we attempt to validate this finding in external cohorts and to probe for causal links between SM levels and chronic kidney disease (CKD).

Materials and methods: We computed an estimate for the causal effect of circulating SM level on the risk of CKD via bidirectional two-stage Mendelian randomization (MR) using the MR-Egger method on genome-wide association studies acquired from the MR Base. First, we acquired the genetic instruments to SM from a multinational European pan-cohort of 13,307 individuals. Second, we acquired the association between the genetic instruments and chronic kidney disease from 117,465 individuals from 48 European cohorts.

Results: The MR estimate for the causal effect of SM on CKD was -1.217 (SE=0.3483; p=0.07303). Alternative methods yielded weaker effect estimates but were consistent with the sign of the effect. In the reverse analysis, the effect of CKD on SM was -0.03059 (SE=0.1651; p=0.8701).

Conclusion: We found a statistically weak causal effect, where a decrease of one standard deviation in the level of circulating SM means a 70 % higher risk of CKD in the general population. Although not statistically significant in the general population, the causal effect was in clear agreement with our previous findings from a more focused longitudinal cohort of 700 persons type 1 diabetes. Furthermore, we confirm that abnormal SM levels are more likely to cause CKD rather than the reverse. Based on these combined results, we suggest that the sphingolipid pathway could serve as an effective preventive target of kidney disease in high-risk groups, such as persons with long-standing type 1 diabetes.

Disclosure: **T. Suvitaival:** None.

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Heparin cofactor II prevents the development of albuminuria in patients with diabetes

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Background and aims: Diabetic nephropathy is an important microvascular complication of diabetes. Protease-activated receptors (PARs) are activated by endogenous serine proteases such as thrombin and it has been reported that activation of PARs promotes the development of glomerular diseases via enhancement of inflammation. Heparin cofactor II (HCII), which is a serine protease inhibitor (serpin), specifically inhibits thrombin action by binding to dermatan sulfate proteoglycans in various tissue matrices. We have shown that HCII prevents the development of cardiovascular diseases and ameliorates insulin resistance via attenuating thrombin-mediated PAR activation. Taken together, we hypothesized that low plasma HCII activity is associated with the development of diabetic nephropathy.

Materials and methods: Plasma HCII activity was measured on the basis of antithrombin activity in the presence of dermatan sulfate with the use of the Stachrom® HCII assay kit (Diagnostica Stago). Spot urine albumin and liver-type fatty acid-binding protein (L-FABP) expressed as a ratio to the level of urinary creatinine (uACR, uL-FABPCR) were determined in 310 Japanese patients (176 males and 134 females) with diabetes mellitus. The degree of association between urinary biomarkers (uACR and uL-FABPCR) and each variable including sex, age, BMI, SBP, serum lipid parameters, UA, creatinine, HbA1c, plasma HCII activities, history of current smoking, hypertension, and dyslipidemia were determined by means of multiple regression analyses. A cross-sectional analysis was performed at baseline to determine whether plasma HCII activity was associated with uACR, log-transformed uACR and uL-FABPCR (n=310). In addition, a longitudinal analysis was performed to determine whether plasma HCII activity was associated with the annual change of uACR (uACR: [uACR (log-transformed uACR) at 1 year after baseline data collection - uACR (log-transformed uACR) at baseline] (n=201)). Our study followed the institutional guidelines of each hospital and was approved by each hospital's Institutional Review Board. Prior informed consent was obtained from all patients according to the Declaration of Helsinki.

Results: Mean plasma HCII activity in all participants was 93.8 ± 17.7%. The levels of plasma HCII activity declined with age. The level of uACR was significantly and positively correlated with uL-FABPCR (R^2=0.280, p< 0.001). Log-transformed uACR also showed a significant and positive correlation with uL-FABPCR (R^2=0.280, p< 0.001). Multivariate regression analysis including confounding factors showed that plasma HCII activity independently contributed to the suppression of uACR and log-transformed uACR (p=0.035 and p=0.008, respectively) but not uL-FABPCR (p=0.527). In addition, plasma HCII activity was significantly and inversely associated with annual increment of uACR and log-transformed uACR after adjustment of confounding factors (p=0.003 and p=0.032, respectively).

Conclusion: The results indicated that HCII can be a novel protective factor against the development of diabetic nephropathy.

Disclosure: **T. Hara:** None.

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Factors associated with occupational sitting in type 1 diabetes

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Background and aims: Excessive sitting and other sedentary behavior are associated with the incidence of many chronic diseases and mortality, independent of physical activity. Particularly occupational sitting is suggested to be an important risk factor. However, the role of excessive sitting in type 1 diabetes has not been previously studied. Therefore, our aim was to study clinical characteristics and risk factors associated with excessive occupational sitting in type 1 diabetes.

Materials and methods: This cross-sectional study included 1,704 individuals with type 1 diabetes (1289 without albuminuria, 218 with microalbuminuria, 150 with macroalbuminuria, and 47 with renal replacement therapy) as a part of the Finnish Diabetic Nephropathy Study. Out of these individuals, 48.8% were men, mean age was 38.9 ± 10.1, mean duration of diabetes 22.3 ± 11.7 years, median occupational sitting time 4.0 (1.5-6.0) hours, BMI 25.5 ± 3.8 kg/m^2, systolic blood pressure 133 ± 17 mmHg, and HbA_{1c} 8.1 ± 1.3%. Occupational sitting time and leisure-time physical activity was assessed at baseline by a validated self-report questionnaire. Long occupational sitting was categorized as the highest tertile (>5.5 h) versus the two lower tertiles. Participants with unclassified kidney status and participants with a short working day (<6 h) were excluded.

Results: Participants with >5.5 h daily occupational sitting were older (39.6 ± 8.9 vs 35.8 ± 9.4 years, p =0.002), had a longer duration of diabetes (22.3 ± 12.0 vs 18.7 ± 11.0 years, p =0.008), lower HbA_{1c} (7.9 ± 1.0 vs 8.2 ± 1.3% p =0.005), had more often a normal albumin excretion rate (80.6% vs 70.3%, p <0.001), were more often highly educated (59.5% vs 32.3%, p <0.001), were less frequently smokers (14.8% vs 26.4%, p <0.001), and less frequently highly physically active during leisure-time (13.6% vs 20.1%, p <0.001). In a multiple logistic regression analysis, occupational sitting time of >5.5h was associated with the highest educational level [OR 3.26, 95% CI (2.44-4.34), p <0.001]. Other variables that were positively and independently associated included normal albumin excretion rate [OR 1.45, 95 % CI (1.12-1.87), p = 0.005], and age [OR 1.02, 95 % CI (1.01-1.03), p =0.005]. Variables that were negatively and independently associated included smoking [OR 0.56, CI (0.43-0.74), p <0.001] and high leisure-time physical activity [OR 0.57, 95% CI (0.39-0.80), p <0.001].

Conclusion: Higher educational level, normal albumin excretion rate, and age were found to be independently and positively associated with >5.5h occupational sitting time, while current smoking, and high amounts of leisure-time physical activity were negatively associated with >5.5h occupational sitting time. These findings might play an important role to guide future prospective studies exploring excessive sitting as an independent risk factor for diabetic complications and mortality in type 1 diabetes.

Supported by: Finska Läkaresällskapet, Perkléns Stiftelse, Finnish Diabetic Association

Disclosure: **M. Seppälä:** Grants; Finska Läkaresällskapet, Perkléns Stiftelse, Finnish Diabetes Association.

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Association between central obesity and rapid decline of eGFR in type 1 diabetes

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Background and aims: Body mass index (BMI) has been causally related to albuminuria in people with type 1 diabetes (T1D). However, the impact of central obesity on the decline of the estimated glomerular filtration rate (eGFR) in this population is unknown. Therefore, we aimed to evaluate the association between central obesity estimated by the waist-height ratio (WHtR) and the decline of eGFR in adults with T1D.

Materials and methods: From the FinnDiane Study, 3245 adults with T1D were included in this analysis. Individuals with end-stage renal disease (ESRD) at baseline were excluded, otherwise followed until their last known eGFR or ESRD, whatever came first. All eGFR values were derived from the CKD-EPI equation. Baseline eGFR was measured within 6 months from the baseline visit and at least three eGFR measures during at least two years of follow-up were required for eGFR slope calculation. Finally, the eGFR slope was determined by regressing eGFR on time and using the slope of the regression line as the eGFR slope. Individuals were grouped into two categories: rapid eGFR decline (slope ≤ -3 ml/min/1.73m^2/year) and slow decline (> -3 ml/min/1.73m^2/year). Binary logistic regression was used to assess the association between WHtR (scaled by 10) and rapid decline of eGFR (the outcome) in three models: unadjusted, minimally adjusted for sex, age of onset and duration of diabetes, and a final model additionally adjusted for systolic blood pressure, HbA_{1c}, triglycerides, total cholesterol, albuminuria stage, smoking, baseline eGFR and the use of renin-angiotensin-aldosterone system inhibitors. Finally, to evaluate the impact of central obesity on the outcome by BMI categories, a logistic regression analysis was done between WHtR and the outcome by baseline BMI categories (low BMI <18.5kg/m^2, normal BMI ≥18.5 and <25, overweight BMI ≥25 and <30, obese BMI ≥30).

Results: From a total of 3245 individuals, 503 (15.5%) had a rapid eGFR decline during a median follow-up of 12.0 (7.1, 15.0) years. The median eGFR slope of individuals in the rapid decline group was -4.79 ml/min/1.73m^2/year compared to -0.59 ml/min/1.73m^2/year in the slow decline group (p<0.001). At baseline, those who experienced a rapid eGFR decline were more likely to be men, were older, had a longer duration of diabetes, lower eGFR, higher WHtR, despite a similar BMI compared to the slow decline group. The WHtR was associated with rapid eGFR decline in the unadjusted [OR 1.56, 95%CI (1.34,1.81)] and minimally adjusted model [1.47, (1.26, 1.73)], but not after adjusting for all covariates. By BMI categories, after adjusting for all covariates, the WHtR was not associated with the outcome in low and normal BMI groups. However, it was strongly associated with the rapid decline of eGFR in overweight individuals [1.74, (1.08,2.78)] and the same trend was seen in obese individuals [1.51, (0.80, 2.89)].

Conclusion: Central obesity increases the odds of having a rapid eGFR decline in adults with T1D and elevated BMI. Therefore, WHtR may be a useful tool to identify individuals at higher risk of rapid progression of diabetic kidney disease in this population.

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Correlation of urine copper level and microalbuminuria in type 2 diabetic patients

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Background and aims: Chronic complications of glucose metabolism disorders might also be associated with alterations in the levels of some trace elements like copper. t has been also suggested that dysregulation of renal Cu homeostasis may be a key event eliciting development of diabetic nephropathy, and selective Cu(II) chelation can protect against pathogenic mechanisms that lead to diabetic nephropathy and might be clinically useful in the treatment of early-stage diabetic kidney disease. The objective of the study was to evaluates the correlation of urine copper level and microalbuminuria in type 2 diabetic patients.

Materials and methods: In this observational study, Type two diabetic (DMT2) patients who were referred to our diabetes clinic, were enrolled based on the following criteria: history of DMT2 for at least one year, lack of urinary tract infection and lack of heart failure. After obtaining signed

written consent, 24-hours urine samples were obtained from patients for measurement of microalbumin and creatinine. Patients were categorized in case and control group based on the presence or absence of albuminuria. The urine copper of patients was measured by atomic absorption spectrophotometry.

Results: 94 T2DM patients participated in the study, 50 patients with microalbuminuria as case group and 44 patients without microalbuminuria as control group. 68% of the participants in the case and 60% of the control group were female. Mean FBS (mg/dL) in normoalbuminuria and microalbuminuria were 119.56 ± 40.43 and 168.82 ± 40.39 ($P < 0.01$), and mean PPBS (mg/dl) in microalbuminuria were 168.7 ± 49.43 and 241.52 ± 69.64, respectively ($P < 0.001$). The mean HbA1c (%) in normoalbuminuria and microalbuminuria were 6.37 ± 0.74 and 7.87 ± 1.62, respectively ($P < 0.01$). Mean (CI 95%) urinary copper levels were 37.12 (14.51−58.61) and 14.61 μcg /L (10.17−19.37) in the case and control groups, respectively ($P = 0.003$). Also, regarding to HbA1c level, there was not a significant difference of mean urinary copper in patients with HbA1c less and greater than 8%in each group ($P = 0.07$). There was no significant difference of urinary copper level between two sexes. There was no correlation between age and urinary copper.

Conclusion: The present study shows diabetic patients with microalbuminuria have increased urinary copper excretion, however does not exclude the potential toxic effects of this high copper excretion on the progression of diabetic nephropathy.

Disclosure: **S. Pattanaik:** None.

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Adaption of oxidative phosphorylation machinery compensates hepatic lipotoxicity in early stages of fatty liver

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Background and aims: Non-alcoholic fatty liver (NAFL) is one of the most common secondary complications of type 2 diabetes. Early stages of NAFL are associated with increased mitochondrial function which was observed to drop significantly during the progression of the disease. This study aims to identify the yet unknown molecular mechanisms behind changes in mitochondrial function in NAFL pathology.

Materials and methods: Main pathways of glucose and fatty acid metabolism were analyzed in primary hepatocytes from mice with transgene-induced hepatic steatosis and hepatic insulin resistance, namely alb-SREBP-1c mouse model, compared to C57Bl6 control animals. Mitochondrial function was assessed by extracellular flux analysis in primary hepatocyte culture and isolated mitochondria from liver tissue. Mitochondrial proteome was analyzed by mass-spectrometry followed by data-independent analysis.

Results: In alb-SREBP-1c hepatocytes de novo lipogenesis is increased twofold compared to control cells ($p < 0.001$), resulting in mild intrahepatic lipid accumulation in these animals. Further in alb-SREBP-1c hepatocytes, glycolysis rate is more than 3-fold higher ($p = 0.002$) and fatty acid oxidation is reduced to 77% ($p = 0.004$), while fatty acid uptake, as well as gluconeogenesis and glycogen production remain unchanged compared to controls. Mitochondrial function in hepatocytes from alb-SREBP-1c animals is significantly increased already at basal level (1.6-fold, $p < 0.001$), with 2.4-fold higher maximal respiration ($p < 0.001$) and 1.5-fold higher ATP production rate ($p < 0.001$) compared to controls. Mitochondrial copy number remains unchanged and no changes in fuel oxidation preferences are observed compared to control in primary hepatocytes. Specifically, complex II-driven oxidative phosphorylation (OXPHOS) is significantly increased in isolated alb-SREBP-1c mitochondria ($p < 0.001$). Analysis of mitochondrial proteome reveals that composition of mitochondrial OXPHOS complexes I, II and III are significantly changed in alb-SREBP-1c animals, e.g. in complex I compounds of ND1-modul, i.e. Fe-S-protein Ndufs6 ($p < 0.01$), ND2-modul , i.e. Fe-S-protein Ndufs5 ($p < 0.01$) and NADH dehydrogenase subunit Mt-nd2 ($p < 0.01$), in complex II heme cluster protein Sdhd ($p < 0.01$) and in complex III the Fe-S-protein Uqcrfs1 ($p < 0.01$) are upregulated up to 70%.

Conclusion: The model investigated represents the early stage of fatty liver with mild hepatic steatosis and hepatic insulin resistance. In this study we show an increase and adaptation in mitochondrial OXPHOS at early stages of fatty liver to overcome lipid overflow. Mitochondrial OXPHOS is likely fueled by a high glycolysis rate, and the adaption to ectopic lipids specifically occurs by altered composition of respiratory chain complexes I, II, and III, as well as OXPHOS enzymes. In conclusion, these results indicate that hepatic metabolism adapts towards higher mitochondrial efficiency by specific modulation of OXPHOS machinery to rescue increased hepatic lipotoxicity in early stage of fatty liver.

Disclosure: **P. Fahlbusch:** None.

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Iron aggravates hepatic insulin resistance in the absence of inflammation in a novel db/db mouse model with iron overload

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Background and aims: The molecular pathogenesis of late complications associated with type 2 diabetes mellitus (T2DM) is not yet fully understood. While high glucose levels indicated by increased HbA1c only poorly explain disease progression and late complications, a pro-inflammatory status, oxidative stress and reactive metabolites generated by metabolic processes were postulated to be involved. Individuals with metabolic syndrome (MetS) frequently progress to T2DM, whereby 70% of T2DM patients show non-alcoholic fatty liver disease (NAFLD), the hepatic manifestation of MetS, and insulin resistance (IR). Epidemiological studies have shown that T2DM and steatosis are associated with alterations in iron metabolism and hepatic iron accumulation. Excess free iron triggers oxidative stress and a switch towards a macrophage pro-inflammatory status. However, so far it remains unclear if hepatic iron accumulation plays a causative role in the generation of IR and T2DM or whether it is merely a manifestation of altered hepatic metabolism. To address this open question, we have generated and characterized a mouse model of T2DM with IR, steatosis and iron overload.

Materials and methods: $Lepr^{db/db}$ mice hallmarked by T2DM, IR and steatosis were crossed with $Fpn^{wt/C326S}$ mice with systemic iron overload to generate $Lepr^{db/db}$ /$Fpn^{wt/C326S}$ mice. The resulting progeny was characterized for major diabetic and iron-related parameters.

Results: We show that features associated with T2DM in $Lepr^{db/db}$ mice, such as obesity, steatosis or insulin resistance reduce the degree of tissue iron overload in $Fpn^{wt/C326S}$ mice, suggesting an 'iron resistance' phenotype. By contrast, we observe increased serum iron levels that strongly exceed those in the iron-overloaded $Fpn^{wt/C326S}$ mice. Increased hepatic iron levels induce oxidative stress and lipid peroxidation and aggravate insulin resistance, as indicated by diminished IRS1 phosphorylation and AKT activation. Additionally, in the liver we observe gene response patterns indicative of de novo lipogenesis and increased gluconeogenesis as well as elevated free glucose levels. Finally, we show that iron overload in $Lepr^{db/db}$ / $Fpn^{wt/C326S}$ mice enhances microvascular complications observed in retinopathy, suggesting that iron accumulation can enhance diabetic late complications associated with the liver and the eye.

Conclusion: Taken together, our data show that iron causes the worsening of symptoms associated with the metabolic syndrome and T2DM. These findings imply that iron depletion strategies together with antidiabetic drugs may ameliorate insulin resistance and diabetic late complications.

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Disclosure: **S. Altamura:** None.

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Impact of lipotoxicity on mitochondrial DNA modifications in liver and muscle

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Background and aims: Type 2 diabetes and obesity are associated with a disturbed lipid metabolism and reduction of mitochondrial capacity. This can lead to lipid accumulation in non-adipose tissues to a cell-toxic level and result in a metabolic condition called *lipotoxicity*. Depending on the stage of disease, altered metabolite flux also has an impact on mitochondrial function that is directly linked to gene expression and epigenetic alterations. In addition, the content of mitochondria in cells, mitochondrial DNA (mtDNA) sequence variations and modifications can interfere with mitochondrial function. Due to the fact that certain phenotypes results from interactions of genetic and environmental factors, the aim of this study was to investigate the effect of *lipotoxicity* on mitochondrial function and mtDNA in skeletal muscle and liver.

Materials and methods: Transgenic mice (aP2-SREBP-1c) overexpressing SREBP-1c under control of the aP2 promotor in adipose cells exhibit a syndrome resembling congenital and generalized lipodystrophy in humans and serve as a model for ectopic lipid accumulation in non-adipose tissue. 24 weeks old female aP2-SREBP-1c mice and healthy controls on a C57BL/6 background were used to investigate liver and gastrocnemius muscle biopsies. E*x vivo* analysis of mitochondrial parameters, like mitochondrial capacity and function were analyzed by extracellular flux analysis and enzyme assays in isolated mitochondria. Furthermore, mtDNA modifications and variations were determined by pyrosequencing and next generation sequencing (NGS).

Results: In lipodystrophic liver cells from aP2-SREBP-1c mice, mitochondrial capacity and the amount of mtDNA increased up to 91% compared to controls. Contrary to that, decreased mitochondrial capacity and a 55% reduced amount of mtDNA were determined in muscle of lipodystrophic animals compared to controls. We observed tissue specific methylation of CpG sites in the mitochondrial transcriptional and replication control D-loop which were mainly hypomethylated in lipodytrophic mice compared to controls. Interestingly, one CpG dinucleotide was significantly hypomethylated in lipotoxic skeletal muscle compared to controls. Furthermore, deep-sequencing uncovered an insertion variant with > 29% heteroplasmy in liver and > 38% in skeletal muscle mtDNA, in healthy C57BL/6 control mice, which was not present in tissues derived from lipodystrophic mice.

Conclusion: We show that *lipotoxicity* has tissue-specific effects on mitochondrial function and mtDNA content. Mitochondrial activity and mtDNA ratios compared to nuclear DNA (nDNA) were reduced in skeletal muscle and increased in livers of lipodystrophic mice. These changes were associated with an altered tissue-specific pattern of methylation within the mtDNA D-loop, strongly suggesting that *lipotoxicity* may alter methylation dynamics in mitochondria. Interestingly, we identified a heteroplasmic insertion variant located within the replication origin of the mitochondrial light strand DNA (OriL) which may interfere with replication rates. Our results indicate a role of genomic flexibility in mtDNA for preserving metabolic health.

Disclosure: **A. Nikolic:** None.

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Yg1699, a novel dual systemic SGLT1 and SGLT2 inhibitor, decreases blood glucose and improves kidney function in primates with diabetic kidney disease

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Background and aims: Diabetic kidney disease (DKD) is the most common cause of chronic kidney disease and end-stage renal failure. It is associated with large social and economic burden, and there exists large unmet need to halt the progressive course of the disease. The complexity of the pathogenesis and the lack of a suitable disease model are the main impediments for the DKD drug development. Nonhuman primate models

have great natural advantages as disease models for drug development because of their similarities to human pathophysiology. In this study, we evaluated the efficacy of YG1699, a novel dual systemic SGLT1 and SGLT2 inhibitor in male cynomolgus monkeys (CM) with diabetic nephropathy.

Materials and methods: 13 male CM were selected from a KBI High-Fat Diet monkey colony meeting the following criteria: obese BMI ≥30 kg/m^2), elderly (age range 10-19 years), diabetic (A1C >9%), with Nephropathy (GFR 20-59 ml/min/1.73m^2 and UACR 30-1200 mg/g). Animals were treated with YG1699 or Vehicle. The YG1699-treated monkeys were initially dosed daily at 8 mg/kg for 7 days followed by incremental dose levels of 10, 12, 14 and 20 mg/kg every 7 days (Total treatment 5 weeks). Prespecified endpoints included mean change from baseline (CFB) in: Fasting Serum Glucose (FSG) 2-hour postprandial glucose (PPG); Urinary Glucose Excretion (UGE); HbA1c; inulin-GFR and Urinary Albumin to Creatinine Ratio (UACR).

Results: CFB data is presented as Mean (±SD). CFB in FSG was lower for YG1699 compared to Vehicle at every timepoint measured during treatment, with week 4 values of -199 (87) mg/dL for YG1699 and -73 (25) for Vehicle. CFB in PPG was measured at week 4 of treatment and lower for YG1699 -130 (116) mg/dL compared to Vehicle -66 (85). CFB in UGE was generally higher for YG1699 than Vehicle during treatment, with week 2 CFB values of 13.8 (14.2) grams for YG1699 and -9.8 (12.9) grams for Vehicle. The CFB reduction in HbA1c was of greater magnitude for YG1699 compared to vehicle at every timepoint measured during treatment, with week 4 CFB values of -5.4 (1.1) % for YG1699 and -3.0 (0.9) for Vehicle. CFB in inulin-GFR was measured at week 4 and was higher for YG1699 at 18.5 (19.7) ml/min/1.73m^2 compared to Vehicle at 2.3 (4.7). This increase in CFB GFR was maintained during the washout period. CFB reduction in UACR was of greater magnitude for YG1699 compared to Vehicle at every time point measured during treatment, with week 4 values of -10.7 (173.0) mg Albumin / g Creatinine for YG1699 and 88.2 (45.3) for Vehicle. animals treated with YG1699 developed soft stools that resolved during washout.

Conclusion: These results are consistent with the mechanism of action of the dual systemic SGLT1 and SGLT2 inhibitor YG1699 and are supportive of a potential for renoprotection in this NHP model of T2D with DKD.

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Disclosure: **C. Li:** Employment/Consultancy; Employee. Stock/Shareholding; Stock.

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GLP-1 receptor agonists attenuates extracellular matrix secretion via inhibiting HMGB1 signalling in rat mesangial cells

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Background and aims: GLP-1R agonists can exhibit a direct renoprotective effect in patients with DN beyond the hypoglycemic effect, but the underlying mechanisms remain unknown. Recent studies have suggested that high mobility group box 1 (HMGB1) contributes to the development and progression of DN. In this study, we assessed whether GLP-1R agonists acts through HMGB1 and explored its downstream signaling pathways in DN.

Materials and methods: Rat Glomerular mesangial cells (GMCs) were divided into four groups: normal control (NG), normal control with 10 nmol/L exendin-4 treatment (GLP-1 analog) (NGE), high glucose (HG), and high glucose with 10 nmol/L exendin-4 treatment (HGE) groups. GMCs were transfected with GLP-1R-siRNA using Lipofectamine ^{TM}2000 and divided as HG, HGE, HGE + GLP-1R-siRNA, or HGE + scrambled siRNA. FN and type IV collagen (COL-IV) were evaluated by enzyme-linked immunosorbent assay (ELISA). GLP-1R, HMGB1, TGF-β1, phosphorylated and total extracellular signal-regulated kinases (ERK), c-Jun NH2-terminal kinases (JNK), p38 mitogen-activated protein kinases (p38MAPK), and NF-κB p65 were measured by western blot analysis.

Results: FN and COL-IV were higher in the HG group than NG group (P<0.05). Compared with NG group, downregulation of GLP-1R and upregulation of HMGB1 and TGF-β1 were observed in the HG group (P<0.05). Additionally, NF-κB p65, the phosphorylation levels of ERK, JNK, and p38MAPK were all significantly elevated in the HG group (P<0.05). All these effects were blunted by Exendin-4 treatment (P<0.05), except of phosphorylated p38MAPK (P>0.05). Moreover, the protective effects of Exendin-4 were abolished by GLP-1R-siRNA transfection (P<0.05).

Conclusion: These results suggest that GLP-1 receptor agonists attenuates the ECM secretion of GMCs induced by high glucose. The potential mechanism involves it binding to and activating GLP-1R, which prevents ECM production by inhibiting HMGB1 and its signaling pathways.

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The role of Nox4-ROS in driving obesogenic bone marrow mesenchymal stem cell phenotype in mice

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Background and aims: Increased accumulation of bone marrow adipose tissue (BMAT) in obesity is associated with increased bone fragility and heightened fracture risk. BMAT arises from bone marrow mesenchymal stem cells (BMSCs) which contribute to bone homeostasis, giving rise to a variety of cells, including adipocytes and osteoblasts. We have previously found that obesity induces a pro-adipogenic phenotype in BMSCs accompanied by accelerated senescence and reactive oxygen species (ROS) production. A key ROS producer, NADPH oxidase 4 (Nox4), contributes to the differentiation of many cell types and has been shown to be a major ROS producer in insulin-stimulated adipocytes. Therefore, we hypothesize that Nox4-produced ROS drive the deleterious BMSC senescent phenotype associated with poor bone status in high fat diet (HFD)-induced obese mice.

Materials and methods: To test this hypothesis, NOX4 -/- and WT male mice were fed standard chow or HFD (60 % kcal fat) for 5 months. Glucose tolerance, food intake and body composition using nuclear magnetic resonance were measured monthly (insulin tolerance was measured at baseline, at 3 months, and at sacrifice). The mice were dissected following 5 months of HFD feeding and the bone phenotype was evaluated by microCT and bone turnover markers were measured by ELISA. In addition, gene expression profiles of Nox4 in BMSC from lean and obese subjects were collected.

Results: Nox4 expression was significantly increased in BMSCs from obese patients compared to lean subjects (~120% vs. ~100% [n=10], p<0.05) and in bones obtained from HFD fed WT mice compared to chow fed WT mice (~1.75x [n=4], p<0.05). In addition, the gene expression profile of Nox4 in different compartments of bone showed that

BMSCs are the major contributor of Nox4 expression in the bone marrow. Thence, we examined WT and Nox4-/- mice in obesogenic conditions and found, in response to 15 weeks of HFD, WT mice demonstrate higher body weight gain and increased fat mass compared to Nox4-/- mice (45.3 g [n=12] vs. 32.2 g [n=5], p<0.0001; 28.1% vs. 16.8%, p<0.0001). Lastly, we observed a statistical trend toward improved glucose tolerance in Nox4 HFD mice compared to WT HFD at 3 and 15 weeks (3 wks, p=0.09; 15 wks, p=0.09) and in insulin tolerance at 12 weeks (p=0.08).

Conclusion: Increased Nox4 expression in human obese BMSCs and bones of HFD fed mice confirmed the contribution of Nox4 to oxidative stress microenvironment within bone marrow. Additionally, Nox4-/- mice possess a metabolic phenotype with anti-adipogenic features. These data indicate that deletion of Nox4-ROS may protect BMSCs from pro-adipogenic phenotype, which may concomitantly enhance bone formation. In this case, targeting Nox4 therapeutically may be a salient strategy to mitigate the hypermetabolic phenotype linked to obesity associated bone fragility.

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Supplementation of methylglyoxal in drinking water does not affect the cerebral microvasculature and cognitive function in non-diabetic mice

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Background and aims: Diabetes is associated with cerebral small vessel disease (cSVD) and cognitive decline yet the underlying mechanism is poorly understood. Methylglyoxal (MGO), a by-product of glycolysis and a major precursor in the formation of advanced glycation end products (AGEs), is increased in individuals with diabetes and is associated with microvascular dysfunction. We previously showed that MGO and MGO-derived AGEs are increased in brain tissue of diabetic rats. The aim of this study was to investigate whether increased levels of circulating MGO as observed in diabetes, can cause cerebral microvascular dysfunction and cognitive impairment.

Materials and methods: 2-3 months old male C57Bl/6J mice were treated with MGO (50mmol/l, drinking water) or not (control) for 3 months (n=17 per group). Mouse cognition and behaviour was tested before treatment and at 6 and 13 weeks of treatment. Working memory, anxiety, short-term and long-term spatial learning and memory were tested using the Y-maze task, elevated zero maze task, object location task and Barnes maze task, respectively. After sacrifice, MGO and AGEs in plasma and brain were measured by UHPLC-MS/MS. Plasma inflammatory markers were assessed by ELISA. Cortical microvessels were isolated and used for gene expression analysis.

Results: Plasma MGO was increased 2-fold ($p<0.0001$) and free plasma MGO derived hydroimidazolone-1 (MG-H1) and Nε-(1-carboxyethyl)lysine (CEL) were increased 1.2-fold ($p=0.01$) and 1.7-fold ($p=0.01$), respectively in the MGO group, while other AGEs were unchanged. In brains of MGO-treated animals, there was a 1.4-fold and a 1.1-fold increase in free MG-H1 ($p=0.02$) and CEL ($p=0.001$) in comparison to controls. In both plasma and brain, there were no differences observed in protein bound AGEs between MGO treated and control group. The behaviour and cognitive function remained unchanged in the MGO group vs controls. MGO did not change the expression of the plasma inflammatory markers IFN-γ, IL-10, IL-1β, IL-6, TNF-α and chemokine (C-X-C motif) ligand 1. In isolated cortical microvessels, expression of inflammatory genes vascular cellular adhesion molecule 1, intercellular adhesion molecule 1, sirtuin 1 and the receptor for AGE, were unchanged.

Conclusion: Plasma MGO and MGO-derived AGEs were increased by supplementation of MGO in drinking water to a level comparable to that in diabetes. Although this was accompanied by increased levels of free MGO-derived AGEs in the brain, this was not associated with microvascular inflammation in the cortical microvessels and cognitive impairment. This indicates that circulating MGO by itself does not lead to microvascular inflammation nor cognitive decline and that the endogenous formation of MGO in diabetes, rather than circulating MGO, may be of importance in cerebral microvascular dysfunction and cognitive impairment.

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Disclosure: **E. Berends:** None.

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Upregulation of hepatic ß-oxidation-linked mitochondrial respiration in experimental models of diabetes and fatty liver disease

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Background and aims: Diabetes mellitus (DM) is tightly associated with non-alcoholic fatty liver disease (NAFLD) and augments its progression to steatohepatitis (NASH), fibrosis, and hepatocellular carcinoma. Mitochondrial function is frequently impaired in DM, but might differentially alter at different stages of NAFLD. Thus, we examined alterations in hepatic mitochondrial respiration in a murine model of combined DM and NAFLD.

Materials and methods: Two-days old male C57BL/6j mice received streptozotocin (STZ) or vehicle (placebo, PLC) and then high-fat diet (HFD) or continued regular chow diet (RCD) from week 4 to 16, yielding 4 models (n=8 each): obesity [PLC+HFD], DM [STZ+RCD], NASH [STZ+HFD] and control [PLC+RCD]. Histology and immunohistochemistry were performed to describe NAFLD, including the NAFLD activity score (NAS) derived from steatosis, lobular inflammation and hepatocellular ballooning. Mitochondrial respiration was assessed in liver tissues using high resolution respirometry (HRR) with different substrates protocols. Hyperinsulinemic-euglycemic clamps with labeled glucose tracer was used to assess insulin sensitivity. Citrate synthase activity (CSA) served as a surrogate marker for mitochondrial content.

Results: Blood glucose levels was >200% higher in DM and NASH ($p<0.0001$ vs control). The NAS (mean±SEM) was not different in obese (1.38±0.32; $p>0.99$), tended higher in DM (2.50±0.42; $p=0.063$) and markedly elevated in NASH (3.25±0.45; $p<0.01$) compared to control (1.00±0.27). Main contributors to NAS were steatosis and hepatocellular ballooning in NASH ($p<0.001$ and $p<0.05$ vs control) and hepatocellular ballooning in DM ($p<0.01$ vs control). Maximal uncoupled β-oxidation-associated respiration was 1.6-, 1.7-, and 1.9-fold higher in obesity, DM and NASH compared to control (all $p<0.01$). Mitochondrial density was decreased in both DM and NASH ($p<0.05$ vs control), whereas β-oxidation-dependent mitochondrial leak control ratio was not different between all groups. Insulin-stimulated glucose disposal was 44%, 53% and 66% lower in obesity, DM, and NASH (both $p<0.0001$ vs control).

Conclusion: Higher respiration per mitochondrion after hyperglycemia and high-fat diet feeding might reflect adaptive response of hepatic mitochondria to various metabolic insults. Inability for further augmentation of hepatic mitochondrial respiration might contribute to NAFLD progression to NASH.

Disclosure: **B. Dewidar:** None.

SO 54 Pathogenic mechanisms of complications

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Continuous glucose monitoring parameters are related to serum levels of non-enzymatic glycation products in patients with type 1 diabetes

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Background and aims: Increased glucose variability (GV) has been found to be an independent risk factor for microvascular and macrovascular complications of diabetes in a number of clinical studies. However, the role of GV in pathogenesis of diabetic complications needs further research. Non-enzymatic glycation is considered as one of the most important biochemical processes linking hyperglycemia and diabetic vascular disease. At the same time, the effect of GV on glycation has not been studied yet. In this work we assessed the relationships between serum levels of non-enzymatic glycation products and continuous glucose monitoring (CGM) derived time in range (TIR) and GV parameters in patients with type 1 diabetes.

Materials and methods: One hundred and thirty patients, 55 M/75 F, from 18 to 70 years of age (median 32.5 years), HbA1c 4.7-13.4% (median 7.9%), were included in the study. The control group included 30 subjects with normal glucose tolerance matched by sex and age. Mean glucose, TIR (3.9-10 mmol/L), time above range (TAR), time below range (TBR), standard deviation (SD), coefficient of variation (CV), mean amplitude of glucose excursions (MAGE), lability index (LI), J-index, 2-hour continuous overlapping net glycemic action (CONGA), M-value, low blood glucose index (LBGI), high blood glucose index (HBGI), and mean absolute glucose (MAG) were derived from CGM records. Serum levels of glycated albumin (GA), pentosidine, advanced glycation end products (AGEs) and their receptors (RAGEs) were determined by ELISA.

Results: The levels of GA, pentosidine and AGEs were increased significantly in patients with diabetes as compared to control group ($p<0.0001$, $p=0.03$ and $p=0.01$ respectively). The concentrations of RAGEs showed no significant differences between the two groups. In patients with diabetes, GA, pentosidine and AGEs demonstrated weak positive correlations with HbA1c, mean monitored glucose, TAR, SD, MAGE, CONGA, LI, J-index, M-value and HBGI, but no correlation with CV and LBGI were found. All studied glycation products have shown negative correlations with TIR. In addition, pentosidine and AGEs correlated positively with MAG; pentosidine demonstrated negative correlation with TBR. No association was observed between RAGEs and CGM parameters. In the models of stepwise multiple regression analysis, mean glucose and TIR, but not GV indices, were the best predictors of the glycation product levels. Specifically, mean monitored glucose was the most important predictor of pentosidine (beta=-0.479, R2=0.11, $p=0.006$), while TIR was the best predictor of AGEs (beta=-0.3, R2=0.09, $p=0.0008$). The concentrations of glycation products did not differ between patients with high and low CV (>36% and $\leq$36% respectively). Wherein, GA, pentosidine and AGEs were significantly higher in patients with TIR $\leq$70% when compared with those with TIR >70% ($p=0.01$, $p=0.004$ and $p=0.002$ respectively).

Conclusion: In patients with type 1 diabetes, CGM-derived mean glucose and TIR demonstrate closer associations with serum levels of non-enzymatic glycation products then GV parameters.

Supported by: RSF (20-15-00057)

Disclosure: **V.V. Klimontov:** None.

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Assessment of healthy people and type 2 diabetes patients on skeletal muscle with T1ρ MRI of calf muscle

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Background and aims: The aim of this study was to investigate skeletal muscles in type 2 diabetes(T2DM) patients and healthy people by T1ρ MRI of Calf Muscle.

Materials and methods: In all, 72 patients were involved in this study. 72 patients were divided into four groups: Long Standing Diabetes Group (Long, *n*=15) and age-matched control group (Elderly, *n*=11), New-onset Diabetes Group (New, *n*=26) and age-matched healthy volunteers (Control, n=20).The lower limb images of everyone in the group was examined by 3.0T whole-body MR scanner, and T1ρrelaxation times of soleus muscle(SOL) and tibialis anterior muscle(TA) was recorded. The age, fasting blood glucose(FBG), cholesterol(CHO), triglyceride(TG), and creatinine(Cr) of the included patients were recorded and analyzed.

Results: The average T1ρ relaxation times of the SOL in the Long, New, Control and Elderly were 41.89±6.41 ms, 34.11±1.98 ms, 32.10±1.18 ms and 38.35±1.67 ms respectively (Long vs New: P<0.0001, Long vs Elderly: P<0.05, New vs Control: P<0.01). The average T1ρ relaxation time of the TA in the Long, New, Control and Elderly were 39.24±7.21 ms, 32.76±2.48 ms, 31.38±2.33 ms and 33.86±1.18 ms respectively. (Long vs New: P<0.0001, Long vs Elderly: P<0.01, New vs Control: P<0.05). Linear correlations were observed between the T1ρ relaxation times of the TA, SOL and the duration of diabetes (R2=0.4198, P<0.0001; R2=0.3257, P<0.0001). Linear correlations were also observed between the T1ρ relaxation time of the TA, SOL and the fasting blood glucose (R2=0.2527, P<0.0001; R2=0.071, P<0.001). Multiple linear regression analysis revealed that age and FBG were two important variables associated with average T1ρrelaxation times of SOL of both lower limbs, with partial regression coefficients of 0.109 and 0.519 respectively (both P < 0.05). Multiple linear regression analysis also showed that age,Cr and Long T2DM were three important variables associated with average T1ρrelaxation times of TA of both lower limbs, with partial regression coefficients of 0.057,0.061 and 4.514 respectively (all P < 0.05)

Conclusion: Our study showed that the T1ρrelaxation times of TA and SOL in newly and long-term diabetic patients is increased, suggesting that the skeletal muscle changes have occurred in both early and late diabetic patients, and may further increase with the progression of the disease.

Disclosure: **L. Guo:** None.

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Dynamics of hexokinase-2 linked glycolytic overload mediating endothelial cell dysfunction in high glucose concentration in vitro

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Background and aims: Metabolic dysfunction of endothelial cells in hyperglycemia contributes to the development of vascular complications of diabetes. Recent studies have revealed that increased glucose consumption mediating metabolic dysfunction is caused by glucose-induced stabilization of hexokinase-2 (HK2) to proteolysis, increasing flux through glycolysis without increased activity of glycolytic enzymes. This produces a wave of increased intermediates in early-stage glycolysis, stimulating increased formation of methylglyoxal and advanced glycation endproducts (AGEs), mitochondrial membrane hyperpolarization and oxidative stress, hexosamine and protein kinase C pathways. In this study, we investigated the dependence of glucose-induced stabilization of HK2 to proteolysis on glucose concentration, duration of exposure to high glucose concentration, correlation with increased glycolytic flux, rate of return of HK2 to basal levels with switch to normal glucose concentration and response to glyoxalase 1 inducer, *trans*-resveratrol-hesperetin combination (tRES-HESP).

Materials and methods: Human aortal endothelial cells (HAECs) were cultured under an atmosphere of air with 5% CO_2, 100% humidity, at 37°C in human large vessel endothelial cell growth medium with growth supplement and antibiotics according to the manufacturer's instructions; used during passages 4 - 6 which maintains the primary endothelial phenotype. Cultures had 4.1 - 20 mM glucose for 6 - 72 h, with and without 5 μM tRES-HESP. HK2 protein abundance was assessed by Western blotting, normalized to ß-actin; and glucose consumption was determined by assay of glucose at the start and end of cultures, normalizing flux to cell number.

Results: HK2 increased progressively with time in HAEC cultures with high glucose concentration, compared low glucose concentration (20 mM vs 4.1 mM glucose), maximizing after 12 h. HK2 abundance was increased maximally by 37% whereas total glucose consumption was increased by 59% (6.77 ± 0.12 to 10.75 ± 0.21 μmol/day/million cells; P<0.001, n = 3). HK2 abundance increased from 4.1 mM to 20 mM glucose, increasing *ca.* 2.4% per mM glucose. HK2 abundance correlated with initial glucose concentration and flux of glucose consumption (r = 0.96 and r = 0.85, respectively; P<0.001; n = 12, *Pearson*). Increased HK2 abundance returned to basal levels only after 48 h in low glucose concentration. tRES-HESP prevented increased HK2 abundance, increased glucose consumption and metabolic dysfunction of HAECs in high glucose concentration.

Conclusion: We conclude that HK2 abundance increases with increased glucose concentration in the clinical range in diabetes. Increased HK2 mediates glycolytic overload and metabolic dysfunction of HAECs in high glucose concentration and tRES-HESP prevents this by normalizing HK2 proteins. The slow return of high HK2 abundance to basal levels in low glucose after exposure to high glucose concentration may contribute to carryover of damaging effects of hyperglycemia from postprandial to fasting phases. tRES-HESP may provide effective treatment of endothelial dysfunction linked to hyperglycemia in diabetes.

Supported by: PJT

Disclosure: **P. Thornalley:** None.

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Fructosamine 3-kinase: activity and polymorphisms of important deglycation enzyme in patients with diabetes

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Background and aims: Fructosamine 3-kinase (FN3K) and FN3K-related protein (FN3K-RP) are important intracellular enzymes involved in deglycation, and thus reduce glycation burden in chronic hyperglycemia in patients with diabetes. The aim of the study was to assess the activity of these enzymes and evaluate two most important FN3K polymorphisms in relation to chronic microvascular changes in diabetes.

Materials and methods: In total, 107 patients were included - 42 with Type 1 diabetes (T1DM) (aged 56±13 yrs) and 65 with Type 2 diabetes (T2DM) (aged 65±11). FN3K and FN3K-RP activity was measured in erythrocytes by HPLC. FN3K rs1056534 and rs3848403 polymorphisms were assessed by RT PCR and Taqman genotypization. Apart from routine biochemical and anthropometrical data, all patients were tested for microvascular complications.

Results: Patients with T1DM had worse diabetes control than with T2DM (63±10 vs. 56±14 mmol/mol; p<0.05). Similarly, retinopathy was more frequent in T1DM than T2DM (64 vs. 9 %). On the contrary, neuropathy or nephropathy was more common in T2DM, 9 vs. 21 % and 17 vs. 35 %, respectively. Genotype frequencies of both polymorphisms were similar in both T1DM and T2DM (*rs1056534 - CC:* 6 and 21 %, *GG:* 53 and 37 %, *CG:* 41 and 42 %, χ^2 test *ns; rs3848403 - TT:* 15 and 29 %, *CC:* 23 and 13 %, *CT:* 62 and 58 %, χ^2 test *ns*). Relative frequencies of mutant alleles were also comparable. Patients with microvascular complication (neuropathy, nephropathy, or retinopathy) had similar frequency of these alleles. FN3K and FN3K-RP activity were comparable in presence of any microvascular complication or even all three microvascular (neuropathy, retinopathy, nephropathy) complications together (FN3K: 19±4 vs. 18±5 mU/gHb, *ns*; FN3K-RP: 98±23 vs. 97±37 mU/gHb, *ns*). In general, mutated C allele of *rs3848403* was associated with lower FN3K activity (18±4 vs. 22±4 mU/gHb; p<0.005), while this was not observed in *rs1056534*. There was not any association observed between glycated hemoglobin and FN3K or FN3K-RP activity.

Conclusion: FN3K and FN3K-RP activities were comparable in patients with or without microvascular complications. Similarly, the frequencies of studied FN3K polymorphisms were not significantly different. The *rs3848403* polymorphism was associated with decreased activity of deglycation FN3K, however this was not related to more frequent microvascular complications. It seems probable, that a (in)balance between proglycation and deglycation mechanisms is more important in the development of microvascular complications, than an absolute deglycation activity.

Supported by: Progres Q25, RVO 64165

Disclosure: **J. Skrha jr.:** None.

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Indicies of uric acid metabolism as marker of anabolic - catabolic balance in men and women with type 2 diabetes with different phenotypes

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Background and aims: In type 2 diabetic (T2D) patients, different phenotypes can be observed which vary in the degree of abdominal obesity, uricemia levels, and lipid spectrum disorders. **Aims:** To identify clinical phenotypes of T2D by analyzing serum uric acid (UA) levels and UA urinal excretion depending of gender and level of obesity in patients.

Materials and methods: 47 patients (25 men, 22 women), aged 30 to 82 years with T2D were included. Anthropometric parameters, % of total fat and water, the visceral fat (VF) level determined by bioelectric impedance. Serum concentration of UA, creatinine and 24-hour UA urinary excretion were determined, the clearance and fractional excretion of UA were calculated. The activity of purine reutilization by the "salvage pathway", carried out by an enzyme hypoxanthine guanine phosphoribosyltransferase (HGPRT), evaluated by the ratio of the urine molar concentrations of UA to creatinine.

Results: Groups of men and women (m/w) who differed in the level of visceral fat (VF) (<12 in women, VF> 12 in men) were considered separately. In each group, subgroups were divided according to the level of body mass index (BMI): 1) non-obese: BMI <30 kg/m^2; n = 20, m/w = 8/12); 2) obese (BMI≥30 kg/m^2; n = 27, (m/w = 17/10). All patients had HbA1c levels > 7.5%, with significantly higher total fat content and visceral fat level in the subgroups of obese patients compared to the non-obese subgroups. The level of uricemia did not exceed normal range for both men and women. Obese women, compared with non-obese women, had significantly increased UA clearance (13.2 ±0.7 vs 11.1 ± 0.5 ml/min, P <0.05) and fractional urate excretion (12.4 ± 0.5 % vs 10.6 ± 0.7%, P <0.05). It can be assumed that in both gropes the level of uricemia maintained within normal limits due to high UA excretion. The level of uricemia in obese men (298.9 ± 25.3 μmol/l) was significantly higher compared to that in non-obese men (364.8 ± 10.8 μmol/l). This is combined with relatively low clearance and fractional excretion of UA (which characterizes the degree of tubular urate reabsorption), leads to less urine excretion of UA and causes an increase of uricemia. But the mechanisms of the letter could differ significantly depending on the degree of obesity. In obese men, the HGPRT level (0.48 ± 0.04 vs 0.30 ± 0.02) was significantly higher than in the non-obese subgroup. Decreased activity of the enzyme HGPRT may cause an increase in urate elimination, as we observed in the subgroup of obese men, compared to the obese subgroup. Thus, the predominant cause of higher uricemia in obese men may be a relative deficiency in the activity of the anabolic enzyme HGPRT, which leads to decrease in purine reutilization and is manifested in increased urinary urate elimination. In obese men, on the contrary, along with the increase in UA reabsorption, the highest HGPRT activity was observed, which characterizes the activation of the anabolic purine reutilization pathway which may course accumulation of total and visceral fat.

Conclusion: The results indicate a difference in the state of anabolic-catabolic balance in men and women with the obese phenotype (with domination of anabolic processes, leading to activation of salvage pathway, and accumulation of fat) and non-obese phenotype (with domination of catabolic pathways that cause the breakdown of purines to the end product, UA).

Disclosure: **A.A. Shuprovich:** None.

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Proton pump inhibitor induced hypomagnesaemia and mortality: a mediation analyses of the diabetes care system cohort

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Background and aims: Chronic proton pump inhibitor (PPI) use and hypomagnesemia are common in patients with type 2 diabetes (T2DM). Since PPI use can lead to hypomagnesemia and magnesium (Mg) levels are inversely associated with CVD and mortality, it is conceivable that Mg is a mediator of the association between PPI use and mortality. Therefore, we investigated serum Mg as a mediator of the association between PPI use and all-cause mortality and fatal and non-fatal CVD in T2DM.

Materials and methods: This study is performed in a subsample of the Diabetes Care System cohort, a prospective and dynamic cohort of T2DM patients in primary care. Serum Mg was measured in participants with stored blood samples collected between 2008-2014. Baseline PPI use and other prescriptions were determined the year preceding the measurement of Mg. Primary outcome is all-cause mortality and secondary outcome is combined fatal and non-fatal CVD, with a follow-up from measurement of Mg until January 2020. Due to a non-linear association of Mg with mortality, the Mg was dichotomized (cut-off > 0.77 mmol/l). We performed causal mediation analyses based on logistic regression and accelerated failure time models (effect estimate expressed as survival time ratio (STR)). Multivariable models adjusted for age, sex, baseline CVD, blood pressure, BMI, laboratory values and medication associated with PPI use, T2DM severity and mortality.

Results: Of the 4037 participants, 1079 (26.7%) were using PPI the year preceding Mg measurement and mean serum Mg was 0.80 mmol/l (SD 0.08). Hypomagnesemia was present in 362 (9.0%) of the participants. During a median follow-up of 7.0 years [IQR 6.4-11.1], 711 (17.6%) participants died and 608 (15.6%) fatal and non-fatal cardiovascular events occurred. PPI use was associated with lower Mg levels (OR 1.28, 95%CI 1.10-1.50) and a shorter survival time (STR 0.86, 95%CI 0.76-0.97) in multivariable adjusted models. PPI use was also associated with higher incidence of fatal and non-fatal CVD, but non-significantly after adjustment for co-medication (STR 0.86, 95%CI 0.71-1.06). Mg explained 4.1% of the effect of PPI use on all-cause mortality, with a shorter survival time for participants with PPI use, compared to those without PPI use through the lowering effect of PPI use on Mg (STR 0.99, 95%CI 0.98-1.00). For the secondary outcome of combined fatal and non-fatal CVD, the Mg explained 8.4% of the total effect after adjusted for co-medication (STR 0.98, 95%CI 0.97-<1.00).

Conclusion: In this T2DM cohort, PPI use is associated with lower Mg and higher mortality. However, the association between PPI use and all-cause mortality and fatal and non-fatal CVD is only minimally mediated through lower Mg levels.

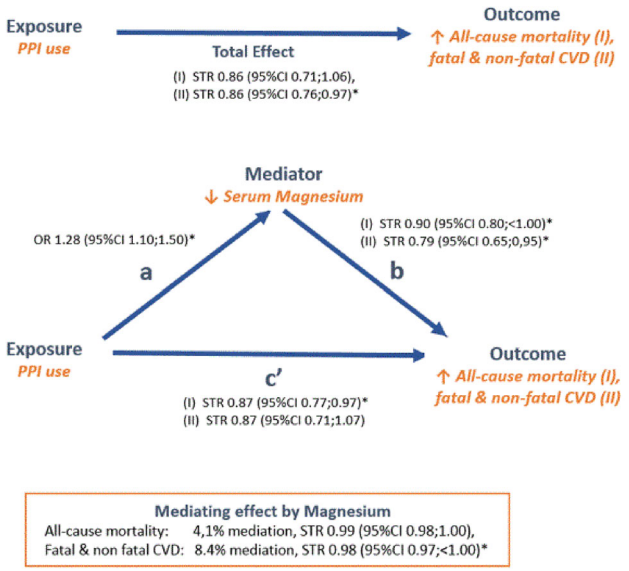

Figure 1. Mediation figure

PPI, Proton pump inhibitor; CVD, cardiovascular disease; STR, survival time ratio (a STR < 1 is reflecting a shorter survival, i.e. higher risk for mortality).* significant (p <0.05)
Total effect = the exposure-outcome association without considering mediation; a-path = association between PPI use and magnesium; b-path = association between magnesium and outcome corrected for determinant (PPI); c'-path = natural direct effect, reflecting the association between PPI use and mortality adjusted for serum magnesium,

Clinical Trial Registration Number: ISRCTN26257579

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Disclosure: **E.A. Vermeulen:** None.

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Pancreas volume in patients with type 1 diabetes: What does it depends on?

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Background and aims: The endocrine and exocrine parts of the pancreas are classically considered two separate organs. This artificial division of one organ into two different parts limits research and, therefore, knowledge of the endocrine-exocrine interaction of the pancreas, both in terms of health and disease. There is an opinion that T1D is a combined endocrine-exocrine disease in which the loss of functional beta cell mass is most clinically apparent. The aim of our study was to identify the pancreas volume (PV) based on MRI and its dependence on the duration of T1D, glycemic control or b-cell function and the severity of the autoimmune process

Materials and methods: 74 patients with T1D were included in the study. Patients were divided in 5 groups, according to disease duration: «a» - up to 1 year (n=11), «b» - 1-5 (n=16), «c» - 5-10 (n=15), «d» -10-15 (n=13), «e» - more than 15 years (n=19). PV was identified by Magnetic resonance imaging (MRI) - magnetic resonance cholangiopancreatography (MRCP). Also determined the prevalence of the pancreatic β-cells autoantibodies (tyrosine phosphatase-like IA-2 (IA-2A) and zinc T8 (ZnT8A)), fasting C-peptide levels and HbA1c. The results presented as median and interquartile range - M [Q25%;Q75%].

Results: All results presented in Table 1. All examined patients with T1D had lower PV than control subjects (p<0,005). PV declining more and more during the duration of T1D (p<0,005).

Conclusion: The data obtained indicate lower PV based on T1D duration. Lower fasting C-peptide levels was associated with the lower PV. We didn't find any correlation between PV and the patients age, BMI and HbA1C levels. We determined positive ZnT8Ab and IA-2Ab levels in all groups, but there is no correlation with PV.

	«a»	«b»	«c»	«d»	«e»	Control subjects
Age, years	26 [21;35]	24 [20;32]	27 [25;33]	31 [24;47]	29 [25;38]	28 [25;31]
BMI, kg/m^2	23,9 [19,9;26,1]	22,7 [20,1;25,9]	21,5 [19,8;25,6]	23,4 [21,1;25,3]	23,6 [21,7;26,9]	23,7 [21,4;26,9]
PV, ml	50,8 [34,6;54,9]	38,1 [31,7;39,3]	33,1 [23,6;37,1]	21,3 [19,2;27,5]	20,8 [19,6;27,3]	71,6 [67,5;79,3]
ZnT8Ab, U/ml	99,3 [31,1;108,3]	23,5 [15,4;30,6]	53,7 [6,7;89,3]	21,3 [10,0;54,7]	12,7 [5,2;71,6]	4,1 [2,9;8,7]
IA-2Ab, U/ml	63,5 [41,2;92]	55,1 [14,2;239,5]	77,1 [2,4;265,7]	33,6 [11,2;101,5]	11,1 [4,1;87,8]	5,3 [4,1;7,9]
Fasting C-peptide, ng/ml	1,04 [0,51;1,23]	0,7 [0,19;0,0,97]	0,71 [0,09;0,85]	0,01 [0,01;0;01]	0,01 [0,01;0,01]	2,23 [1,76;3,13]
HbA1C, %	9,3 [7,9;11,4]	8,8 [6,9;10,1]	8,2 [7,5;9,9]	7,8 [7,1;10,4]	8,1 [7,7;9,5]	5,3 [5,1;5,6]

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Disclosure: **M. Ragimov:** None.

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Nodular goiter increases a risk of neurovascular complications in adult patients with type 1 diabetes

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Background and aims: Nodular goiter is common in the general population. Recently, several studies have been published on the association between impaired glucose metabolism and thyroid volume and the presence of thyroid nodules. The study aimed to assess the relationship between nodular goiter presence and the clinical feature and occurrence of chronic complications in type 1 diabetes patients.

Materials and methods: The study population comprised 126 consecutive European Caucasian participants with type 1 diabetes (T1DM) including 57 (45.2)% men. Median participants` age was 31 [interquartile range (IQR): 26-36] years and T1DM duration was 14 (IQR: 9-18) years.46 (36.5)% participants were diagnosed with at least one neurovascular complication [retinopathy - 43 (34.4)%, diabetic kidney

disease - 11 (8.7)%, autonomic neuropathy - 12 (10)% and peripheral neuropathy 12 (10)%]. At the time of enrollment, 15 % of participants were diagnosed with hypothyroidism. Neck ultrasonography (US) was performed to assess the morphology of the thyroid gland. A thyroid nodule was defined as a lesion with a diameter large than 3 mm.

Results: Thyroid nodules were found in 18 patients (14%). We found no statistically significant differences in smoking status, HbA1c, body mass index (BMI), waist/hip ratio (WHR), thyroid lobes volumes, the estimated glucose disposal rate (eGDR), visceral fat index (VFI) in the study group vs. patients without thyroid nodules. Nonetheless, patients with thyroid nodules were older [43.5 (35-57) vs. 30 (25-35) years; $P = 0.0002$], with women prevalence [78% vs. 51% of women; $P = 0.03$] and had longer T1D duration [19.5 (15-31) vs. 13 (9-17) years; $P = 0.001$]. Additionally, group with nodules had lower free triiodothyronine (FT3) [2.76 (2.66-2.89) vs. 3.09 (2.82-3.36) pmol/L; $P = 0.004$] and lower estimated glomerular filtration rate (eGFR) [83.39 (75.84-104.72) vs. 99.16 (89.10-111.09) mL/s/1.73m^2; $P = 0.01$] in comparison with group without nodules. Occurrence of neurovascular complications in all [(61.1 vs 32.4) %, P=0.02], autonomic [(26.7 vs 7.6) %, P= 0.04] and peripheral neuropathy [(33.3 vs 4,6) %, P=0.001] was higher in group of patients with thyroid nodules. Multivariate linear logistic regression analysis indicated thyroid nodules (odds ratio [OR], 27.73; 95% confidence interval [CI], 1.68-458.88; $P = 0.02$], diastolic blood pressure (DBP) [OR, 1.20; 95% CI, 1.00-1.44; $P = 0.04$] and T1D duration [OR], 1.17; 95% CI, 1.02-1.32; $P = 0.02$] as predictors of peripheral neuropathy occurrence after adjustment for sex, age, HbA1c, BMI, systolic blood pressure (SBP), FT3.

Conclusion: The occurrence of thyroid nodules in adults with type 1 diabetes was associated with a higher incidence of neurovascular complications

Disclosure: **A. Rogowicz-Frontczak:** None.

SO 55 Not so sweet: cancer and diabetes

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A CT-Scanner to detect cancer in subjects hospitalised for uncontrolled diabetes and weight loss

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Background and aims: Unexpected weight loss is a non-specific symptom of cancer. Cancers of the pancreas, liver, kidney and lung are detectable by a CT-scanner, and more frequent among subjects with diabetes. We aimed to know how many cases could be detected in poorly controlled, weight-loosing, subjects with diabetes and what were their clinical characteristics

Materials and methods: 275 patients admitted for uncontrolled diabetes with unexpected weight loss were included. They were interviewed, examined, had blood and urinary samples and all the subjects had a contrast-injected CT-scanner of chest, abdomen and pelvis.

Results: The 275 patients were mainly men (63.3%), 58.7±12.9 years old, with 5 (0-13) years durations of diabetes, poorly controlled: mean HbA1c at 10.7±2.9%. Most of them (62.2%) had chronic diabetic complications: 16.0% had a diabetic retinopathy, 38.1% had a neuropathy, 32.1% had a diabetic kidney disease and 20.1% had a macroangiopathy. Their BMI was 26.8±6.0kg/m^2 at the inclusion and the maximal BMI during their life had been 32.7±7.3kg/m^2. The CT-scanner led to the diagnosis of cancer in 18 patients (6.5%): 8 pancreas, 5 lung, 3 liver and 2 kidney. These patients did not have more diabetes complications than the others, or a greater recent weight loss than the others, however they had lost more from their maximal weight: -21.7kg±12.4 vs -15.1kg ±11.2 (p=0.02). This difference remained significant after adjustments for age, gender and HbA1c (HR: 1.043, 95%CI: 1.009-1.0079). A more than -20 kg weight loss was related to cancer after adjustment for age, sex and HbA1c:HR = 3.343 (95% CI: 1.080-10.347). The HbA1c were lower in patients with cancer than without cancer: 9.4 ± 2.0% vs 10.8 ± 3.0% (p = 0.047). The HbA1c were negatively related to the recent weight loss (B = -0.305, p<0.001), but not to the long-term weight loss.

Conclusion: Among 275 patients admitted for uncontrolled diabetes and weight loss, the CT-scanner of the chest, abdomen and pelvis allowed the diagnosis of 18 cancers: 6.5%. The patients with cancer had a greater long-term weight loss adjusted for age, gender and HbA1c.

	Total	Cancer	No cancer	p
N	275	18	257	
Age (years)	58.7±12.9	63.8±11.5	58.3±12.9	0.081
Women, n (%)	101 (36.7%)	7 (38.9%)	94 (36.6%)	0.807
HbA1c at inclusion (%)	10.7±2.9	9.4±2.0	10.8±3.0	**0.047**
BMI at inclusion,(kg/m²)	26.8±6.0	26.3±4.5	26.8±6.1	0.715
Maximal BMI during life (kg/m²)	32.7±7.3	34.2±6.0	32.5±7.3	0.348
Weight loss during the previous 3 months,(kg)	-5.0±3.7	-4.5±2.8	-5.0±3.8	0.514
Weight loss from maximal weight, kg	-15.6 ±11.4	-21.7 ±12.4	-15.1±11.2	**0.02**
More than -20kg weight loss from maximal weight, n (%)	56 (23.8%)	7 (41.2%)	49 (22.5%)	**0.081**
Diabetes complications, n (%)	153 (62.2%)	10 (66.7%)	143 (61.9%)	0.790

Table 1 : Comparison of subjects with versus without Cancer

Disclosure: **M. Barbet-Massin:** None.

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The effect of the Danish National colorectal screening program on detecting cancer for patients with diabetes

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Background and aims: Colorectal cancer (CRC) is one of the few cancer types for which individuals with diabetes (both type 1 and 2) have a substantially higher incidence and higher mortality compared to individuals without diabetes. The objectives of this study were to evaluate to what extent individuals with diabetes participate in the Danish National CRC screening program (free of charge, fecal immunochemical test), and to estimate the effect of screening invitations on the number of CRC diagnoses for individuals with and without diabetes.

Materials and methods: This retrospective cohort study was based on national health registers describing all residents in Denmark aged 50-72 years (N=1.273,094) linked with data from the Danish National CRC screening programme. Participation in the screening programme is free of charge and includes immunochemical detection of blood in self-administrated faecal samples. Individuals were randomly invited to the first round of screening between 2014-2017. In total, 665,266 Danes had received an invitation by May 1 2016, whereas 607,828 were invited later. Among those invited, the likelihood of participating for patients with and without diabetes where compared while adjusting for age and gender. The intention-to-treat effect of the invitation was assessed by the likelihood of being diagnosed with cancer between invited and not yet invited individuals.

Results: In total, there was 1,273,094 eligible individuals for screening invitation. Of them, 108,101 (8.5%) had diabetes, 53,827 were invited before May 2016 and 54,274 were not yet invited. Of the invited 57,4% of the individuals with diabetes participated, compared to 65,7 % in the remaining population; giving an adjusted risk difference at 9.2% (95%CI, 8.8%-9.6%). The likelihood of being diagnosed with CRC was 1.2‰ (95%CI, 1.1-1.3) for not yet invited individuals without diabetes. In general, screening invitations increased the likelihood of being diagnosed with CRC by 1.5‰ (95%CI 1.3-1.6), and among individuals with diabetes, screening invitations increased the likelihood by an additional 0.7‰ (95%CI, 0.1-1.2‰). This means, that a person with diabetes invited to screening had a likelihood of being diagnosed with CRC at 2.1‰ (95%CI, 1.5-2.8).

Conclusion: Individuals with diabetes had almost 10% lower adherence to the national CRC screening compared to the remaining screening population (50-72 year). Even though the participation rate was lower among patients with diabetes in the invited group, the effect of the invitation was significantly higher than the effect of invitation in individuals without diabetes. Offering CRC screening to individuals with diabetes has the potential of securing a life with diabetes with less illness. Attention to adherence is needed to achieve the full benefit.

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Disclosure: **T. Laurberg:** None.

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Cancer cachexia is associated with beta cell dysfunction: cross-sectional study in patients with pancreatic cancer

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Background and aims: While pancreatic ductal adenocarcinoma (PDAC) is associated with incident diabetes, the causality is still debated. Cancer cachexia (CC) is an inflammatory, multi-system condition that develops early in the course of PDAC and it contributes to glycaemic derangements. We aimed to explore whether insulin resistance and/or beta cell dysfunction relates to development of cancer cachexia syndrome

Materials and methods: Cross-sectional study comparing patients with PDAC with (CC+, n=16) and without cachexia (CC-, n=26). Patients with resectable PDAC were recruited before being enrolled for surgery and any chemotherapy. CC was diagnosed using validated score by Evans et al based on weight loss, reduced food intake, functional impairment and systemic inflammation. An age and BMI matched control group (C, n=23) comprised of patients with benign pancreatic lesions. In a fasting state indirect calorimetry to assess substrate utilization and OGTT (75g glucose) to assess glucose, insulin, c-peptide, NEFA, glycerol and BCAA changes over 120min, were performed. Indices of insulin secretion (insulinogenic index, AUCs, disposition index) and insulin resistance (insulin sensitivity index, ISI) were calculated

Results: Sixty-five patients were enrolled over 24 months with comparable age across groups. Patients CC+ had higher REE and had lost more weight (CC+ 12.2 vs CC- 6.7 vs C 4.0 kg, p=0.003). Both first phase (IGI: CC+ 0.32±0.4; CC-0.34±0.4; C 0.79±0.7 μU/mL*mg/dL, p=0.03) and second phase ($iAUC_{insulin}/iAUC_{glucose}$ CC+ 6.2±8.2; CC- 7.0±6.9; C 17.4±16, p=0.03) of insulin response in CC+ were decreased, despite the substantially larger weight loss and the same trend was found when insulin response was adjusted for insulin sensitivity (oral disposition index: CC+ 19.0±25.9; CC- 23.4±21.9; C 53.2±58.8; p=0.02). Whole body insulin resistance as well as lipolytic response was similar across groups. Lower fasting BCAA (CC+ 345 vs CC- 411 vs C 417, p=0.03) and trend towards decreased suppression by hyperinsulinemia was observed in CC+.

Conclusion: Despite the more pronounced body weight loss in CC+ group, there is a signal of beta cell dysfunction but not insulin resistance contributes to impaired glucose-insulin homeostasis in PDAC and cancer cachexia

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Immune infiltration of CD8+ T cells in patients with diabetic pancreatic cancer reduces the malignancy of cancer tissues

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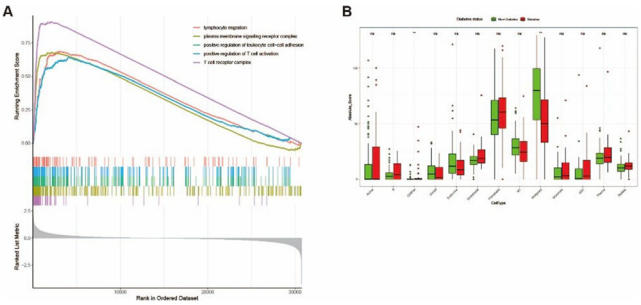
Background and aims: Although the functional damage of the diabetic pancreas can affect the postoperative recovery of pancreatic cancer patients, there is no significant difference in the prognosis of pancreatic cancer patients with a history of diabetes and ordinary pancreatic cancer patients. There is still no practical theory to explain this phenomenon. The purpose of this study is to find out the difference between diabetic pancreatic cancer and typical pancreatic cancer through the analysis of the tumour immune microenvironment of pancreatic cancer tissues, thereby

revealing the heterogeneity of the two types of pancreatic cancer patients and addressing these two different types of pancreatic cancer design personalized immunotherapy programs.

Materials and methods: The mRNA expression profile data of 141 cases and 51 cases with clinical data of diabetes status were obtained from the TCGA database and the GEO database, respectively. The CRA001160 data set was obtained in the TISCH database, which contained 57443 cells' single-cell expression profile sequencing data. The DESeq2 was used to analyze the differential expression of diabetic and non-diabetic patients. The ClusterprofileR was used to perform GSEA. The Seurat was used to process single-cell expression profile sequencing data. The Cibersortx was used to construct a feature matrix of single-cell sequencing data and to deconvolve Bulk-RNAseq data to obtain each pancreatic cancer patient's tumour invasion score.

Results: The results of GSEA showed that lymphocyte migration, plasma membrane signalling receptor complex, positive regulation of leukocyte cell-cell adhesion, positive regulation of T cell activation, T cell receptor complex were significantly enriched in diabetic pancreatic cancer patients. Compared with regular pancreatic cancer patients, patients with diabetic pancreatic cancer had a higher degree of CD8+ T lymphocyte infiltration, and the corresponding absolute score of malignant cells was lower.

Conclusion: The activation of inflammatory-related signalling pathways in diabetic pancreatic cancer patients increases the immune infiltration of CD8+ T cells in cancer patients and reduces the development of malignant tumour tissues. These findings help us further understand the immune microenvironment of patients with diabetic pancreatic cancer, suggesting that our immunotherapy based on CD8+ T lymphocytes may positively affect patients with pancreatic cancer. These studies still need to be supported by more experimental data.



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Assessment of glucose metabolic disorders in management of prostate cancer with GnRH agonists

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Background and aims: Follow-up study to assess glucose disorders in patients with local prostate cancer (pT3N0M0) who were treated with agonists of gonadotropin-releasing hormone (aGnRH). 102 patients were enrolled to the study, 99 subjects were followed up till the study completion. The mean age was 69±8,64 years old.

Materials and methods: The study of fasting plasma glucose (FPG), glycated hemoglobin (HbA1c) was performed at baseline, after 3, 6 and 12 months ADT. Flash glucose monitoring systems were installed in ten patients with elevated glycemia who received ADT with GnRH agonists for at least 6 months, allowing them to obtain ambulatory glycemic profile data. Glucose variability indices (AUC, CONGA, JINDEX) had calculated.

Results: Achievement of low threshold values of testosterone was accompanied by an increase in the levels of FPG and HbA1c over 12 months of ADT regardless of baseline age, BMI, WC, with a maximum increase during the first 3 months of treatment, and progressively after 6 months and 12 months. FPG (mmol/l) basic, 3, 6 and 12 months, respectively 5,18±0,91; 5,67±0,93; 5,77±0,96; 5,90±1,12 (for all differences $p \leq 0.001$). HbA1c (%) 5,36+0,66; 5,66±0,83; 5,77±0,82; 5,89±0,83 (for all differences $p \leq 0.001$). The proportion of patients with prediabetes is increasing, according to ADA criteria (5,6-6.9 mmol/l) after 3 months was 36%, after 6 months - 41%, and after 12 months 66% of patients. And also change in AGP, manifested by an increase in the total daily glycemic load (mean AUC 172,49 ($p \leq 0.001$), glycemic variability (mean CONGA 6,817 ($p \leq 0.001$), and an increase in postprandial glycemia (JINDEX 22,945 ($p \leq 0.001$).

Conclusion: Long-term androgen deprivation therapy with GnRH agonists leads to an early, progressive, clinically significant deterioration of glucose metabolism regardless of baseline age, BMI, WC. Features of AGP are increase of glucose variability and total glycemic load predominantly, and peak glycemic values rise.

Disclosure: **E. Gritskevich:** None.

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Seeing the brain through the eye: relationship between lower retinal thickness and altered grey matter structure

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Background and aims: Retinal thickness, measured by Optical Coherence Tomography (OCT), has been associated with cerebral atrophy in different systemic diseases. In type 1 diabetes (T1D), however, retinal thickness and its association with brain structure are poorly studied, whereas (proliferative) vasculopathy is the most described retinal complication. Thus, we evaluated local neuroretinal alterations and its correlation with grey matter structure in T1D.

Materials and methods: Thirty-nine T1D patients with proliferative retinopathy (44.69 ± 7.35 years, 24 [61.5%] females), 32 without clinically manifest microangiopathy (37.43 ± 9.13 years, 23 [71.9%] females), and 42 controls (36.69 ± 10.95 years, 26 [61.9%] females) underwent OCT of both eyes measuring retinal thickness within the ETDRS region of the central, the parafoveal, and perifoveal area of the macula and MR-imaging. Linear regression was used to determine differences in retinal thickness, adjusted for age, sex, systolic blood pressure, and depression symptoms. FSL-PALM was used to calculate associations with grey matter surface area, thickness, and volume, corrected for multiple voxel testing (Family Wise Error [FWE]), 3 grey matter modalities and 3 retinal areas.

Results: No differences were found in retinal thickness of the left and right eye justifying the use of the mean of both eyes. Lower parafoveal retinal thickness was found in all T1D patients (253.9 ± 21.0 μm) versus controls (266.6 ± 13.4 μm; all $p < 0.002$). Post-hoc analysis showed that those with proliferative retinopathy (247.2 ± 23.1 μm) had lower retinal thickness compared to both controls and patients without clinically manifest microangiopathy (262.1 ± 14.7 μm; all $p < 0.001$). In all T1D patients, an association was found between lower parafoveal retinal thickness and lower grey matter surface area in the left occipital cortex. This correlation extended into the bilateral inferior temporal/fusiform, superior, middle and orbitofrontal cortices ($p_{FWE} < 0.05$).

Conclusion: This study showed retinal thinning in T1D, driven by those with microangiopathy. Neuroretinal thinning was correlated with lower surface area of the primary visual cortices, extending into the temporal visual stream and higher-order frontal-occipital pathways. This shows that retinal thickness is related to an extensive network of structural grey matter in this group of T1D patients.

Supported by: DCTI

Disclosure: **R.B. Rodrigues:** None.

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Retinal microperimetry: an useful tool for the monitoring of the cognitive function in patients with type 2 diabetes

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Background and aims: The current guidelines for the management of type 2 diabetes (T2D) recommend annual screening for cognitive impairment in patients >65 years. The most widely used test for screening is the Minimental State Evaluation Test (MMSE). However, MMSE has limitations and the accurate evaluation of cognitive status is based on complex neuropsychological tests, which makes their incorporation into the current standard of care for the T2D population infeasible. Our group has previously showed that retinal microperimetry is useful for detecting cognitive impairment in patients with T2D, but there is no information regarding its usefulness as a periodic monitoring tool for assessing cognitive decline. On these bases we aimed to explore the role of retinal microperimetry in the follow-up of the cognitive function of the patients with T2D>65 years.

Materials and methods: Prospective observational study which comprised 100 consecutive patients >65 years with T2D, without known cognitive impairment, attended at the outpatient clinic of our center between March 2018-October 2019. The study was conducted according to the tenets of the Helsinki Declaration and the Ethic Committee of our center approved all procedures. All the patients underwent a complete neuropsychological evaluation (RBANS) in order to recruit patients with mild cognitive impairment (MCI), that further were evaluated using retinal microperimetry (sensivity dB, gaze fixation parameters: P1%, P2%, BCEA63, BCEA95) and MMSE at baseline and 12 month follow-up.

Results: A total of 59 patients with MCI were identified. A significant decline in MMSE score was observed at 12 month of follow-up (25.74 ±0.9 vs. 24.71±1.4; p=0.001). Regarding the microperimetry, we did not find significant changes in retinal sensitivity after 12-month of follow-up. By contrast, all gaze fixation parameters showed significant worsening at the end of the follow-up period as reflected by **Table 1**.

Conclusion: Retinal microperimetry is a useful tool for the monitoring cognitive decline of mild cognitive impairment in T2D patients>65 years. Gaze fixation seems a more sensitive parameter for follow-up in 12 month period of time than retinal sensitivity. This results support the concept that gaze fixation and retinal sensitivity are mediated by different brain neural circuits.

Table 1

Microperimetry parameters (MAIA)	Basal	12-month follow-up.	p value
Retinal Sensitivity (dB)	18.36±0.8	17.52 ±0.8	0.2753
Fixation stability P1 (%)	68.29 ± 3.4	58.12 ± 3.9	0.0045
Fixation stability P2 (%)	84.24 ± 3.5	77.06 ± 3.1	0.0040
BCEA63 (°²)	3.67± 0.6	4.90 ± 0.7	0.0287
BCEA95 (°²)	30.99± 4.8	41.42± 6.2	0.0402

Disclosure: **A. Rojano Toimil:** None.

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The impact of type 1 diabetes on brain volumes in neurologically asymptomatic adults

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Background and aims: The impact of type 1 diabetes on brain cortical and white matter volumes is disputed. There are contradicting reports on how age at disease onset affects brain growth - with some studies reporting a negative effect of onset in early childhood while others found a larger impact when diabetes starts in adolescence. We aimed to assess how age at onset of type 1 diabetes affects brain volumes, and, in addition, study whether type 1 diabetes and diabetes-related factors associate with brain atrophy in neurologically asymptomatic adults with type 1 diabetes.

Materials and methods: This cross-sectional sub-study of the Finnish Diabetic Nephropathy Study includes 191 participants with type 1 diabetes and 30 healthy sex- and age-matched controls, mean age 39.8 (33.0-44.8) and 38.4 (32.2-42.9) years, respectively. All participants underwent a thorough clinical work up, brain MRI and imaging analysis as well as segmentation using FreeSurfer 6.0. We examined cerebral cortical and white matter volumes. Absolute volumes were divided by intracranial volume, giving volume fractions which correct for individual size differences and include a measure of atrophy.

Results: Participants with type 1 diabetes had no significant differences in cortical or white matter volume fractions compared to healthy controls. Disease onset before seven years of age was associated with lower cortical (-4.0%, p=0.04), white matter (-5.4%, p=0.01) and intracranial (-5.1%, p=0.01) volumes compared to those with later onset, but not with lower volume fractions. Among participants with type 1 diabetes, 39% showed signs of cerebral small vessel disease and 16% had albuminuria. We observed no differences in volume fractions according to presence or absence of signs of cerebral small vessel diseas or albuminuria. Furthermore, we found no associations between volume fractions and metabolic- or diabetes-related traits, such as diabetes duration, HbA_{1c}, BMI, blood pressure, dyslipidemia, creatinine, or estimated glucose disposal rate.

Conclusion: Early onset of type 1 diabetes is associated with negative effects on attained brain size, but not with brain atrophy. Even though signs of cerebral small vessel disease were common in the type 1 diabetes group, we found no connection with smaller brain volumes in these neurologically asymptomatic adults. Neither metabolic nor diabetes-related traits associated with brain atrophy.

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Reduced cortical gyrification in middle-aged patients with type 1 diabetes is related to lower white matter integrity and worse cognitive performance

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Background and aims: Type 1 diabetes mellitus (T1DM) has been linked to poorer cognitive functioning and cerebral structural changes, although cortical gray matter has not been consistently related to cognitive decrements. Cerebral gyrification, which is important for cognition and is associated with white matter connectivity, has not yet been studied in T1DM patients. Our aim was to investigate local gyrification in T1DM patients and possible associations with cognitive performance and white matter (WM) integrity.

Materials and methods: Fifty-one T1DM patients with (T1DM-MA^+), 53 patients without microangiopathy (T1DM-MA^-), and 49 controls underwent magnetic resonance imaging and neuropsychological assessment. Gyrification was calculated using FreeSurfer 6.0 using the local gyrification index (lGI). Between-group differences were determined using FSL-PALM adjusted for age, sex, systolic blood pressure, and multiple comparisons (Family Wise Error [FWE]). Probabilistic tractography, fractional anisotropy, and apparent diffusion coefficient were determined in MRtrix3. Linear regression in SPSS25 was used to calculate correlations and mediation.

Results: In all T1DM as one group, lGI was reduced bilaterally in occipital and parietal regions, extending into the temporal and superior frontal gyri ($P_{FWE} < 0.05$). This effect was driven by the T1DM-MA^+ group ($P_{FWE} < 0.05$), although a trend towards lower left superior parietal lGI was found in T1DM-MA^- patients (PFWE = 0.053). Reduced lGI was moderately related to poorer overall cognitive abilities ($\beta = 0.219$, $P = 0.025$), as well as to lower WM integrity of the bilateral arcuate fasciculi (left: $\beta = 0.232$ $P = 0.018$; right: $\beta = 0.222$, $P = 0.025$) and forceps major ($\beta = 0.217$, $P = 0.028$), which connect the regions of lower lGI. Reduced WM integrity of these 3 tracts significantly mediated the relationship between lower lGI and poorer cognitive performance in all T1DM patients ($P < 0.05$).

Conclusion: Reduced parieto-occipital lGI was found in T1DM, which was most pronounced in patients with peripheral microangiopathy. Local GI was associated with poorer overall cognitive functioning, but this relationship was mediated by lower WM integrity of tracts connecting the cortical regions showing lower lGI. This study provides additional insights into the relationship between cortical gray matter and cognitive functioning.

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Disclosure: **J. dos Santos Silva:** None.

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Cerebral and peripheral microcirculation in type 2 diabetes and obesity, influence of neuropathy and C-peptide

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Background and aims: Microcirculation is damaged in diabetic patients and it has also been observed in obesity. Damage to microcirculation affects both cerebral and peripheral microvessels and is one of the main pathogenetic factors in the development of neuropathy. Neuropathy may also develop in obesity. C-peptide ameliorate microcirculation and vascular endothelial growth factor (VEGF) is an angiogenic factor. Our main

aim was to investigate the the cerebral and peripheral microcirculation, peripheral neuropathy and to find any association between them and with C-peptide and VEGF level in obesity and type 2 diabetes.

Materials and methods: Participants (diabetic group: 16 female and 24 male, mean age: 50.9±6.9 year, BMI: 32.9±5.1 kg/m^2; obesity group: 18 female and 14 male, mean age: 51.4±1.0 year, BMI: 38.8±6.0 kg/m^2) were recruited from the obesitology and diabetology outpatient department of Internal Medicine, University of Debrecen and were involved just after a written consent accepted by the local ethical committee was obtained. Tc99m HMPAO dynamic SPECT/CT studies were performed to assess cerebral and peripheral microcirculation. Neurometer was used to determine neuropathy and results were given as CPT (current perception threshold) on the basis which three groups of patients - severe, mild and no neuropathy - were created. Non-parametric Spearman correlation tests with FDR corrections were used for statistical analysis.

Results: Leg perfusion was significantly lower in the diabetic group ($p<0.001$) and it correlated significantly with BMI (rho=0.36). According to the presence and severity of neuropathy a significant difference in lower limb microcirculation was detected independently of diabetes and obesity. Surprisingly the results in the severe neuropathy group were only non-significantly decreased compared to patients without neuropathy ($p=0.18$). However, a significant difference between mild neuropathy and no neuropathy group, nevertheless between mild and severe neuropathy group was revealed ($p=0.036$ and $p=0.042$ resectively). In mild and severe neuropathy groups diabetic patients had significantly worse circulation than obese patients ($p=0.033$ and $p=0.041$ respectively). There were no significant differences in hemispheral and regional brain perfusion neither between T2DM and obese patients nor among neuropathy groups. C-peptide level was non significantly lower in mild and higher in severe neuropathy patients compared to those without neuropathy, but significant difference between mild and severe groups was found ($p=0.0066$). VEGF was significantly elevated in severe neuropathy patients compared to no neuropathy group ($p=0.049$). Lower limb microcirculation correlated significantly with C-peptide level ($p<0.05$, rho: 0.29) but not with VEGF level. There was also positive correlation between C-peptide level and cerebral microcirculation ($p<0.05$, rho: 0.27).

Conclusion: C-peptide highly and positively contributes to the changes in lower limb microcirculation in patients with neuropathy. Cerebral microcirculation was not altered in our study, but positive correlation with C-peptide level was found.

Disclosure: **M. Káplár:** None.

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Neuroprotective effects of Zfra 1-31 peptide against high glucose-induced mitochondrial and autophagy defects

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Background and aims: Type 2 diabetes (T2D) is a global epidemic and a major risk factor for cognitive decline and dementia, particularly Alzheimer's disease (AD). Thus, it is of utmost importance to elucidate the mechanisms underlying diabetes-associated neurodegeneration in order to identify new therapeutic targets and/or biomarkers that will allow an early and accurate diagnosis and personalized therapies. Previous studies from our laboratory clearly show that mitochondria are a mechanistic link between T2D and AD. However, the cascade of events associated to mitochondrial anomalies in T2D-associated neurodegeneration and AD remain elusive. In this study, we aimed to evaluate the effect of Zfra 1-31, a specific inhibitor of the putative tumor suppressor WW domain-containing oxidoreductase (WWOX), in neuronal cells exposed to high glucose focusing on its interaction with mitochondria. WWOX is known to participate in several molecular interactions, signaling and apoptotic pathways in many diseases however, the possible involvement of WWOX in hyperglycemia-associated neurodegeneration has never been explored.

Materials and methods: We performed studies in differentiated SH-SY5Y human neuroblastoma cells exposed to high glucose for 48h and treated (or not) with Zfra1-31. Several parameters were analyzed: cell viability, mitochondrial membrane potential ($\Delta\Psi m$) and mitochondrial reactive oxygen species (ROS) production. More, WWOX activation (through phosphorylation at tyr33 residue), mitochondrial biogenesis and dynamics (fission and fusion), as well as, autophagy-related proteins were evaluated by western blot.

Results: High glucose caused an ~75% increase in the levels of activated WWOX (phosphorylated at tyrosine 33), promoted mitochondrial dysfunction characterized by a ~32% decrease in $\Delta\Psi m$ and a 27% increase in ROS production resulting in a ~20% increase in cell death ($p<0.05$ in all parameters when compared with control group). Of note, the activation of WWOX preceded mitochondrial dysfunction and cell death. More, high glucose promoted a ~21% decrease in mitofusin 1 and ~38% decrease in NADH-ubiquinone oxidoreductase chain 1 levels ($p<0.05$), despite the ~70% ($p<0.05$) increase in nuclear respiratory factor 1 and ~55% ($p<0.001$) increase in mitochondrially encoded cytochrome c oxidase I levels, which suggest that the activation of mitochondrial biogenesis try to compensate for the existing mitochondrial anomalies. Moreover, high glucose caused an ~40% decrease in Phosphoinositide 3-kinase class III and Autophagy-Related 7 protein levels ($p<0.05$) suggesting a compromised autophagic clearance particularly at nucleation and elongation phases. Importantly, inhibition of WWOX with Zfra 1-31 prevented the above-mentioned alterations promoted by high glucose.

Conclusion: Altogether our observations show that WWOX activation underlies high glucose-induced neuronal cells damage and death. Moreover, our results support the therapeutic potential of Zfra 1-31 for hyperglycemia/diabetes-associated neurodegeneration.

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Disclosure: **C. Carvalho:** None.

SO 57 All you need to know about atherosclerosis and diabetes

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Safety, efficacy and feasibility of a modified variable rate intravenous insulin infusion regime in treating hyperglycaemia in acute coronary syndrome

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Background and aims: Hyperglycaemia at presentation with acute coronary syndromes (ACS) is a strong and independent risk marker for adverse outcomes post ACS, irrespective of prior diabetes status. Limited evidence suggests a survival advantage from managing admission hyperglycaemia (blood glucose >11mmol/L) in ACS with intravenous insulin infusion. However, hindered by concerns of hypoglycaemia (blood glucose <4.0mmol/L) and pulmonary oedema, less than half of patients with diabetes and only a fifth without a prior diabetes diagnosis receive such infusions when eligible. We examined safety, efficacy and feasibility of the modified, weight-adjusted variable rate intravenous insulin infusion (VRIII) regime, adapted from the TITAN-ACS study, in its management of a real-world population with admission hyperglycaemia and ACS.

Materials and methods: We evaluated care of consecutive patients referred to the in-patient diabetes team following admission to the coronary care unit (CCU) with ACS at a major UK tertiary cardiac centre from January to November 2020. A previous quality improvement project on the management of hyperglycaemia in ACS led to a change in our guidance from the use of a standard VRIII to a modified version. The guidance recommends a weight-adjusted, VRIII regime [administered with 5% Dextrose (30ml/hour) and potassium chloride (40 mmol/L) solution] to be commenced on admission and continued for 24 hours. 36/60 patients, who received VRIII were examined for metabolic and cardiac complications, and 30-day all-cause mortality as a whole and as subgroups based on diabetic status.

Results: As shown in Table 1, the average age was 62.8 (± 13.9 standard deviation), predominantly of male gender (66.7%), White European ethnicity (75%) with known diabetes mellitus (72.2%). Median admission glucose was 16.0 (IQR 14.6-20.2) which improved to 8.6 (IQR 6.9-10.0) after a median 27 hours (IQR 22-37) of VRIII. VRIII was associated with only 1 episode of mild hypokalaemia with no hypoglycaemic or arrhythmic events found. The rates of in-patient pulmonary oedema and 30-day all-cause mortality were 16.7% and 11.1%, respectively. These observations on the efficacy and safety of VRII were evident across the subgroups of patients with and without a prior diagnosis of diabetes (Table 1).

Conclusion: This real-world analysis provides further support of the efficacy, safety and feasibility of the use of the modified, weight-adjusted VRIII regime in managing acute hyperglycaemia in patients with ACS, particularly of ST elevation myocardial infarction (STEMI). No episodes of hypoglycaemia or other adverse events were noted. With increasing prevalence of diabetes and significant advancements in ACS practice, more recent randomised controlled trials are needed to assess the advantage of VRIII in improving patient outcomes across the subtypes of ACS in this contemporary treatment era.

Table 1. Characteristics and outcomes in patients presenting with ACS and acute hyperglycaemia treated with VRIII

Characteristics	All, n=36	Known diabetes, n= 26	Not known diabetes, n= 10
Age (years) (mean ± SD)	62.8 ± 13.9	63.8 ± 14.1	60.0 ± 13.8
Men (%)	24 (66.7)	15 (57.7)	9 (90)
White European (%)	27 (75)	18 (69.2)	9 (90)
STEMI (%)	31 (86.1)	21 (80.8)	10 (100.0)
HbA1c on admission (mmol/mol) (mean ± SD)	78.5 ± 20.9	80.5 ± 19.6	74.0 ± 24.1
Glucose levels on admission (mmol/L) (median, IQR)	16.0 (14.6-20.2)	16.6 (14.6-19.6)	15.5 (14.8-21.0)
Glucose levels at the end of infusion	8.6 (6.9-10.0)	9.0 (6.9-11.1)	7.4 (6.9-8.6)
Hypokalaemia during infusion (%)	1 (2.8)	1 (3.9)	0 (0.0)
Pulmonary oedema (%)	6 (16.7)	5 (19.2)	1 (10.0)
Death (%)	4 (11.1)	4 (15.4)	0 (0.0)
Arrhythmias (%)	2 (5.6)	2 (7.7%)	0 (0.0)

Disclosure: **A.L. Liarakos:** None.

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Estimated risk of atherosclerotic cardiovascular disease and heart failure in type 2 diabetes without previous cardiovascular events

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Background and aims: Therapeutic algorithms of type 2 diabetes mellitus (T2DM) suggest that in patients with very high/high cardiovascular disease (CVD) risk, glucagon-like peptide-1 receptor agonists (GLP1-RAs) are preferred when atherosclerotic cardiovascular disease (ASCVD) predominates and sodium glucose transporter 2-inhibitors (SGLT2-i) are preferred when heart failure (HF) predominates, leaving open therapeutic indications when the CVD history is silent or uncertain.

Materials and methods: We used an observational and cross-sectional approach to assess the CVD risk profile of 1089 consecutive patients with T2DM without established previous or current CVD history attending our diabetes clinic from July 2012 to October 2017. We employed the 10-year ASCVD risk calculator and the QDiabetes calculator for the 10-year HF risk.

Results: The 10-year risk of ASCVD was higher than 10-year risk of HF (20.8±13.8% vs 10.5±7.5%; $p<0.01$) but they were highly correlated ($r=0.743$; $p < 0.001$). In the effort to establish the features of the extreme risk profiles, we segregated our population in quintiles of ASCVD/HF risk ratio. Patients with a relatively highest risk of HF (Q1) were more frequently women, had more severe obesity, longer duration of diabetes, worse glycemic control and were more frequently on insulin therapy than those with relatively highest ASCVD risk (Q5) ($p<0.01$ for all comparisons).

Conclusion: In T2DM without history of CVD events the estimated risk of ASCVD and of HF assessed using commonly available calculators are strongly associated but the risk of ASCVD is consistently higher than the risk of HF.

Disclosure: **R. Cannistraci:** None.

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Coronary calcification and HbA_{1c} in the north Staffordshire cohort of the UK-based national targeted lung health check

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Background and aims: The Targeted Lung Health Check (TLHC) pilot project at the University Hospitals of North Midlands included patients who are at risk of lung cancer to undergo CT thorax (more than a 2% Liverpool Lung Project (LLP) and 1.5% Prostate, Lung, Colorectal and Ovarian (PLCOm2012) risk of lung cancer within the next 5 years). To maximize benefit, the local team undertook additional health checks including: 1. Measuring HbA1c if it had not been previously checked by their general practitioner (GP). 2. Assessment of coronary calcification on CT scan. Both are surrogates for coronary heart disease (CHD) risk. This analysis aimed to evaluate whether those patients whose GPs did not measure HbA1c would run a similar CHD risk (based on both above-mentioned surrogates).

Materials and methods: Inclusion: All patients from the participating 6 general practices, aged 55-74 years with past/current smoking history.

Exclusion: Current cancer patients and those who had already received a chest CT within the last 12 months were outside the remit of this screening program. Statistical analysis: Chi-squared test, using the OpenEpi online stats calculator.

Results: Coronary calcification was evaluated in a total of 316 patients from this 12-month period (04/2019-03/2020). • Coronary calcification was detected in 264 patients (83.5%); including 123 (83.1%) of those who had their HbA1c checked by the GP and 141 (83.9%) who had it checked by the TLHC (p=0.422). • Coronary calcification severity: o GP-instigated HbA1c: Calcification was mild in 43 (35.2%), moderate in 47 (38.5%) and severe in 32 (26.2%) patients. o TLHC-instigated HbA1c: Calcification was mild in 62 (44.3%), moderate in 46 (32.9%) and severe in 32 (22.9%) patients. o There was no statistical difference between the two groups (p=0.329). • Number of coronaries calcified: o GP-instigated HbA1c: Calcification involved one coronary in 18 (14.8%), two coronaries in 26 (21.3%), three coronaries in 41 (33.6%) and four coronaries in 37 (30.3%) patients. o TLHC-instigated HbA1c: Calcification involved one coronary in 25 (18.1%), two coronaries in 32 (23.2%), three coronaries in 37 (26.8%) and four coronaries in 44 (31.9%) patients. o There was no statistical difference between the two groups (p=0.661). • Calcification in participants with raised HbA1c (both 42-47 and ≥ 48): Coronary calcification was prevalent in both GP- (85.7%) and TLHC (80.6%)-instigated HbA1c.

Conclusion: Coronary calcification was very common in this cohort, with >80% prevalence in patients with raised HbA1c. There was no statistically significant difference in the severity of calcification or umber of coronaries calcified between those who had the GP instigating their HbA1c and those who had it under the TLHC. These results provide evidence for:1. The need for proactive diabetes screening in routine care, to avoid missed cases with dysglycaemia.2. The benefits in extending the remit of health checks, e.g. the TLHC, to include surrogates for CHD (HbA1c and coronary calcification) to inform more proactive prevention in these cases.

Disclosure: **S. Raza:** None.

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Disse insulin resistance index is a predictor of hospital outcome of coronary artery bypass grafting in patients with diabetes type 2, prediabetes and normoglycaemia

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Background and aims: To study markers of insulin resistance and their relationship with preoperative status and hospital complications of coronary artery bypass grafting (CABG) in patients with type 2 diabetes (T2D), prediabetes and normoglycemia.

Materials and methods: We included in the study 708 consecutive patients who underwent CABG in 2011-2012. In the absence of previously established diabetes mellitus and borderline fasting hyperglycemia (6.1-6.9 mmol/L (110-125 mg/dL) for venous plasma), an oral glucose tolerance test was performed. Patients were divided into 2 groups - with carbohydrate metabolism disorders (CMD), n=266 and normoglycemia, n=442). Free fatty acids, fasting insulin in plasma were determined, and the indices insulin resistance were calculated. Postoperative complications and their relations with markers of insulin resistance were analyzed.

Results: Screening before coronary artery bypass grafting increased the number of patients with established type 2 diabetes from 15.2% to 24.1%, the number of persons with prediabetes - from 3.0% to 13.4% (p=0,004), the total number of persons with any established disorders of carbohydrate metabolism (MCI) from 18.2% to 37.5% (p=0,032). More than a third of all T2D (36.8%) and the overwhelming majority of prediabetes cases (78.0%) were revealed during an additional preoperative study of the glycemic status. Incidence of significant hospital complications (25.2% vs 17.0%, p=0.007), progression of renal failure in CKD (4,9 % vs 2.0 %, p=0.021), multiple organ failure (4.5% vs 1.7%, p=0.039), extracorporeal homeostasis correction (3.7% vs 1.1%, p=0.020), significant complications of the sternal wound (6.3% vs 2.9%, p=0.018), and urgent surgery on the arteries of the lower extremities (1.5% vs 0%, p = 0.039) was greater in CMD patients. A logistic regression analysis was performed to identify predictors of the endpoint "stay in the hospital after CABG for more than 10 days, or an unfavorable outcome." According to the result of multivariate analysis, the Disse index became a significant predictor of this endpoint in several regression models, adjusted for age, gender, functional class of heart failure, overweight, left atrial size, T2D (odds ratio (OR) 1.060 in one of the models; 95% confidence interval (CI) 1.016-1.105; p = 0.006). Also, in multivariate analysis, the independent predictors of the end point were: female sex, age, body mass index, duration of cardiopulmonary bypass, left atrial size, end diastolic size of the left ventricle, type 2 diabetes, level of free fatty acids (OR 3.335; 95% CI 1.076-10.327 ; p = 0.036). Fasting glucose, insulin, lipid, QUICKI, and Revised-QUICKI levels were not associated with hospital prognosis even at the one-way analysis step.

Conclusion: Screening for CMD before CABG significantly increased the number of the patients with prediabetes and type 2 diabetes. In the group with CMD, there were more frequent hospital complications of CABG. The Disse Index and free fatty acids was an independent predictors of long hospital stay and/or poor outcome.

Disclosure: **N. Bezdenezhnykh:** None.

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Glucose variability is associated with an adverse vascular profile but only in the presence of insulin resistance in individuals with type 1 diabetes

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Background and aims: Glucose variability (GV) has been implicated in the development of vascular complications in type 1 diabetes (T1D) but data have been inconsistent. We hypothesised that the detrimental effect of high GV in people with T1D is mainly evident in those with concomitant insulin resistance.

Materials and methods: A secondary analysis was conducted on pretreatment data from 107 patients with T1D enrolled on four randomised controlled trials. Continuous glucose monitoring data over a period of 7-days provided GV metrics, including standard deviation (SD) and coefficient of variation (CV), as well as time in range (TIR), defined as glucose between 3.9-10.0 mmol/l. HbA1c, body mass index and presence of hypertension were used to calculate estimated glucose disposal rate (eGDR), an insulin resistance marker, using an established formula. We applied an unsupervised, data-driven cluster analysis to classify patients into 3 GV clusters. High insulin resistance was defined as eGDR<6 mg/kg/min. The relationship between eGDR and thrombosis markers including fibrinogen level and plasminogen activator inhibitor-1 level (PAI-1) with GV were investigated.

Results: Of 107 patients, 48, 40, and 19 patients were assigned into low, intermediate, and high GV clusters, respectively. There was no difference in age among 3 clusters (median [IQR] 27.2 [23.3, 30.8] vs 30.5 [24.9,

32.8] vs 29.2 [26.5, 33.6] years, p=0.125). Fibrinogen levels were 1.5 [1.2, 1.8] in low GV cluster rising to 2.4 [1.6, 2.9] and 4.05 [2.8, 4.5] mg/ml, in intermediate and high GV clusters, respectively (p<0.001). PAI-1 levels demonstrated similar patterns at (0.83 [0.58, 1.14], 1.36 [0.89, 1.93] and 2.25 [1.69, 2.44] ng/ml, for low, intermediate and high GV clusters, respectively (p<0.001). When the analysis was conducted according to eGDR (<6 or ≥6 mg/kg/min), as a marker of high and low insulin resistance, the correlations between thrombosis markers and GV were only observed in those with low eGDR as demonstrated in Figure 1. Moreover, the lowest GV cluster was associated with the highest eGDR (8.7 [6.85, 9.6]) compared with intermediate and high GV clusters which showed eGDR of 6.4 [4.5, 9.2] and 4.2 [2.85, 4.9] mg/kg/min, respectively (p<0.001).

Conclusion: Higher GV is associated with increased fibrinogen and PAI-1 levels in young adults with T1D but only in those displaying features of insulin resistance, assessed as eGDR<6 mg/kg/min. Therefore, high GV may only have detrimental vascular effects in the presence of insulin resistance. Future longitudinal outcome studies are required to analyse the effects of GV on development of vascular complication in T1D individuals with and without insulin resistance.

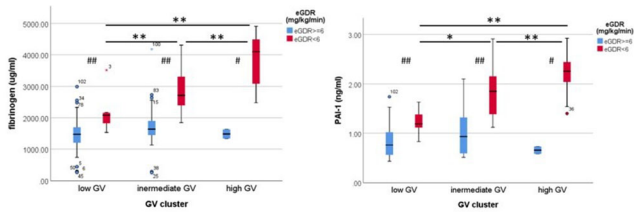

Figure 1 Thrombosis biomarkers by glucose variability (GV) clusters in conjunction with estmated glucose disposal rate (eGDR) cut-off values less or more than 6 mg/kg/min . The *left panel* shows fibrinogen levels, whereas the *right panel* demonstrates plasmingogen activators inhibitor-1 (PAI-1) levels of 107 individuals with type 1 diabetes with different degrees of GV and eGDR (low GV, n=48; intermediate GV, n=40; high GV, n=19). Between-cluster comparison: *p<0.05, **p<0.01; with-in cluster comparison: #p<0.05, ##p<0.01.

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Disclosure: **N. Kietsiriroje:** None.

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The impact of obstructive sleep apnoea on macro- and micro- vascular complications in patients with type 1 diabetes: a population-based retrospective cohort study

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Background and aims: Obstructive sleep apnoea (OSA) is associated with increased risk of cardiovascular disease (CVD), microvascular complications and mortality in patients with Type 2 diabetes (T2D). T1D is also associated with increased risk of OSA. However, whether the coexistence of T1D and OSA increases the risk of incident diabetes-related complications is unknown. Therefore, we aimed to assess the impact of OSA on the macro- and micro- vascular complications in T1D.

Materials and methods: We utilised the Health Improvement Network (THIN), a large UK primary care database, to conduct a retrospective cohort study. Each patient with OSA and T1D (exposed) was matched for age (± 3 years), sex, and body mass index (BMI) (± 3 kg/m^2) to four patients with T1D without OSA (unexposed). Patients with the outcome of interest at baseline were excluded. The study period was from 1 January 1990 to 31 August 2019. A CVD composite of ischaemic heart disease, stroke or transient ischaemic attack, and heart failure was the primary outcome. Secondary outcomes were the individual components of the composite outcomes and microvascular complications. Cox regression was used to calculate hazard ratios using Stata IC version 15.

Results: 483 exposed and 1,139 matched controlled were included. For the whole cohort, mean (SD) age was 50 (14) years, 1,287 (79%) were men, and mean BMI was 31.6 (6.1) kg/m^2. Median [IQR] HbA1c was 67.0 [58.0 to 78.1] mmol/mol for exposed and 66.1 [56.3 to 78.0] mmol/mol for the unexposed. The adjusted hazard ratio (aHR) of composite CVD in the exposed vs. unexposed was 1.92 (95% CI: 1.27 to 2.92; p <0.01). The aHR of incident heart failure was 2.65 (1.42 to 4.94; p <0.01), and ischaemic heart disease was 2.24 (95% CI: 1.31 to 3.86; p <0.01). The aHR of peripheral vascular disease was 2.68 (95% CI: 1.23 to 5.87; p =0.01), and for Chronic kidney disease was 1.54 (1.05 to 2.28; p =0.03). Incident AF was more likely among the exposed compared to the unexposed but this was not statistically significant [HR: 2.09; (0.95 to 4.60), p=0.07).

Conclusion: In patients with T1D, OSA was associated with a significant increase in incident CVD, chronic kidney disease, heart failure, ischaemic heart disease, and peripheral vascular disease. Hence, clinicians should have low threshold to suspect OSA in T1D and to implement effective CVD prevention strategies.

Table 1: Cox proportional hazards models for risk of incidents macro- and microvascular outcomes

Outcome	Crude HR	Adjusted HR
CVD	2.17 (1.47 to 3.22)**	1.92 (1.27 to 2.92)**
-Heart failure	3.23 (1.81 to 5.76)**	2.64 (1.42 to 4.94)**
-Stroke or transient ischaemic attack	0.97 (0.52 to 1.80)	
-Ischaemic heart disease	2.50 (1.51 to 4.14)**	2.24 (1.31 to 3.86)**
Peripheral vascular disease	3.23 (1.54 to 6.79)**	2.68 (1.23 to 5.87)*
Hypertension	0.79 (0.48 to 1.32)	
Atrial fibrillation	2.30 (1.11 to 4.76)*	2.09 (0.95 to 4.60)
Diabetic foot disease	1.10 (0.70 to 1.71)	
Chronic kidney disease	1.69 (1.17 to 2.43)**	1.54 (1.05 to 2.28)*
Sight-threatening retinopathy	1.29 (0.93 to 1.78)	

Adjusted HR: adjusted for baseline age categories, sex, BMI categories, smoking status, Townsend categories (social deprivation), ethnicity, diabetes duration, HbA1c categories, albumin-to-creatinine ratio (ACR) categories, drinking status, and estimated glomerular filtration rate (eGFR) categories; ** : <0.01; * : <0.05.

Disclosure: **Z. Alshehri:** None.

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Cerebral small vessel disease does not associate with blood glucose control in neurologically asymptomatic individuals with type 1 diabetes

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Background and aims: We observed recently that a third of neurologically asymptomatic individuals with type 1 diabetes (T1D) showed signs of cerebral small vessel disease (cSVD). The aim of this study was to determine whether medium- or long-term blood glucose control or glycaemic variability are different in those with and without cSVD.

Materials and methods: We enrolled 189 participants (47.1 % men; median age 40.0, IQR 33.0-45.2 years) with T1D (median diabetes duration of 21.7, IQR 18.3-30.7 years). None of the individuals had signs of neurological or end-stage kidney disease. Three or more HbA_{1c} values (median count 16, IQR 10-23) were collected over the course of 10 years before a visit including a clinical examination, biochemical sampling, and brain MRI. For long-term glucose control, HbA_{1c}-$mean_{overall}$, coefficient of variation (HbA_{1c}-CV), and average real variability (HbA_{1c}-ARV) were calculated for each subject. Further, HbA_{1c}, fructosamine (FA), and glycated albumin (GA) were measured reflecting blood glucose control during a timespan of 1 to 2 months and 2 to 3 weeks, respectively. cSVD consisted of cerebral microbleeds (CMBs), white matter hyperintensities (WMHs), and lacunar infarcts, and were graded from brain MRI.

Results: Signs of cSVD were present in 66 (34.9%) participants. CMBs were present in 45 (23.8%) participants distributed as follows: 144 had no CMBs, 33 had one to two CMBs and 12 had three or more CMBs. HbA_{1c}-$mean_{overall}$ (8.3 [± 0.9] % vs. 8.1 [± 1.0] %, $p = 0.141$), HbA_{1c}-CV (6.7 [5.5 - 8.7] % vs. 7.6 [5.7 - 9.9] %, $p = 0.245$), and HbA_{1c}-ARV (0.5 [0.4 - 0.6] vs. 0.5 [0.3 - 0.7], $p = 0.953$) did not differ in individuals with signs of cSVD compared to those without. No differences in HbA_{1c} (8.2 [7.6-8.9] % vs. 8.0 [7.3-8.8] %, $p = 0.259$), FA (2.6 [2.4 - 2.9] mM/l vs. 2.5 [2.3 - 3.0] mM/l, $p = 0.587$) or GA (97.2 [73.9 - 117.8] nM/ml vs. 89.6 [76.3 - 115.9] nM/ml, $p = 0.704$) were observed in individuals with and without cSVD. Further, no differences in any of the blood glucose variables and cSVD stratified for CMBs or WMHs were detected. Neither were numbers of CMBs associated with the studied measures. Additionally, after dividing the studied variables into quartiles, no association with cSVD was observed.

Conclusion: We observed no association between blood glucose control and cSVD in neurologically asymptomatic individuals with type 1 diabetes. This finding was unexpected considering the large number of signs of cerebrovascular pathology in these people after two decades of chronic hyperglycaemia and warrants a deeper look into their metabolism.

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Disclosure: **J. Inkeri:** None.

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Assessment of metabolic risk following initial presentation to transient ischaemic attack clinic

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Background and aims: Diabetes as well as Transient ischaemic attacks (TIAs) are both well known risk factors for stroke development with recent management of type 2 diabetes care focussing on cardiovascular risk reduction. The aim of this study was to assess the metabolic risk of patients presenting to transient ischaemic attack (TIA) clinic over a five-year follow-up period thereby supporting more rigorous screening of this subgroup.

Materials and methods: A retrospective cohort captured 207 consecutive patients presenting to TIA clinic across a multisite district general hospital in the UK between 1st January 2014 and 1st January 2015. Information was collected from electronic records over the subsequent five years including: HbA1c; lipid profile; further presentation to clinic; relevant comorbidities and mortality.

Results: 207 patients (90 Male: 117 Female, age 63.8 ± 16.6 years) were assessed, 19.8% (41) of whom had type 2 diabetes. 38.6% of the total clinic population (80 patients) had a clinically confirmed TIA or stroke. Of this subgroup of 80 patients, (43 Male: 37 Female, age 70.8 ± 12.4 years) 28.8% (23) had type 2 diabetes, and 8.8% (7) were at risk of type 2 diabetes. 13.0% (3) of those with diabetes, 28.6% (2) at risk and 66% (33) without diabetes did not have HbA1c recorded within six months of presentation.Only 4.3% (1) and 8.6% (2) patients with diabetes and clinically confirmed TIAs did not have total cholesterol and low-density lipoprotein (LDL) measured within six months of presentation, respectively. By comparison, 30% (15) and 36% (18) of patients without diabetes did not have these measured. 46% of patients without diabetes who did have LDL recorded had a level higher than 3.0 mmol/L.Patients with diabetes were more likely to have a subsequent TIA or stroke (p = 0.043). Mortality rate was significantly higher in those who presented initially with TIA or stroke (p = 0.0451).

Conclusion: To the authors' knowledge this is the first data set to assess patients presenting to TIA clinic for metabolic risk factors. One in five of all patients presenting to TIA clinic and just under one in three patients with clinically confirmed TIA had known type 2 diabetes. Only a minority of patients presenting to TIA clinic were screened for underlying diabetes or had an up to date Hba1c check. Total cholesterol and LDL was found to be monitored more consistently in patients with diabetes than without. This highlights the need for universal screening for diabetes (and other metabolic risk factors) in all TIA clinic referrals to promote treatment optimisation.

Disclosure: **V.C. Shaw:** None.

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Prediction of chronic kidney disease in people with diabetes

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Background and aims: People with diabetes in general have a higher risk of developing Chronic Kidney Disease (CKD). The early detection of CKD in people with diabetes helps their health care providers to adjust their therapy to slow down the disease progression to higher stages, prevent complications, and reduce cardiovascular-related conditions. It also takes a great financial burden off the insurance companies in the long run by way of future treatment costs. We have developed a model that uses the historical data from the past 2 years of people with diabetes (PwD) and predicts the chance of them having CKD in the next 3 years.

Materials and methods: Our model employed the XGBoost (eXtreme Gradient Boosting) algorithm. It used more than 860,000 PwD data from the IBM EHR (Electronic Health Records) database for training and optimizing the model parameters. It was then independently verified using more than 500,000 PwD data from the CPRD (Clinical Praxis Research Datalink) database from the UK, and close to 140,000 PwD data from the INPC (Indiana Network for Patient Care) database from the US. We considered an incident of CKD based on the appearance of a relevant ICD code. The major benefit of the XGBoost algorithm in the context of risk prediction based on EHR data is that there is no need for imputation of the missing values.

Results: We evaluated the performance of the Roche XGBoost model for CKD in terms of the area under the receiver operating characteristic curve (AUC). While we obtain comparable results for the US-based data sets (prediction model: $AUC_{Explorys}$=0.83, AUC_{INPC}=0.84), we observe a performance decrease for the validation on CPRD data (AUC_{CPRD}=0.74). However, on all data sources our XGBoost model shows a superior performance when compared to appropriate benchmark algorithms from literature.

Conclusion: The XGBoost model for CKD disease prediction has a superior performance over benchmark models from literature.

Disclosure: **N. Afshar:** None.

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The ceramide- and phosphatidylcholine- based Coronary Event Risk Test 2 (CERT2) and cardiovascular mortality in men and women with type 2 diabetes

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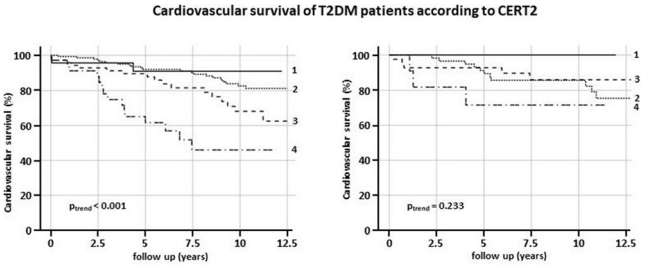
Background and aims: The recently introduced Coronary Event Risk Test version 2 (CERT2) is a validated cardiovascular risk predictor score that uses circulating ceramide and phosphatidylcholine concentrations. We here aimed at investigating the power of CERT2 to predict cardiovascular mortality in 280 male and 121 female patients with type 2 diabetes (T2DM).

Materials and methods: Prospectively, we recorded 55 cardiovascular deaths in men and 19 in women during a mean follow-up time of 7.6±3.6 and 8.1±3.4 years respectively.

Results: Overall, cardiovascular survival decreased with increasing CERT2 risk categories (figure 1). In Cox regression models, CERT2 significantly predicted the incidence of cardiovascular mortality in male patients with T2DM (unadj. HR 1.82 [1.39-2.37] per standard deviation; p<0.001), the unadj. HR in women was 1.36 [0.83-2.22]; p=0.228). After adjustment for age, BMI, current smoking, LDL cholesterol, HDL cholesterol, hypertension, and statin use the HR in men was 1.73 [1.31-2.29]; p<0.001) and in 1.40 [083-2.36]; p=0.210 women. Interaction terms CERT2 x gender were non-significant both in univariate analysis (p=0.354) and after multivariate adjustment (p=0.359).

Conclusion: We conclude that sex does not significantly impact the association of CERT2 with cardiovascular mortality in patients with T2DM.



The Kaplan Meier plot indicates the cardiovascular survival according to the CERT2 risk categories ranging from low risk (1) to very high risk (4) for men (left) and women (right).

Disclosure: **A. Leiherer:** None.

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Type 2 diabetes, chronic kidney disease and major cardiovascular events in patients with established coronary artery disease

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Background and aims: Both type 2 diabetes (T2DM) and chronic kidney disease (CKD) confer a high risk of cardiovascular disease (CVD), and these conditions frequently coincide. The aim of this study was to investigate the single and joint effects of T2DM and CKD on major cardiovascular events (MACE) in a high-risk population of patients with established coronary artery disease (CAD).

Materials and methods: We prospectively investigated 1460 patients with angiographically proven CAD over 10.4±4.8 years, of whom 454 (30.8%) had T2DM and 251 (17.1%) had CKD.

Results: MACE occurred more frequently in T2DM patients than in non-diabetic subjects (40.4% vs 28.7%, p<0.001) and in patients with CKD (eGFR < 60ml/min/1.73m^2) than in those with an eGRF ≥60ml/min/1.73m^2 (51.6% vs 28.3%, p<0.001). When both, T2DM and CKD were considered, 863 subjects had neither T2DM nor CKD, 346 had T2DM

but not CKD, 148 did not have diabetes but had CKD, and 103 had both T2DM and CKD. When compared with the incidence of MACE among patients with neither T2DM nor CKD (25.3%), MACE occurred more frequently in patients with T2DM who did not have CKD (35.8%; p<0.001) as well as in non-diabetic patients with CKD (47.6%; p<0.001) and occurred most freuently in patients with both, T2DM and CKD (57.4%; p<0.001), in whom the incidence of MACE was higher than in those with T2DM but not CKD (p<0.001) or those without T2DM but with CKD (p=0.025); the incidence of MACE was higher in non-diabetic CKD patients than in T2DM patients who did not have CKD (p=0.041). In Cox regression analysis, T2DM (HR=1.46 [1.20-1.78]; p<0.001) and CKD (HR=1.81 [1.45-2.27]; p<0.001) were mutually independent predictors of MACE after multivariate adjustment.

Conclusion: We conclude that T2DM and CKD are mutually independent risk factors for MACE in patients with established CAD. CAD patients with both CKD and T2DM are an extremely high risk for MACE.

Disclosure: **L. Sprenger:** None.

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Combined dulaglutide-dapagliflozin treatment improves vascular dysfunction and albuminuria vs DPP4 inhibitors independently of glycaemic control

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Background and aims: Dulaglutide, a glucagon-like peptide-1 receptor agonist (GLP-1RA), and dapagliflozin, a sodium-glucose cotransporter-2 inhibitor (SGLT-2i), each have favorable effects on diabetic nephropathy and macrovascular diabetic complications. The aim of this study is to determine whether their combination exerts greater improvements on arterial stiffness, endothelial function and albuminuria in type 2 diabetes mellitus (T2DM) compared to treatment with dipeptidyl-peptidase 4 inhibitors (DPP-4is).

Materials and methods: Overall 37 patients with T2DM were included in our study. 21 patients were transitioned from DPP-4is to dulaglutide and dapagliflozin and were followed immediately prior (baseline) and 4 months after the initiation of the combination. 16 patients, matched for sex, age and glycemic control, remained on treatment with DPP-4is (control group). In each visit we measured a) Carotid-femoral PWV b) central systolic blood pressure (cSBP) c) perfused boundary region (PBR) of the sublingual arterial microvessels, d) urinary albumin-to-creatinine ratio (UACR), e) glycosylated hemoglobin (HbA1c).

Results: Both groups had similar cardiovascular markers, UACR and HbA1c at baseline (p>0.05). After a 4-month treatment period, patients on dulaglutide/dapagliflozin combination improved HbA1c (7.9±1.5% vs 6.59±0.6%, p<0.001), PBR (2.3±0.3 vs. 2.1±0.2 μm, p<0.05), PWV (11.9 ±0.3.5 vs. 10.9±2.2m/s, p<0.05), cSBP (128.5±23.6 vs. 121.1±15.7 mmHg, p<0.05) and UACR (413.66±352.57 vs. 248.06±203.5 mg/g, p<0.001). There were no statistically significant differences in PBR (2.1 ±0.3 vs. 2.2±0.3 μm, p>0.05), PWV (10.7±3.4 vs. 12±3.3m/s, p>0.05), cSBP (125.4±21.2 vs. 127±20.1 mmHg, p>0.05) and UACR (240.8 ±103.6 vs. 204.9±119.6 mg/g), in patients who remained on DPP-4is, despite improvement of HbA1c (8.2±1.9% vs 7.3±1.3%, p<0.01).

Conclusion: The combination of dulaglutide and dapagliflozin as an add-on treatment to metformin improves arterial stiffness, endothelial glycocalyx and albuminuria after four months treatment compared to DPP-4is in patients with T2DM .

Disclosure: **E. Korakas:** None.

SO 59 Vascular complications: mechanisms and risk factors

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Diabetic retinopathy and skin tissue advanced glycation end products are biomarkers of vascular events in type 2 diabetic patients: results of the prospective precised study

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Background and aims: Vascular events (VE) are the main cause of mortality in patients with type 2 diabetic patients (PwT2D). However, the risk of VE is not homogeneous in PwT2D and, therefore, an early identification of diabetic patients at high risk of developing VE remains a challenge. The aim of this study is to evaluate whether the presence of diabetic retinopathy (DR) and accumulation of advanced glycation end products (AGEs) in subcutaneous tissue can help to identify those patients at high risk of VE.

Materials and methods: It was a prospective case-control study comprising 200 subjects with T2D with no history of clinical cardiovascular disease and 60 non-diabetic controls, matched by age and sex. The inclusion period began on September 2014 and finished on June 2017. We collected basal features of the subjects, classical cardiovascular risk factors (i.e. age, sex, hypertension, dyslipidemia and coronary artery calcium score [CACs]), presence and degree of DR, and the accumulation of advanced glycation end products in subcutaneous tissue using the AGE reader™ device (DiagnOptics Technologies). We followed these subjects until December 2020, collecting any coronary, cerebrovascular or peripheral arterial event.

Results: After a follow up of 4.35 ± 1.43 years, a total of 24 VE were registered. There was no significant difference regarding age and gender between PwT2D and the control group. The number of VE was higher in PwT2D than in the control group (12.3% vs. 1.75%). When analyzing the risk factors we found that apart from classic risk factors such as age, gender and CACs, PwT2D with VE presented a higher prevalence of DR (47.8% vs. 24.4%; p=0.018) and AGEs in subcutaneous tissue (63.15% vs 26.71% of values in the higher tertile, p=0.001).

Conclusion: As we expected, patients with T2D have significantly more vascular events than non-diabetic subjects. Apart from the classic factors such as age, sex and CACs, we observed that the presence of DR and a high levels of AGEs in subcutaneous tissue were predictors of vascular events.

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Disclosure: **A. Planas Vilaseca:** None.

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Type 2 diabetes and risk of major cardiovascular events in peripheral artery disease versus coronary artery disease patients

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Background and aims: The prevalence of type 2 diabetes (T2DM) is higher in peripheral artery disease (PAD) than in coronary artery disease

(CAD) patients, and PAD overall confers higher cardiovascular risk than CAD. How the incidence of major cardiovascular events compares between PAD and CAD patients when analyses are stratified by the presence of type 2 diabetes (T2DM) is unclear and is addressed in the present study.

Materials and methods: We prospectively recorded major cardiovascular events and death over 10.0±4.7 years in 923 patients with stable CAD, of whom 26.7% had T2DM and in 292 patients with PAD, of whom 42.1% had T2DM. Four groups were analyzed: CAD patients without diabetes (CAD/T2DM-; n=677), CAD patients with T2DM (CAD/T2DM+; n=246), PAD patients without diabetes (PAD/T2DM-; n=169) and PAD patients with T2DM (PAD/T2DM+; n=123).

Results: When compared to the incidence of MACE in CAD+/T2DM- patients (25.1%), it was significantly higher in CAD+/T2DM+ patients (35.4%; p<0.001), in PAD+/T2DM- patients (30.2%; p=0.022) and in PAD+/T2DM+ patients (47.2%; p<0.001). Patients with both PAD and T2DM in turn were at a higher risk than CAD+/T2DM+ or PAD+/T2DM- patients (p=0.001 and p<0.001, respectively). The incidence of MACE did not differ significantly between PAD+/T2DM- and CAD+/T2DM+ patients (p=0.413). Compared to patients with CAD, Cox regression analyses after multivariate adjustment showed an adjusted hazard ratio of 1.46 [1.14-1.87], p=0.002 for the presence of PAD. Conversely, T2DM increased the risk of MACE after multivariate adjustment in CAD and PAD patients (adjusted HR 1.58 [1.27-1.98], p<0.001).

Conclusion: In conclusion, our data show that T2DM and the presence of PAD are mutually independent predictors of MACE. Patients with both PAD and T2DM are at an exceedingly high risk of MACE.

Disclosure: **C. Saely:** None.

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The role of visceral and subcutaneous adipose tissue distribution determined by CT in the development of subclinical atherosclerosis in type 2 diabetes and obesity

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Background and aims: Abdominal obesity and type 2 diabetes mellitus are important risk factors for cardiovascular disorders. Visceral adipose tissue (VAT) means greater cardiometabolic risk than subcutaneous adipose tissue (SAT). Carotid artery intima-media thickness (cIMT) indicates subclinical atherosclerosis and should also be considered as a cardiovascular risk factor. The aim of this study was to investigate the association between body fat distribution, especially the VAT/ SAT ratio (VSR) and anthropometric parameters and to examine the effect of VSR on metabolic condition and cIMT of patients diagnosed with obesity and type 2 diabetes.

Materials and methods: We included 42 patients with obesity (BMI: 37.6 kg / m^2, mean age: 52.1 years) and 50 patients with type 2 diabetes (BMI: 33.6 kg / m^2, mean age: 50.7 years) in our study. Quantification of visceral and subcutaneous fat tissue was performed in axial planar CT images taken at the level of LI vertebra. Within the slice, different regions of adipose tissue were segmented after manually selecting ROIs (region of interest). cIMT was determined by B-mode doppler ultrasound. Lipid panel, HbA1c and fasting plasma glucose were also measured. Non-parametric Spearman correlation tests with FDR corrections were used for statistical analysis.

Results: For the overall group of patients our study showed significant positive correlation between the amount of visceral and more strongly of subcutaneous fat tissue and body mass index (BMI) (p<0.01 and p<0.001 respectively). Consequently we found a significant negative relationship of VSR with BMI (rho=-0.45, p<0.001). We revealed that VSR was increasing significantly with age (p<0.05) and these changes in VSR were accompanied by significant elevation of blood glucose and HbA1c levels (rho=0.28, p <0.01 and rho=0.26, p<0.05 respectively). In obese patients in paralel with increasing VSR higher triglycerid and lower HDL-C values were detected (rho=0.45, p<0.01 and rho=-0.39, p<0.05 respectively). Regarding subclinical atherosclerosis significantly higher mean and maximum IMT in association with increasing VSR was proved not only in diabetic but even more prominently is obese patients (p<0.01 and p<0.001 respectively).

Conclusion: Increasing BMI is accompanied by more prominent fat tissue accumulation in the subcutaneous than in the visceral region. Elevation of VSR has negative impact on blood glucose control and in obesity on HDL-C and triglycerid levels. The positive correlation of VSR with cIMT indicates the role of VAT in the development of subclinical atherosclerosis.

Disclosure: **R. Esze:** None.

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Elevated serum transferrin may play a vasoprotective role in individuals with type 2 diabetes

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Background and aims: Individuals with type 2 diabetes mellitus (T2D) show alterations in iron metabolism hallmarked by elevated serum iron and ferritin levels. Severely elevated iron levels (e.g. transferrin saturation>60%) promote endothelial dysfunction and atherosclerosis in patients with the iron overload disease hereditary haemochromatosis and corresponding disease models. In this study, we investigated if mildly elevated iron levels in T2D contribute to endothelial dysfunction and atherosclerosis.

Materials and methods: Participants from the HEIST-DIC study (225 with T2D, 37 with prediabetes, and 106 nondiabetic controls) were analyzed. Serum iron indices were analyzed for associations with indices of vascular dysfunction (intercellular adhesion molecule-1 (ICAM1) and vascular-cell adhesion molecule-1 (VCAM1)) and atherosclerosis (carotid intima-media thickness (cIMT) and calculated pulse-wave velocity (cPWv)). We also histologically analyzed blood vessels from 12 individuals with diabetes and 12 nondiabetic controls for iron deposition. Furthermore, serum samples from 40-42 weeks-old lean and diabetic (db/db) mice with and without iron oveload ($Fpn^{wt/C26S}$) were analyzed.

Results: Among the iron indices in the T2D cohort, ferritin and transferrin levels were elevated, while transferrin saturation was decreased and iron levels were not different. The histological analysis of blood vessels did not show any overt iron deposition in T2D. In the univariate regression analysis, ferritin levels were significantly associated with cIMT (r=0.037; P=0.007) and cPWv (r=0.051; p<0.001) while VCAM1 was significantly associated with transferrin (r=-0.247; p=0.027). In the multivariate regression, serum ferritin was no longer associated with cIMT (r=0.01; P=0.468), or cPWv (r=0.016; P=0.162). However, serum transferrin retained its significant negative association with VCAM1 (r=-0.269; P=0.026). Interestingly, serum transferrin also correlated negatively with hsTroponinT (r=-0.045; P=0.024), eGFR (r=0.001; P=0.004) and LDL-cholesterol levels (r=-0.07; P=0.005). By contrast, a comparison of

lean and diabetic Fpn^{C326S} mice revealed significantly higher serum iron and transferrin levels, but the levels of ICAM, VCAM, and VEGF were not different.

Conclusion: In summary, we find that mice and humans with diabetes show perturbations in circulating iron indices as evidenced by higher serum iron, ferritin, and transferrin levels. Of note, elevated iron levels in T2D still remain within the physiological range and do not affect vascular function. This strongly suggests that an aggravation of atherosclerosis depends on the presence of non-transferrin-bound-iron that is generated when the transferrin saturation exceeds 60%, as demonstrated previously. We propose that elevated serum transferrin levels in patients with diabetes type 2 may act as a physiological high-affinity chelator that inhibits the appearance of free iron. Such buffering of circulatory iron may protect vessels from damage.

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Disclosure: **A. Ruban Agarvas:** None.

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Long-term palmitate treatment attenuates endothelial barrier via increased mitochondrial reactive oxygen species and malondialdehyde

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Background and aims: Obesity and associated hyperlipidemia are the major risk factors for peripheral insulin resistance and type 2 diabetes. Whereas endothelial insulin resistance is manifested by oxidative stress, increased endothelin-1 and lower NO production, little is known whether it alters the endothelial barrier. Malondialdehyde (MDA) is an established cardiovascular marker of increased oxidative stress and lipid peroxidation, but how it affects endothelial barrier is also unknown. We explored whether long-term palmitate treatment elicits oxidative stress and MDA production to deteriorate insulin signaling and barrier integrity of human vascular endothelial cells.

Materials and methods: HUVECs were treated with various concentrations (0.3-1 mM) of albumin-conjugated palmitate for several days and subjected to time-lapse microscopy, on-line measurements of transendothelial electric resistance (TER), FITC-dextran diffusion assay, reactive oxygen species (ROS, by DCF-DA) and NO (by DAF-AM) detection, and western blots to assess insulin signaling and MDA-modified proteins. To compare palmitate effects to those of hyperglycemia, cells were alternatively treated with high glucose (28 mM), or briefly by exogenous MDA or methylglyoxal (MGO, 50-250 uM for up to 5 hrs).

Results: High-palmitate (0.8 mM) exerted bi-phasic effect on the baseline TER of HUVEC monolayers, while high glucose had no effect. Palmitate initially augmented TER via increased basal NO production by eNOS, yet blunting its activation by insulin (i.e., causing insulin resistance). Subsequently, palmitate attenuated the palmitate-induced TER, as compared to the untreated cells, and this was associated with gradual accumulation of ROS and MDA, both prevented by the mitochondria-targeted antioxidant SkQ1. Exogenous MDA dose-dependently disrupted TER, increased FITC-dextran diffusion, and augmented MDA-modified proteins, while MGO had no such effects. The deteriorating effect of MDA was mirrored by reduced insulin activation of eNOS and NO production, although the basal insulin cascade and eNOS activities were not affected.

Conclusion: These results suggest that palmitate effects on vascular endothelium are time-dependent and switch from initial NO-mediated protection of the barrier to its impairment by the build-up levels of ROS and MDA, which becomes deleterious to endothelial barrier independent of basal NO production.

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Disclosure: **A.V. Vorotnikov:** None.

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Vascular complications in patients with type 2 diabetes and associated factors in Maghreb (Algeria and Tunisia cohort of the DISCOVER study programme)

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Background and aims: Patients with type 2 diabetes mellitus (T2D) are at high risk of vascular complications whose burden at early stages of T2D progression is not well described in Maghreb. This report aimed to assess the prevalence of microvascular and macrovascular complications and their risk factors in Algeria and Tunisia cohort from the DISCOVER program; a prospective, observational study of 15,992 patients with T2D initiating second-line therapy, conducted across 38 countries.

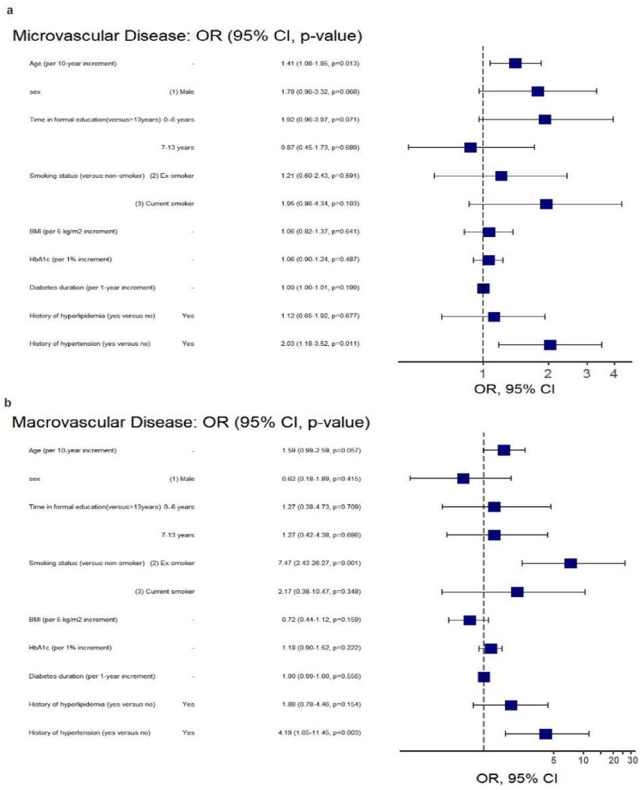
Materials and methods: Baseline cross-sectional analysis of Algeria and Tunisia cohort, describing the prevalence of microvascular and macrovascular complications and their associated factors in T2D patients initiating 2nd line therapy in real world settings. Data were collected using a standardized case report form. Prevalence of microvascular and macrovascular complications at baseline were assessed crude and were standardized for age and sex. Multivariable analysis was used for the factors associated with complications' prevalence.

Results: 504 patients were included from 29 investigational sites. Mean age was 55.2 years SD[10.3 years] and 53.4% were Male. At baseline mean time since T2D diagnosis was 5.8 years SD[4.8 years] and mean HbA_{1c} was 8.5% SD[1.57%]. T2D first line therapy was 77.4% Metformin monotherapy, and 10.1% Metformin + Sulfonylurea. The crude prevalence of microvascular and macrovascular complications was 21.6% and 7.1% respectively. The most reported microvascular complications were albuminuria (7.5%), peripheral neuropathy (7.5%) while the most reported macrovascular complication was coronary artery disease (5.2%). Hypertension and hyperlipidemia were present in 36.1% and 26% of cases respectively. 31.5% of patients were taking statin and 24.4% were on aspirin. The age-and sex-standardized prevalence of microvascular complications was 19.95% (95% CI 18.93%-21.02%) and the age- and sex-standardized prevalence of macrovascular complications was 5.78% (95% CI 5.21%-6.41%). Factors significantly associated with microvascular complications were age (per 10-year increment) (OR, 1.14; 95% CI, 1.08-1.85) and history of hypertension (OR,2.03; 95% CI, 1.18-3.52). Factors significantly associated with macrovascular complications were history of smoking (OR, 7.47; 95% CI,2.43-26.27) and history of hypertension (OR,4.19; 95% CI, 1.65-11.45).

Conclusion: Even at an early stage of T2D progression, vascular complication were substantially present which highlights the importance of early

cardiovascular assessment, prevention and risk factors modification in T2D management strategies.

Figure. Multivariable analysis of factors associated with a microvascular and b macrovascular complications. BMI body mass index, CI confidence interval, HbA1c glycated hemoglobin, OD odds ratio.



Clinical Trial Registration Number: NCT02322762

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Disclosure: **R. Malek:** None.

SO 60 Hepatic fibrosis: from screening to treatment

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Non invasive blood based biomarkers as screening tools for hepatic fibrosis in subjects with type 2 diabetes

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Background and aims: NAFLD is dramatically increased in parallel with the pandemic of Type 2 Diabetes Mellitus (T2DM). We aimed to assess the performance of the most commonly used non-invasive blood biomarkers for liver fibrosis in subjects with T2DM.

Materials and methods: We investigated 120 consecutive subjects with T2DM attending the Diabetic Outpatient Clinic at an Academic Hospital in Athens, Greece. All had demographic, clinical and biochemical data recorded. Hepatic Steatosis (HS) was estimated by Magnetic Resonance Imaging determined by Proton Density Fat Fraction Software (MRI-PDFF) and defined as the percentage of total liver fat divided by the liver volume. HS of <5% was considered abnormal. Liver Stiffness Measurement (LSM) was estimated by Two Dimensional Shear Wave Elastography (2D SWE) (Supersonic Imagine, Aix-en-Provence, France). The PNPLA3 (I148M) variant was evaluated by standard molecular techniques. FIBROMAX, APRI Index, NAFLD Fibrosis score, BARD score, FIB-4 Index were calculated.

Results: 97 subjects (80.8%) had HS of >5%. Only 16 subjects (14%) had LSM >8.0 kPa. Among APRI score (p=0.0010, NAFLD Fibrosis score (p=0.408), FIB-4 Index (p=0.658), BARD score (p=0.701), FibroTest (p=0.921), FibroTest was diagnostically closer to LSM(SWE). LSM(SWE) was directly correlated with both ActiTest (r=0.405, p≤0.001) and NashTest2(r=0.299, p=0.002). ActiTest predict subjects need to perform LSM(SWE) by 5.632 times (p≤0.001, C.I.3.213-8.051) and NashTest2 by 3.981 times (p≤0.001, C.I.2.398-5.563).

Conclusion: Subjects with T2DM may require predictive models for hepatic fibrosis specifically developed for them.Extrapolation of results from non diabetic population may result in misclassification.

Disclosure: **A. Meritsi:** None.

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Anti-fibrotic potential of a novel long-acting glucagon/GIP/GLP-1 triple agonist (HM15211) in preclinical models of fibrosis

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Background and aims: Fibrosis due to nonalcoholic steatohepatitis (NASH) remains a major cause of liver-related mortality. Since complex biological pathways are involved in fibrosis progression, multi-disciplinary therapeutic approaches should be required to effectively deliver treatment effects on fibrosis. For this purpose, we developed a novel long-acting Glucagon/GIP/GLP-1 triple agonist, HM15211. Here,

we evaluated the anti-fibrotic effect of HM15211 in various animal models of fibrosis.

Materials and methods: In the first study, to induce cholestasis-induced liver fibrosis, anesthetized mice were subjected to the ligation of common bile duct. 2 days after surgical procedures, the mice were administered either with HM15211 or obeticholic acid (OCA) for 2 weeks. In a second study, to induce liver inflammation with fibrosis, thioacetamide (TAA) was concomitantly administered to the mice fed with high-fat and -fructose diet (AMLN/TAA mice) for 16 weeks, and HM15211 was subcutaneously administered during last 8 weeks. In a third study, mice were fed with choline-deficient and high fat diet (CD-HFD mice) for 16 weeks to establish diet-induced NASH and fibrosis model, and HM15211 was subcutaneously administered during last 8 weeks, and acylated GLP-1 or GLP-1/GIP agonist were used as comparative control. At the end of treatment, liver tissues and blood samples were prepared, and the degree of fibrosis was determined by measuring fibrosis related markers and histologic analysis.

Results: In BDL mice, HM15211 treatment showed greater reduction in hepatic hydroxyproline (-21.9 and -43.7% *vs.* vehicle for OCA and HM15211) and fibrosis score (1.7, 1.8 and 1.0 for vehicle, OCA, and HM15211) compared to OCA. In addition, HM15211 treatment was also associated with more reduction in hepatic fibrosis marker gene expression such as TGF-β (-47.8 and -62.8% *vs.* vehicle for OCA and HM15211), α-SMA (-51.6 and -77.7% for OCA and HM15211), and collagen-1α1 (-11.3 and -51.6% for OCA and HM15211). In AMLN/TAA mice, HM15211 treatment not only significantly reduced hepatic (-53.9, -41.4 and -51.9 % *vs.* vehicle for α-SMA, TIMP-1 and collagen1a1 expression) and blood (-49.3, -48.0 and -49.1% *vs.* vehicle for TIMP-1, PIIINP and hyaluronic acid level) surrogate markers for fibrosis, but also showed a significant reduction in hepatic hydroxyproline (-53.1% *vs.* Veh) and sirius red positive area (-70.6% *vs.* Veh). Next, anti-fibrotic effect of HM15211 was further evaluated in CD-HFD mice. Strikingly, greater reduction in hepatic hydroxyproline and collagen contents (-4.2, -10.0, -31.2% vs. vehicle for acylated GLP-1, acylated GLP-1/GIP, HM15211) was observed compared to acylated GLP-1 or acylated GLP-1/GIP in CD-HFD mice.

Conclusion: HM15211 treatment was associated with robust improvement in fibrosis across animal models of fibrosis. Based on these results, HM15211 may be a novel therapeutic option for liver fibrosis in addition to NASH itself. On-going human efficacy study will assess the clinical relevance of these findings.

Disclosure: **J. Kim:** None.

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Anti-fibrotic effect of a novel long-acting GLP-1/GCG/FGF21/anti-cytokine tetra-specific drug (OGB21502) in CCl_4-induced liver fibrosis mice

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Background and aims: Liver fibrosis is an abberant wound healing process that features apotosis of hepatic cells, infiltration of the mononuclear cells and other immune cells, activation of macrophages and hepatic stellate cells, and changes in ECM properties. The complex interaction between different cell types with network of cytokine-induced signaling provokes scarring and sustains the vicious cycle. Given the complexity of the pathophysiology, therapeutic strategies simultaneously targeting multiple aspects of fibrotic liver may provide effective treatment to fibrosis as well as NASH. Therefore, we developed a novel long-acting and multi-target drug (GCGR, GLP-1R, FGFR1/KLB, cytokineR), OGB21502. Glucagon-like peptide-1 (GLP-1) and glucagon (GCG) combination suppresses lipogenesis thereby reducing fat accumulation and pro-inflammatory responses at liver. FGF21, an endogenous metabolic hormone, is a key regulator of carbohydrate and lipid metabolism, with potential anti-fibrotic effects. Anti-cytokine antagonises the pro-inflammatory cytokine playing an important role in the regulation of inflammatory responses. UniStac platform is a dedicated technology to develop drugs with more than three targets. OGB21502 is a UniStac-based novel long-acting multi-target drug that prevents inflammation and fibrosis of liver tissue by simultaneously controlling multiple targets related to apoptosis, macrophage activation, and fibroblast activation. Here, we evaluate the effect of OGB21502 in liver fibrosis using CCl_4-induced mice model.

Materials and methods: To induce liver fibrosis, CCl_4 in corn oil was administered 24 times by I.P. injection to a strain of Balb/c mice for 8 weeks, and OGB21502 was S.C. administered during the last 4 weeks of treatment. Ocaliva was used as comparative control. At the end of treatment, the liver tissue samples were prepared, and the degree of fibrosis was determined by measuring hydroxyproline contents. Additional liver tissue samples were subjected to H&E and Sirius red staining, followed by histological grading. Quantitative PCR analysis was performed to determine the hepatic fibrosis marker for gene expression.

Results: In CCl_4 induced mice (8 weeks induction), OGB21502 treatment led to significant decrease in blood ALT (-38.1% vs. vehicle); hepatic hydroxyproline (-31.5% vs. vehicle) and total bilirubin in blood (-26.4% vs. vehicle). Histopathological analysis indicated that OGB21502 meaningfully reduced fibrosis score (-37.5% vs. vehicle); hepatocyte ballooning score (-50% vs. vehicle) and inflammation score (-16.7% vs. vehicle). To emphasize, while all of the vehicle progressed to cirrhosis, 80% of the population administered OGB21502 at high concentrations had cirrhosis progression inhibition. Also, OGB21502 reduced the level of picosirius red positive area (-32.5% vs. vehicle) and collagen I (-23.7% vs. vehicle). In addition, the level of TGF-β (-27.2% vs. vehicle); α-SMA (-32.3% vs. vehicle); LOLX-2 (-42.2% vs. vehicle) and collagen (-34.4% vs. vehicle) in liver were significantly decreased in the OGB21502 treatment group.

Conclusion: Based on these results, OGB21502 offer therapeutic anti-inflammatory and anti-fibrotic effects. OGB21502, based on UniStac technology, induce synergies of multiple targets, improving NASH and fibrosis.

Disclosure: **M. Kim:** None.

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Therapeutic effect of a novel long-acting GLP-1/GCG/FGF21/anti-cytokine tetra-specific drug (OGB21502) in MCD diet-fed NASH mice model

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Background and aims: Non-alcoholic steatohepatitis (NASH) is a liver inflammation and damage caused by a buildup of fat in the liver. It is characterized by varying degrees of hepatic steatosis, cytoskeletal damage, and lobular inflammation with or without fibrosis. NASH is rapidly emerging as a major public health problem, but there is currently no FDA-approved drug to treat these patients. This has prompted substantial efforts to identify novel pharmacological concepts for correcting the underlying metabolic deficits and thereby alleviate, or prevent, hepatic fibrosis in NASH. In this respect, we developed a novel long-acting and multi-target drug (GCGR, GLP-1R, FGFR1/KLB, cytokineR), OGB21502. Glucagon-like peptide-1 (GLP-1) and glucagon (GCG) suppresses lipogenesis thereby reducing fat accumulation and pro-inflammatory responses at liver. FGF21, an endogenous metabolic hormone, is a key regulator of carbohydrate and lipid metabolism, with potential anti-fibrotic effects. Anti-cytokine ligand antagonizes the pro-inflammatory cytokine, playing an important role in the regulation of inflammatory responses. The UniStac is a dedicated platform to develop

drugs with more than three targets. OGB21502 is a UniStac-based tetra-specific drug in which clinically well-validated targets for NASH are covalently attached to albumin. In the progress of NASH, fat accumulation causes apoptosis of liver cells. In this process, macrophages such as Kupffer cells secrete various cytokines, resulting in tissue inflammation. Apoptosis and tissue inflammation activate hepatic stellate cells (HSC) and fibrosis progresses. OGB21502 is a novel multi-target drug that prevents inflammation and fibrosis of liver tissue by simultaneously controlling liver microenvironment (LME) such as apoptosis, macrophage activation, and fibroblast activation. Here we evaluate the effect of OGB21502 in NASH and fibrosis using MCD diet-fed mice.

Materials and methods: To induce NASH, the mice were fed with methionine-choline deficient diet (MCD) for 8 weeks, and OGB21502 was subcutaneously administered during the last 4 weeks. of feeding. Liraglutide (Saxenda, Eli lilly) and dulaglutide (Trulicity, Eli lilly) was used as comparative control. At the end of treatment, the liver tissue samples were prepared, and the degree of hepatic steatosis and lobular inflammation was determined by measuring transforming growth factor-β (TGF-β) and liver fibrosis related cytokine, respectively. Additional liver tissue samples were subjected to H&E staining, followed by histological grading.

Results: In MCD diet-fed mice (8 weeks induction), OGB21502 treatment led to significant decrease in blood ALT (-45.2% vs. vehicle). Histopathological analysis indicated that OGB21502 meaningfully reduced NAS score (NAFLD activity score, -56.5% vs. vehicle); steatosis score (-57.1% vs. vehicle) and inflammation score (-84.6% vs. vehicle). Compared to vehicle, 100% of OGB21502 dramatically showed "Not NASH" histopathological results. Also, the level of TGF-β (-39.9% vs. vehicle) and α-SMA in liver were significantly decreased in the OGB21502 treatment group.

Conclusion: Based on these results, OGB21502 offer therapeutic anti-inflammatory and NASH improvement benefits. Multi-target approach created by UniStac technology offer a therapeutic potential for NASH and fibrosis.

Disclosure: **R. Kim:** None.

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Growth differentiation factor-15 as a mediating factor in the association between type 2 diabetes and liver fibrosis in NAFLD

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Background and aims: Type 2 diabetes mellitus (T2DM) is a strong risk factor for liver fibrosis in patients with non-alcoholic fatty liver disease (NAFLD). It remains uncertain why T2DM increases the risk of liver fibrosis. Recently, it has been suggested that growth differentiation factor-15 (GDF-15) concentrations increase the risk of liver fibrosis. We aimed to investigate a) if GDF-15 concentrations were a mediating factor in the relationship between T2DM and liver fibrosis and b) what factors linked with T2DM are associated with increased GDF-15 concentrations in patients with NAFLD.

Materials and methods: Ninety-nine patients with NAFLD (61% men, 42.4% T2DM) were studied. Serum GDF-15 concentrations were measured by electro-chemiluminescence immunoassay. Vibration-controlled transient elastography (VCTE)-validated thresholds of ≥8.2 and ≥9.7kPa were used to assess liver fibrosis (≥F2 or ≥F3 fibrosis respectively). Regression modelling, receiver operator characteristic curve analysis and Sobel mediation analyses were used to test associations, risk predictors and mediators respectively.

Results: Patients with NAFLD and T2DM (n=42) had higher serum GDF-15 concentrations [mean(SD): 1271.0(902.1) vs. 640.3(332.5) pg/ml, p<0.0001)], and a higher proportion had VCTE assessed ≥F2 fibrosis (48.8 vs. 23.2%, p=0.01) than those without T2DM. GDF-15 was strongly and independently associated with liver fibrosis (p=0.001), and GDF-15 was the most important single factor predicting ≥F2 or ≥F3 fibrosis (≥F2 fibrosis AUROC 0.75, (95%CI 0.63-0.86), p<0.001, with sensitivity, specificity, positive predictive (PPV) and negative predictive (NPV) values of 56.3%, 86.9%, 69.2% and 79.1% respectively). GDF-15 was a mediating factor in the association between T2DM and ≥F2 fibrosis (Sobel test statistic 3.26, p=0.001). HbA1c concentrations alone explained most (30%) of the variance (p<0.0001) of the variance in GDF-15 concentrations (**Table 1 - model 1**). A model that included HbA1c and other factors associated with T2DM (including metformin treatment) explained 60% of the variance in GDF-15 concentrations (p<0.0001) (**Table 1 - model 2**).

Conclusion: GDF-15 concentrations are a potential mediating factor in the association between T2DM and liver fibrosis in NAFLD. HbA1c concentrations explain a large proportion of the variance in GDF-15 concentrations and these data suggest that an increase in HbA1c concentration associated with T2DM may underpin the increase in GDF-15 concentration that occurs with T2DM.

Table 1 – Multivariable linear regression models explaining variance in serum GDF-15 concentrations.

Independent variables	R-square (R^2)	R^2 change	p-value
Model 1	**0.296**	**0.296**	**<0.00001**
HbA1c (mmol/mol)			
Model 2	**0.60**	**0.304**	**<0.00001**
HbA1c (mmol/mol), age (yrs), AST (IU/l), metformin use (yes), hs-CRP (mg/l) and e-GFR (ml/min/1.73 m^2)			

Sample size, n=99. In all regression models, the dependent variable was the logarithmically transformed serum GDF-15 concentrations (pg/ml). *Abbreviations*: AST; aspartate aminotransferase, hs-CRP; high-sensitivity C-reactive protein, eGFR; estimated glomerular filtration rate and CI; confidence interval.

Clinical Trial Registration Number: NCT01680640

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Disclosure: **J. Bilson:** None.

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Relationship between advanced liver fibrosis using transient elastography and diabetic complications: data in 684 patients from the Angiosafe T2D cohort

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Background and aims: Advanced fibrosis is the main prognostic driver associated with overall mortality in NAFLD. In type 2 diabetes (T2D), prevalence of advanced fibrosis, using vibration-controlled transient elastography (VCTE), has been reported to be as high as 15 to 20%. However, relationship between advanced fibrosis and diabetic complications remains poorly known. The aim of this study was to assess the relationship between advanced fibrosis and the presence of diabetic complications.

Materials and methods: Angiosafe is an ongoing prospective longitudinal cohort (n=3154) initiated in 2016 to study the occurrence of diabetic

complications in T2D patients. From Oct 2019 to Dec 2020, patients from Angiosafe cohort were screened for fibrosis using VCTE (FibroScan 430) during their annual check-up. Patients with other causes of liver disease (alcohol, HCV and HBV) were excluded. NAFLD (steatosis ≥5%) was diagnosed by means of controlled attenuation parameter (CAP) ≥ 250 dB/m. Liver fibrosis was staged according to liver stiffness measurement (LSM) ≥ 8 kPa significant fibrosis (F2-3-4); LSM ≥ 10 kPa advanced fibrosis (F3-4) and LSM ≥ 15 kPa cirrhosis (F4).

Results: 684 patients (men 59%; median age 61 yrs; BMI 28.7 Kg/m²; Waist circumference (WC) 104 cm; HbA1c 7.6 %; AST 28 UI/l; ALT 30 UI/l; GGT 34 UI/l and triglycerides 1.3 g/dL) were enrolled. NAFLD was present in 74.4% of patients, F2-3-4 in 22.5%, F3-4 in 12.4% and F4 in 3.8%. Prevalence of complications was: macrovascular 24.8%, retinopathy 20.5%, neuropathy 39.4% and nephropathy (KDIGO) 38.3%. Prevalence of nephropathy was significantly higher in F3-4 patients as compared to others (52.1% vs. 36.3%, respectively; p<0.02) while it did not differ for macrovascular complications (23.6% vs. 25%; p=0.88), retinopathy (13.9% vs. 21.5%; p=0.16) and neuropathy (43% vs. 38.9%; p=0.54). In multivariate analysis, WC (OR 1.05; p<0.03) and AST (OR 1.02, p<0.05), were independently associated with F3-4.

Conclusion: In a large cohort of T2D French patients, NAFLD and advanced fibrosis were present in 74.4% and 12.4% of patients, respectively. Nephropathy was the only diabetic complication associated with advanced fibrosis. In multivariate analysis, waist circumference and AST were independently associated with advanced fibrosis.

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Disclosure: **T. Vidal Trecan:** None.

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Main determinants of NAFLD based on fibroscan in early stages of glucose intolerance

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Background and aims: Previous studies have suggested an association between sympathetic and parasympathetic activity and non-alcoholic fatty liver disease (NAFLD). In the attempt to gain better insight in this potential association we have explored the association between CAP and metabolic features as well autonomic function in subjects with normal (NGT) and impaired glucose tolerance (IGT).

Materials and methods: Twenty five NGT (age 44.8±9.6 yrs; BMI 32.3 ±6.9 kg/m^2) and 27 IGT (47.6±11.8 yrs; 31.0±6.5 kg/m^2) subjects underwent a 75 g OGTT and a Mixed Meal Tolerance Test (MMTT) for assessment of glucose and insulin secretion. Parameters of beta-cell function and insulin sensitivity were calculated. Autonomic function was assessed by ANX 3.0 monitoring system applying standard clinical tests. CAP was determined by Fibroscan (Echosense) and presence of NAFLD defined as CAP >233 dB/m.

Results: A CAP >233 was found in 72% of NGT and 67% in IGT. Subjects with NAFLD, irrespective of glucose tolerance, had higher BMI and waist circumference; lower insulin secretion and action and lower parasympathetic activity (Table). On a matrix analysis, after adjustment for age and BMI, CAP was positively related to SBP; insulin action and negatively related to parasympathetic activity. Regression analysis showed that AUC-insulinMMTT but not parasympathetic activity remained independently related to NAFLD - OR 24.4, 95% CI: 2.17 - 274.77; p=0.010. On a receiving operating curve analysis a "cut off" value of 15620 uIU/ml^{-1}*180 min^{-1} provided a 75% sensitivity and 75% specificity for CAP >233.

Conclusion: Our results do not support a role for parasympathetic activity in NAFLD. Rather they show that a stimulated hyperinsulinemia may be associated with greater risk of NAFLD irrespective of glucose tolerance.

Parameters	NGT + NAFLD- (N=7)	NGT + NAFLD+ (N=18)	P value (ANOVA)	IGT + NAFLD- (N=9)	IGT + NAFLD+ (N=18)	P value (ANOVA)
Age (years)	40.7±8.5	48.9±9.3	0.035	45.4±14.0	50.4±11.7	0.341
Waist circumference (cm)	88±13	108±10	**0.001**	91±16	109±11	**0.003**
BMI (kg/m²)	26.1±6.5	33.4±5.2	**0.008**	26.7±4.4	33.4±5.0	**0.002**
AUC-insulinOGTT (uIU)	5616(4294-6071)	10322(6496-17344)	**0.004**	9059(5234-11139)	13175(9996-22196)	**0.024**
AUC-insulinMMTT (uIU)	9492(7465-10816)	18130(13728-28846)	**0.003**	13727(10537-18993)	24360(18636-32411)	**0.011**
Insulinogenic Index (uIU/mmol)	0.9(0.60-1.40)	1.2(0.80-1.98)	0.306	0.6(0.50-0.85)	0.95(0.60-2.15)	**0.042**
ISSI-2	256(198-588)	191(153-253)	**0.026**	158(121-198)	178(130-201)	0.597
SBP (mmHg)	123±12	128±14	0.429	119±17	129±14	0.124
PSNS activity at rest (bpm²)	3.9(1.59-6.19)	1.19(0.62-1.59)	**0.008**	1.02(0.49-6.51)	0.75(0.34-1.37)	0.172
PSNS activity during deep breathing (bpm²)	25.80(9.96-44.41)	12.61(4.78-20.69)	0.185	35.96(14.29-92.61)	18.56(2.67-29.12)	**0.049**
PSNS activity during Valsalva maneuver (bpm²)	5.91(1.03-16.29)	7.78(3.11-13.50)	0.353	7.94(4.85-23.93)	3.85(1.11-9.81)	**0.053**

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SO 61 Fatty liver always hides some complications

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Nonalcoholic fatty liver disease, liver fibrosis and cardiovascular disease in the general US population

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Background and aims: Cardiovascular disease (CVD) risk is higher in patients with nonalcoholic fatty liver disease (NAFLD), but is still debated whether this can be attributed to its link with known CVD risk factors or to an independent contribution of liver steatosis and fibrosis. The aim of this study is to investigate the association between NAFLD, CVD and heart failure in the general US population.

Materials and methods: This is an analysis of data from the 2017-2018 cycle of the National Health and Nutrition Examination Survey (NHANES). We included participants older than 40 years with available data on vibration-controlled transient elastography (VCTE) and without hepatitis C, hepatitis B and significant alcohol consumption. Hepatic steatosis and fibrosis were diagnosed by the median value of controlled attenuation parameter (CAP, cut-off 274 dB/m) and liver stiffness measurement (LSM, cut-off 8 kPa), respectively. History of CVD was self-reported and defined as a composite of coronary artery disease (CAD) and stroke/transient ischemic attacks.

Results: Among the 2734 participants included, prevalence of NAFLD was 48.6% (95% CI 45.1-51.4), 316 participants (9.7%, 95% CI 8.1-11.6) had evidence of signifant liver fibrosis and 371 (11.5%, 95% CI 9.5-13.9) had a positive history of CVD. In univariate analysis, patients with CVD had a higher prevalence of steatosis (59.6% vs 47.1%, p=0.013), but not fibrosis (12.9% vs 9.3%, p=0.123). After adjustment for potential confounders in a multivariable logistic regression model including age, race-ethnicity, diabetes, hypertension, cigarette smoke and chronic kidney disease, neither steatosis nor significant fibrosis were independently associated with the prevalence of CVD and HF.

Conclusion: In this cross-sectional population-based study we did not identify an independent association between steatosis and fibrosis and CVD events. Large prospective cohort studies are needed to provide a more definitive answer on this topic.

Disclosure: **G. Perseghin:** None.

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Increased risk of hospitalisations in type 2 diabetes (T2D) with non-alcoholic steatohepatitis (NASH): a prospective study from Hong Kong Diabetes Register

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is common in people with type 2 diabetes (T2D). Non-alcoholic steatohepatitis (NASH) is the active form of NAFLD leading to morbidities including liver cirrhosis and hepatocellular carcinoma. We aimed to examine the clinical outcomes of Chinese T2D with NASH.

Materials and methods: In this prospective cohort of Chinese patients with T2D who had transient elastrography (FibroScan) done at baseline between 2013 and 2014, we examined clinical outcomes, including the incidence and days of hospitalization, liver cirrhosis, hepatocellular carcinoma, decline in renal function measured by estimated glomerular filtration rate (eGFR), chronic kidney disease (CKD, defined as eGFR <60 ml/min/1.73m^2), end-stage renal failure, fatal and non-fatal coronary artery diseases, heart failure, fatal and non-fatal cerebrovascular disease, all hospitalizations, all site cancers and mortality by retrieval of data from electronic medical record (EMR). NASH and NAFLD were defined as liver stiffness measurement (LSM) ≥10kPa and controlled attenuation parameter (CAP) ≥248 dB/m respectively.

Results: Among 1842 T2D who had FibroScan done at baseline, 1734 (296 NASH, 798 NAFLD, 640 normal control) were included in the present analysis (87 had invalid or missing data, 4 were excluded due to alcohol or drug related liver diseases and 17 were excluded due to other liver diseases). After a median follow-up of 6.07 [interquartile range 5.84 to 6.30] years, 2 developed cirrhosis, 6 was diagnosed to have hepatocellular carcinoma and 5 had incident CKD. There was a total of 4331 incident hospitalizations, 31987 days of hospital stay, and 171 death. At baseline, 57.4% NASH were men, with mean age:61.2±12.5 years, body mass index (BMI):29.4±5.2 kg/m^2, fasting plasma glucose (FPG):8.3±2.9 mmol/L, HbA_{1c}:8.0±1.6%, systolic blood pressure (SBP):142±18 mmHg, diastolic blood pressure (DBP):78±12 mmHg, low density lipoprotein cholesterol (LDL):2.2±0.8mmol/L, high density lipoprotein cholesterol (HDL):1.2±0.4mmol/L and median triglyceride (TG): 1.5[1.1 to 2.2]mmol/L. The respective figures for T2D with NAFLD and control were 27.2±3.8 and 24.2±3.4 kg/m^2, 7.9±2.5 and 7.4±2.7 mmol/L, 7.8±1.5 and 7.7±1.7%, 137±18 and 136±20 mmHg, 77±11 and 75±11 mmHg, 2.3±0.8 and 2.3±0.8mmol/L, 1.3±0.4 and 1.4 ±0.4mmol/L, 1.4[1.0 to 2.0] and 1.1[0.8 to 1.5] mmol/L. T2D with NASH had significantly worse metabolic profile, including higher BMI, FPG, HbA_{1c}, SBP, DBP and TG than NAFLD and control (all p<0.05). Using Cox regression analysis, T2D with NASH had significantly higher risk of hospitalizations compared to their counterparts without NASH after adjustment for potential confounders including age, sex, duration of diabetes, alcohol drinking history, albuminuria, HbA1c, lipid and eGFR (hazard ratio, HR: 1.81 [1.2 to 2.74], p=0.005).

Conclusion: T2D with NASH have increased risk of hospitalizations and deserve more clinical attention.

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Subjects with NASH and diabetes have a more unfavourable metabolic profile compared to NAFL with and without diabetes: results from the EPOS study

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Background and aims: Over the last two decades, the high prevalence rates of NAFLD have been paralleling the epidemic of obesity and type 2 diabetes (T2D). The mechanisms that are involved in the progression from steatosis (NAFL) to steatohepatitis (NASH) are not completely elucidated and, although it is known that patients with T2D have a higher prevalence of NASH and liver fibrosis, it has not been clarified how the two diseases interact with each other. Thus, the aim of the study was to evaluate by a machine learning approach how the presence of NASH in

diabetic and non-diabetic subjects was associated with insulin resistance and adipose tissue (AT) dysfunction.

Materials and methods: In 279 histologically characterized NAFLD subjects (166 NASH, 113 NAFL, BMI range 19-40 kg/m^2) from the European NAFLD Registry we measured circulating markers of AT dysfunction i.e., free fatty acid (FFA) and Adipo-IR=FFAxIns, AT dysfunction and inflammation (i.e. leptin, adiponectin, TNF-α and MCP-1), hepatic lipid oxidation (BOH) and lipoprotein metabolism (TG, cholesterol, HDL, ApoA1 and ApoB). In order to classify for metabolic alterations in presence of T2D and NASH, subjects were grouped as: non-diabetic NAFL (n=62) and NASH (n=68), and T2D-NAFL (n=45) and -NASH (n=82), excluding 22 patients with cirrhosis. A Random Forest classifier was used. Age, sex and BMI were used as covariates. The statistical significance of the model was assessed with respect to a null model.

Results: The classification model was able to discriminate the four groups with a good accuracy and statistical significance (p=0.01). Among the variables that mostly contributed to the random forest there were markers of AT dysfunction and inflammation and insulin resistance. We observed higher leptin and TNF-α, and lower adiponectin in NASH vs NAFL and a stepwise trend with decreasing HDL and increasing insulin resistance (HOMA and Adipo-IR) and markers of T2D (HBA1c, glucose) from non-diabetic NAFL and NASH to T2D- NAFL and NASH. BOH that reflects hepatic mitochondrial beta oxidation was increased stepwise from non-diabetic NAFL to T2D-NASH and positively associated to Adipo-IR but it was not a significant variable in the classification model. The model was trained on the 70% of the data, validated through repeated cross validation and tested on the remaining part of the subjects.

Conclusion: The presence of NASH and T2D is associated with a more unfavorable metabolic profile, characterized by worse insulin resistance, AT dysfunction and inflammation while hepatic mitochondrial beta oxidation did not appear to be dysfunctional, indicating once again the importance of AT dysfunction in NASH.

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Non-alcoholic fatty liver disease in overweight subjects related to impaired glucose control and increased insulin resistance

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Background and aims: Obesity, Non-alcoholic fatty liver disease (NAFLD) and the metabolic syndrome share many common pathways. The aim of this study is to observe changes in liver texture and liver function in obese subjects according to their metabolic status over time. Furthermore, this baseline analysis was conducted to investigate several hepatic and metabolic indices in relation to insulin resistance and glucose metabolism in overweight or obese subjects.

Materials and methods: 301 overweight subjects with a BMI > 28 kg/m² with or without type 2 diabetes mellitus (T2DM) were included in this epidemiological survey. Subjects underwent liver elastography (FibroScan®, Echosense, France) to investigate liver fat (CAP) and liver stiffness (E). Blood was drawn for the calculation of $HOMA_{IR}$, AST/ALT ratio, fatty liver index (FLI), the BARD Score, and NAFLD Fibrosis Score. To determine glucose tolerance an oral glucose tolerance test was conducted in subjects without a history of T2DM.

Results: 109 overweight subjects with diabetes mellitus type 2 (T2DM), 42 with impaired (IGT) and 150 with normal glucose tolerance (NGT) were included in the baseline analysis. Subjects with T2DM were slightly older (61.8±9.8; years±SD) compared to IGT (54.4±14.4) or NGT subjects (47.7 ± 12.5). BMI was slightly higher in T2DM subjects (33.9 ±3.7: kg/m² ± SD) compared to IGT (32.6±3.5) or NGT 31.6±2.6) subjects. The characteristics of the liver parameters of interest for the three different metabolic groups are presented in Table 1. While liver fat and elasticity were only slightly elevated in NGT as compared to IGT, both parameters were significantly higher in T2DM (p<0,05). Consistent to these findings, insulin resistance ($HOMA_{IR}$), fatty liver score and NAFLD fibrosis score were slightly higher in subjects with IGT compared to those with NGT, but significantly higher in subjects with T2DM (p<0,05). A BARD index of 2 or more, as indicator of liver fibrosis, was found in 97.2 % of subjects with T2DM compared to 63.4 % in IGT and 71.1 % in NGT.

Conclusion: In overweight subjects, our results confirm an elevation in liver fat and stiffness in line with a deterioration in liver function scores in relation to increasing insulin resistance and a loss in glucose control. Ongoing follow up of our patients after one and two years is expected to provide further information about the progression of the disease in relation to the change in metabolic control over time.

	NGT	IGT	T2DM	P NGT vs IGT	p NGT vs. T2DM	p IGT vs. T2DM
CAP (dB/m)	6.4 (4.8, 8.0)	5.5 (4.4, 6.5)	8.6 (7.3, 10.0)	n.s.	0.035	0.038
E (kPa)	277 (268, 285)	284 (262, 307)	324 (312, 336)	n.s.	<0.0001	0.0003
AST/ALT Ratio	0.96 (0.90, 1.01)	0.91 (0.82, 1.01)	0.86 (0.80, 0.91)	n.s.	0.01	0.32
$HOMA_{IR}$	2.7 (2.5, 3.0)	3.51 (2.9, 4.1)	6.9 (6.0, 7.9)	n.s.	< 0.0001	<0.0001
FLI	69 (66, 72)	73 (67, 80)	87 (84, 89)	n.s.	<0.0001	<0.0001
NAFLD Fibrosis Score	-2.2 (-2.3, -2.0)	-1.7 (-2.1, -1.4)	-0.3 (-0.5, -0.1)	0.03	<0.0001	<0.0001

Table 1: Parameters of liver injury in the different metabolic groups; Mean (95% confidence interval)

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Association between non-alcoholic liver fibrosis scoring systems and micro-macrovascular complications

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Background and aims: Non-alcoholic fatty liver disease Fibrosis Score (NFS) and Fibrosis-4 (FIB-4) are tools used in general assesment of advanced hepatic damage risk in primary care. According to the literature diabetic vascular complications is highly associated with the increased risk of hepatic fibrose, and this is linked to liver fibrosis scoring systems. We examined the relationship between micro and macrovascular complications and non-alcoholic fatty liver disease fibrosis systems.

Materials and methods: 293 cases who submitted to the internal medicine clinic in 2019 were assessed prospectively in a cross-sectional manner for the study. 147 of them were found to have metabolic syndrome (a score of 3 or higher on the ATP3 scale), while the rest were classified as the control group. In the classes, the presence of micro and macrovascular complications (with nephropathy, neuropathy, retinopathy, coronary artery disease, cerebrovascular disease, and peripheral artery disease) was assessed. The NFS and FIB-4 scores were calculated and then compared. NFS was calculated by using age, AST, ALT, and platelet count. FIB-4 was calculated by using age, BMI, AST and platelet count. For the multivariate analysis, the possible factors identified with univariate analyses were further entered into the logistic regression analysis to determine independent predictors of micro - macrovascular disease outcome.

Results: In patients with metabolic syndrome, microvascular complications and micro-macrovascular complications increased as NFS increased (p = 0.002, p<0.05, respectively). When the Fib 4 score was used, there

was no meaningful correlation (p = 0.63). Furthermore, the increase in microvascular complications was linked to an increase in NFS score in patients without metabolic syndrome in the control group (p = 0.017) CI:1.297 (1.048-1.606). The involvement of metabolic disease and the NFS score were found to have a strong correlation (p<0.05). NFS was found to increase with the number of metabolic syndrome criteria (p<0.05).

Conclusion: A multivariate logistic regression analysis revealed that significant predictors for macrovascular disease were age, metabolic syndrome and NAFLD scores.

		Sig.	Exp(B)	95% C.I.for EXP(B)	
				Lower	Upper
Step 1[a]	Age	.161	1.024	.991	1.058
	The presence of metabolic syndrome	**.027**	1.971	1.080	3.599
	NFS	.155	1.192	.936	1.518
	Constant	.049	.141		
Step 2[a]	The presence of metabolic syndrome	**.027**	1.972	1.080	3.600
	NFS	**.017**	1.297	1.048	1.606
	Constant	.002	.552		

Disclosure: **H. Ataoglu:** None.

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Congestive heart failure and the metabolic syndrome are mutually independent predictors of non-alcoholic fatty liver disease

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is associated with both the metabolic syndrome (MetS) and congestive heart failure (CHF). The MetS is highly prevalent in CHF patients; however, the single and joint associations of the MetS and CHF with NAFLD have not been investigated yet. This issue therefore is addressed in the present study.

Materials and methods: We investigated 202 patients with CHF and 670 controls who did not have signs or symptoms of CHF and in whom significant coronary artery disease was ruled out angiographically. The presence of NAFLD was determined using the validated fatty liver index (FLI).

Results: The prevalence of the MetS was 61.9% in CHF patients and 45.7% in controls (p<0.001). FLI values and prevalence rates of NAFLD (FLI≥60) in non-CHF subjects without MetS were 40±25 and 25.0%, respectively. They were significantly higher in non-CHF, but MetS patients (71±22, p<0.001 and 69.3%, p<0.001, respectively), in CHF patients without MetS (54±24, p<0.001 and 42.9%, p=0.002, respectively) and in CHF patients with MetS (76±20, p<0.001 and 82.4%, p<0.001, respectively). In multivariate analysis of covariance, the MetS and CHF proved to be mutually independent predictors of FLI after adjustment for age, sex, BMI, LDL-C, history of smoking and hypertension (F=296.94; p<0.001 and F=21.68; p<0.001, respectively); concordantly, the MetS and CHF independently predicted the presence of NAFLD in logistic regression analyses, with adjusted odds ratios of 6.67 [4.83-9.21]; p<0.001 and 2.52 [1.67-3.79]; p<0.001, respectively.

Conclusion: We conclude that CHF and the MetS are mutually independent predictors of NAFLD.

Disclosure: **B. Mutschlechner:** None.

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Current type 2 diabetes, rather than previous gestational diabetes, is associated with liver disease in U.S. women

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Background and aims: The aim of this study is to investigate the relative contribution of previous gestational diabetes mellitus (GDM) and current type 2 diabetes (T2D) on the development of liver fibrosis, the strongest predictor of end-stage liver disease.

Materials and methods: This is an analysis of data from the 2017-2018 cycle of the National Health and Nutrition Examination Survey. We included women age ≥20 years that had delivered at least one live birth and had available data on vibration-controlled transient elastography (VCTE). Hepatic steatosis and fibrosis were diagnosed by the median value of controlled attenuation parameter (CAP) and liver stiffness measurement (LSM), respectively.

Results: Among the 1699 women included in the study, 144 (10.1%, 95% CI 7.7-13.2) reported a previous diagnosis of GDM. Women with previous GDM were younger, had a higher BMI and waist circumference, a higher prevalence of T2D, lower systolic blood pressure and were significantly older at the time they had the last live birth. Univariate analysis did not show a significant difference between women with and without a prior history of GDM in terms of both steatosis (44.8% vs 39.4%, p=0.464) and fibrosis (7.5% vs 7.6%, p=0.854). Multivariable logistic regression analysis showed that BMI, γ-glutamyltranspeptidase levels, T2D (OR 2.96, 95% CI 1.48-5.93, p<0.01), HBV and HCV infection were associated with higher odds of significant fibrosis, but previous GDM showed a neutral effect.

Conclusion: The results of this analysis confirm that women with GDM should be screened and followed for the development of T2D, but those that do not go on to develop T2D might not experience a poor hepatic prognosis.

Disclosure: **S. Ciardullo:** None.

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Non-alcoholic fatty liver indices and their association with glucose metabolism in pregnancy

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Background and aims: Previous studies indicated that hepatic steatosis indices can be used to accurately stratify the risk for non-alcoholic fatty

liver disease (NAFLD), with the advantage of being non-invasive and easy to assess. Therefore, they are particularly useful for NAFLD classification in pregnancy. Recent publications showed that the proposed indices are highly predictive for the later development of gestational diabetes mellitus (GDM) as well. This study aims to extend these results by providing a detailed examination of their association with parameters of glucose metabolism during gestation.

Materials and methods: A total of 103 women were included in this study and underwent metabolic characterisation before 20 weeks of gestation including fasting assessment of glucose, insulin and C-peptide. The study participants were classified according to the hepatic steatosis index (HSI) into low (HSI-G1, n=18), intermediate (HSI-G2, n=47) and high risk (HSI-G3, n=38) for NAFLD. In addition, women were classified by use of the fatty liver index (FLI) as an alternative method. At 26 weeks of gestation (IQR: 25 to 27 weeks) all mothers received an oral glucose tolerance test (75g-OGTT) with five measurements of glucose, insulin and C-peptide to assess dynamic parameters of glucose metabolism including the predicted M-value (PREDIM, representing insulin sensitivity) as well as the oral disposition index (ISSI-2, representing β-cell function).

Results: Both NAFLD indices were closely associated with insulin resistance (HSI: rho = 0.71, p<0.001; FLI: rho = 0.69, p<0.001) and compensatory increased insulin release (HSI: rho = 0.54, p<0.001; FLI: rho = 0.57, p<0.001) derived from fasting parameters at early pregnancy. Moreover, HSI-G3 and FLI-G3 subgroups showed significantly impaired insulin action during the OGTT as compared to the G1 and G2 subgroups as well as lower levels of ISSI-2 indicating impaired β-cell function. As a consequence, patients classified as HIS-G3 as well as FLI-G3 presented increased glucose concentrations during the OGTT. Both steatosis indices were associated with the development of GDM (HSI: OR 1.18, 95%CI 1.08 to 1.32, p=0.001; FLI: OR 1.05 95%CI 1.03 to 1.09, p<0.001) as well as with birth weight percentiles of the infants after delivery.

Conclusion: Non-invasive fatty liver indices are associated with higher risk of GDM, which is likely due to impaired insulin action and β-cell dysfunction. As these indices are easily available at early pregnancy, they may provide a useful tool for GDM risk assessment.

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Lipid-lowering in diabetes is not intensified beyond 3-months if coronary angiography with fractional flow reserve (FFR) suggests non-obstructive coronary artery disease

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Background and aims: FFR assessment of coronary artery disease (CAD) distinguishes obstructive vs. non-obstructive CAD in those with at least moderate CAD. Patients with diabetes and either obstructive or non-obstructive CAD are at risk for future ASCVD events. We sought to examine the management of atherogenic lipids (LDL-C and non-HDL-C) in patients with diabetes with obstructive vs non-obstructive CAD following coronary angiography with FFR.

Materials and methods: We reviewed the electronic medical records of patients with diabetes who underwent FFR, instantaneous wave free ratio (iFR), or computed tomographic (CT)-FFR assessment at an academic medical center from 2013-2020. Obstructive CAD was defined as ≥1 lesion with FFR ≤ 0.8, iFR ≤ 0.89, or CT-FFR ≤ 0.8, or by visual assessment. Atherogenic lipids (LDL-C and non-HDL-C) for obstructive and non-obstructive groups were compared at baseline, 3, and 6 mos. using the Mann-Whitney U test.

Results: There were 198 patients with obstructive vs 107 with non-obstructive CAD. Most assessments were performed for a non-acute coronary syndrome indication (n=231 (76%)). Mean (±SD) age was 65 ±11 yrs, with 248 (32%) women, and 235 (77%) white. At baseline, approximately half of the patients were treated with metformin (obstructive CAD: n=97 (49%), non-obstructive CAD: n=45 (41%)), and around 40% were treated with insulin (obstructive CAD: n=87 (44%), non-obstructive CAD: n=44 (41%)). Use of GLP-1 RA or SGLT2i drug classes was low (< 10% in both groups). Baseline A1c was not significantly different between groups (obstructive CAD: 7.57±1.65, non-obstructive CAD: 7.15±1.32 (p=0.08)). LDL-C and non-HDL-C at baseline were not significantly different, and baseline statin use was 78% for obstructive (n=155) vs 73% for non-obstructive CAD (n=78). At 3 months, LDL-C and non-HDL-C decreased in both groups (obstructive CAD: LDL-C -0.3 mmol/L, non-HDL-C -0.2 mmol/L; non-obstructive CAD: LDL-C -0.6 mmol/L, non-HDL-C -0.5 mmol/L (p > 0.05)). However, at 6 mos., LDL-C and non-HDL-C continued to decline in the obstructive group, but increased in those with non-obstructive CAD (LDL-C +0.6 mmol/L (p=0.006), non-HDL-C +1.0 mmol/L (p=0.027)) (Table).

Conclusion: After assessment by FFR, atherogenic lipids are lower at 3-months in both those with obstructive and non-obstructive CAD. However, by 6 months, this effect is lost in those with non-obstructive CAD, and both LDL-C and non-HDL-C increase in this group. Our findings highlight the need to improve atherogenic lipid lowering in all patients with diabetes and CAD (both obstructive and non-obstructive) following angiography with FFR to reduce risk of ASCVD events in this high-risk group of patients. Also, we noted low rates of baseline GLP-1 RA and SGLT2i use in this cohort, highlighting another area for improvement in the treatment of patients with diabetes and CAD.

		Obstructive CAD			Non-Obstructive CAD			
		Atherogenic lipids (mmol/L) (mean±SD)	Change (mmol/L)	Change (%)	Atherogenic lipids (mmol/L) (mean±SD)	Change (mmol/L)	Change (%)	p-value
Baseline	LDL-C	1.9±0.9 (n=124)	--	--	2.1±1.0 (n=62)	--	--	.251
	non-HDL-C	2.7±1.0 (n=128)	--	--	2.7±1.2 (n=64)	--	--	.8
3 months	LDL-C	1.7±0.7 (n=56)	-0.2	-13%	1.5±0.2 (n=21)	-0.6	-28%	.243
	non-HDL-C	2.5±0.8 (n=56)	-0.2	-8%	2.2±1.0 (n=21)	-0.5	-18%	.166
6 months	LDL-C	1.5±0.6 (n=41)	-0.2	-10%	2.1±0.9 (n=25)	+0.6	+39%	**.006**
	non-HDL-C	2.2±0.8 (n=42)	-0.3	-11%	3.2±2.3 (n=24)	+1.0	+44%	**.027**

Table. LDL-C and non-HDL-C following fractional flow reserve (FFR) assessment in those with diabetes and obstructive vs non-obstructive coronary artery disease. Absolute and percent change in LDL-C and non-HDL-C is in comparison to the most recent prior time-point. Groups compared using Mann Whitney U test.

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The rate of decline in nocturnal interstitial glucose concentrations influences some electrocardiogram parameters in type 1 diabetes

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Background and aims: The relationship between the rate of glucose reduction and electrocardiogram (ECG) responses during the nocturnal period within the same participant with type 1 diabetes (T1D) is relatively undefined. This study sought to determine whether the rate of change in interstitial glucose (iG) concentrations influenced ECG parameters during the nocturnal period in people with T1D.

Materials and methods: This was an exploratory, secondary analysis of a randomised, cross-over design involving 10 participants with T1D (M: n=8 F: n=2, HbA_{1c}: 55.7±14.5 mmol/mol (7.2±1.3%), age: 34.5±13.9 years, diabetes duration: 14.4±11.1 years) who on four separate occasions performed 45 minutes of moderate intensity evening exercise (~60% VO_{2max}) followed by an overnight stay in a clinical research facility. Participants were monitored throughout the nocturnal period (00.00-06.00) using 3-lead ECGs (Faros 180) and continuous glucose monitoring (CGM) systems (FreeStyle Libre). Time and duration matched nocturnal CGM data were used to identify periods on two different nights where iG was found to be changing at either a fast or slow rate of change (FAST: -0.04±0.02 vs. SLOW: -0.01±0.01 mmol/l/min, $p<0.001$). ECG data were analysed using Wilcoxon Signed Ranks Test with $p\leq0.05$ accepted for statistical significance. Glycaemic thresholds were hypoglycaemia (≤3.9 mmol/l), euglycemia (4-10 mmol/l) and hyperglycaemia (≥10 mmol/l).

Results: The ECG data are reported within Table 1 for the FAST and SLOW conditions. In FAST: 7/10 participants started and ended with iG values in hyperglycaemic zones, with 1/10 starting in hyperglycaemia and ending in euglycemia, and 2/10 euglycemia-euglycemia. In SLOW: 7/10 started and ended in hyperglycaemia, with 3/10 starting in hyperglycaemia and ending in euglycemia. Delta ECG responses from start to end were similar between the FAST and SLOW conditions (NS).

Conclusion: In people with T1D fast compared with slow rates of change in interstitial glucose affected some nocturnal ECG responses. Though QTc, SDNN and LF/HF were not altered, HR, RMSSD and pNN50% were statistically significantly lower during FAST compared to SLOW reduction in night-time interstitial glucose concentrations.

Parameter	FAST (Median [range])	SLOW (Median [range])	P value
Heart Rate (bpm)	60.9 (43.9-87.1)	61.6 (46.2-81.9)	<0.01
QTc (ms)	408.0 (373.0-427.0)	406.0 (387.0-430.0)	0.660
SDNN (ms)	72.3 (22.0-239.0)	69.9 (29.3-208.0)	0.113
RMSSD (ms)	46.0 (14.7-118.9)	54.3 (12.1-125.4)	<0.01
pNN50%	18.9 (0.0-74.8)	27.5 (0.6-80.1)	<0.01
LF/HF ratio	2.4 (0.0-56.22)	2.2 (0.0-32.5)	0.499

Table 1. Comparison of ECG responses during FAST and SLOW conditions

Disclosure: **M. Smallman:** None.

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Empagliflozin ameliorates obesity-induced cardiac dysfunction via activating SIRT3-mediated autophagy

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Background and aims: Autophagy is a cellular pathway crucial for protecting against cardiac complications and several pathologies such as lipid metabolism and insulin sensitivity by using bulk degradation and recycling of cytoplasmic components. Important links between the regulation of autophagy and obesity including food intake, insulin sensitivity and cardiac dysfunction exist. Several clinical trials of the sodium-glucose co-transporter 2 (SGLT2) inhibitors have reported that heart failure patients receiving SGLT2 inhibitors had lower risks of cardiovascular death, regardless of the presence or absence of diabetes. Empagliflozin (EMPA), a highly selective SGLT2 inhibitor, has favorable effects on ameliorating cardiovascular injury and cognitive dysfunction in obese and type 2 diabetic mice. The primary aim of this study is to unravel the molecular mechanisms by which EMPA affects obesity heart function and its role in the complications associated with autophagy.

Materials and methods: To determine the role of EMPA on cardiac histology and function, as well as its mechanisms, we established an experimental model of obesity by a 12-week high-fat diet (HFD) treatment that develops cardiac dysfunction at a high frequency. C57BL/6J mice were randomized into the following three groups: (i) control (ii) HFD, and (iii) EMPA (10 mg/kg/day)/HFD by the intragastric route for 8 weeks. The cardiac function was detected by echocardiography, and the H-E and Oil-red-O-stained sections were used to show the differing abnormalities in cardiac histology. Hearts and transfected H9c2 cells were harvested for biochemical analysis.

Results: Lower body weight and whole-body fat, as well as improved metabolic disorders, showed in the HFD C57BL/6J mice with EMPA treatment. Moreover, EMPA reduced myocardial damage with the improvement of cardiac function and the decelerated fat accumulation. In addition, EMPA significantly augmented cardiac SIRT3, the key mitochondrial NAD+-dependent deacetylase, regulates the activity of many substrates involving in autophagy. Intriguingly, EMPA treatment enhanced the autophagy-related Beclin1 and Atg5 expression in HFD mice. However, these responses were blunted in siSIRT3 transfected H9C2 cells treated with palmitic acid in response to EMPA. These findings provide a novel mechanism for the cardiovascular protection of SGLT2 inhibitor in obesity-related cardiac dysfunction via regulating SIRT3-mediated autophagy.

Conclusion: Our findings in current study indicate a novel communication between cardiac function and autophagy in HFD mice in response to the treatment of SGLT2 inhibitor. Empagliflozin improved obesity-related cardiac dysfunction via regulating SIRT3-mediated autophagy that may serve as a potential treatment strategy for obesity-related cardiomyopathy.

Supported by: NSFC 81870593

Disclosure: **J. Zhang:** None.

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Efficacy of intravenous ferric carboxymaltose in patients with iron deficiency following acute heart failure, by diabetes status: a subgroup analysis of the AFFIRM-AHF trial

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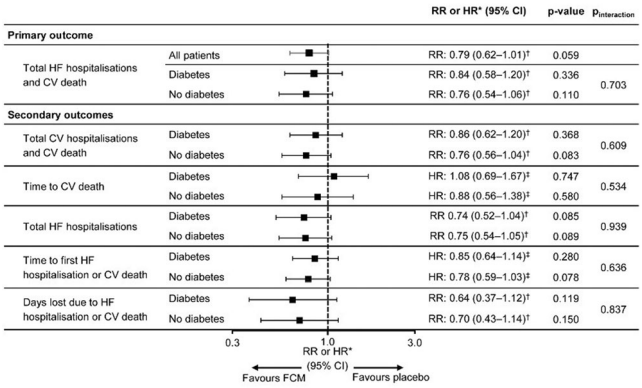
Background and aims: In the AFFIRM-AHF trial, treatment with intravenous (IV) ferric carboxymaltose (FCM) reduced the risk of heart failure (HF) hospitalisations vs placebo in patients with iron deficiency after an acute HF episode. Of these patients, 42% had diabetes. This *post hoc*-analysis explored the effect of FCM on outcomes in AFFIRM-AHF patients with and without diabetes.

Materials and methods: In AFFIRM-AHF, patients with iron deficiency stabilised following hospitalisation for acute HF were randomised to receive IV FCM or placebo. In this *post hoc* analysis, the patients with a documented diabetes status (yes/no) at baseline who received ≥1 dose of study drug and had ≥1 post-randomisation data point were included. The primary outcome was a composite of total HF hospitalisations and cardiovascular (CV) deaths. Secondary outcomes are listed in the **Figure**. Outcomes with FCM vs placebo were evaluated up to 52 weeks in patients with and without diabetes and the interaction of diabetes status with treatment effect was assessed.

Results: Of the 1,108 randomised patients included in the main analysis, 470 (FCM 227; placebo 243) had diabetes and 638 (FCM 331; placebo 307) did not have diabetes. At baseline, 61% of patients with diabetes and 51% of patients without diabetes were male; 58% and 39%, respectively, had ischaemic HF aetiology. In patients with diabetes, the number of primary endpoint events with FCM vs placebo were 142 vs 202 (annualised rate ratio [95% confidence interval (CI)]: 0.84 [0.58–1.20]); in patients without diabetes, the corresponding values were 151 vs 170 (annualised rate ratio [95% CI]: 0.76 [0.54–1.06]); $p_{interaction}$ for diabetes status and treatment effect was non-significant (0.703). Findings were similar for secondary outcomes (**Figure**).

Conclusion: In AFFIRM-AHF, the effect of FCM vs placebo on primary and secondary CV outcomes was similar for patients with and without diabetes; rates and risks were consistently nominally lower with FCM vs placebo.

Figure



*FCM vs placebo. †Annualised event RR analysed using a negative binomial model. ‡HR for treatment difference analysed using Cox regression model. All models adjusted for covariates: sex, age, HF aetiology, HF duration, country, and diabetes status at baseline, and diabetes at baseline*treatment. N=1,108 for all patients. Respective n-values in patients with and without diabetes at baseline were 227 and 331 for FCM and 243 and 307 for placebo. CI, confidence interval; CV, cardiovascular; FCM, ferric carboxymaltose; HF, heart failure; HR, hazard ratio; RR, rate ratio.

Clinical Trial Registration Number: NCT02937454

Supported by: Vifor Pharma

Disclosure: **G. Rosano:** None.

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Effect of saxagliptin on LV structure and function in patients with type 2 diabetes and heart failure: results of measure-HF

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Background and aims: Further investigation was suggested by the SAVOR-TIMI 53 results regarding the risk of hospitalisation for heart failure (HF) and the dipeptidyl-peptidase 4 inhibitor saxagliptin (SAXA), especially for high-risk patients with type 2 diabetes (T2D) and established HF with reduced ejection fraction (HFrEF). The Measure-HF Trial investigated the effect of SAXA on left ventricular (LV) structure, function and N-terminal pro-brain natriuretic peptide (NT-proBNP) in patients with T2D and established symptomatic heart failure with LV ejection fraction (LVEF) <45% (HFrEF) and NT-proBNP >300 pg/mL.

Materials and methods: Patients were randomised to SAXA (n=112), sitagliptin (SITA, n=115) or placebo (PBO, n=121) plus usual care for 24 weeks. Primary endpoint was change from baseline in LV end diastolic volume index (LVEDVi) measured by MRI (core laboratory). Secondary endpoints included changes in LV end systolic volume index (LVESVi), LVEF, LV mass, and NT-proBNP levels. Worsening HF events were adjudicated. Exploratory analyses also evaluated the same endpoints for SITA vs PBO.

Results: Mean age was 65.4 years, 68.9% male, mean LVEF 37.3%, and median NT-proBNP 941 pg/mL. Primary and secondary endpoints demonstrated no difference between SAXA vs PBO (Table). There was no significant difference in the change in NT-proBNP levels between SAXA and PBO groups. The changes in endpoints for SITA were consistent with those of SAXA. Adjudicated HF events were balanced: SAXA (5), SITA (3) and PBO (6).

Conclusion: In patients with T2D and HFrEF, SAXA compared with PBO produced no unfavorable effects on LV structure, function or natriuretic peptide levels.

TABLE: Adjusted mean change from baseline in LVEDVi, LVESVi, LVEF, LVM

Analysis (full data set)	SAXA (N=112)		PBO (N=120) [a]
PRIMARY ENDPOINT			
LVEDVi, change at 24 weeks; mL/m² (95% CI)	0.93 (-4.04, 5.90)		3.53 (-1.18, 8.23)
Difference from PBO (95% CI)[b]		-2.60 (-7.04, 1.85)	
One-sided p-value for non-inferiority vs. PBO		<0.001	
SECONDARY ENDPOINTS			
LVESVi, change at 24 weeks; mL/m² (95% CI)	-0.87 (-5.34, 3.61)		0.76 (-3.47, 5.00)
Difference from PBO (95% CI)		-1.63 (-5.64, 2.37)	
LVEF, change at 24 weeks; % (95% CI)	1.14 (-1.19, 3.48)		1.04 (-1.17, 3.26)
Difference from PBO (95% CI)		0.10 (-2.00, 2.20)	
LVM, change at 24 weeks; g (95% CI)	-2.42 (-7.30, 2.46)		1.19 (-3.44, 5.81)
Difference from PBO (95% CI)		-3.61 (-7.97, 0.76)	

[a] 1 randomized patient did not receive treatment
[b] if the upper limit of the two-sided 95% CI for the adjusted mean treatment difference is less than 10% of the baseline mean value (10.5295 mL/m²) overall patients in the study then SAXA will be considered non-inferior to placebo
LVEF; Left Ventricular Ejection Fraction, LVM; Left Ventricular Mass

Clinical Trial Registration Number: NCT02917031

Supported by: AstraZeneca

Disclosure: **P. Bertram:** Employment/Consultancy; AstraZeneca.

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Oleate prevents palmitate-induced abnormalities in insulin signalling in human cardiac progenitor cells

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Background and aims: myocardial ectopic lipid deposition has been associated with insulin resistance and cardiovascular risk. Palmitate, one of the most abundant saturated fatty acids, has been shown to impair insulin signaling in multiple cell types. Human cardiac progenitor cells (CPC) represent a compartment of multipotent stem cells essential for constant tissue repair and renewal in the adult heart. In this study, the ability of palmitate to impair insulin signaling, and the modulatory effects of oleate, a mono-unsaturated fatty acid, on palmitate-induced abnormalities were investigated in human CPC.

Materials and methods: human CPC were obtained from non-diabetic subjects undergoing cardiac surgery for coronary artery bypass grafting and/or valve surgery. Human CPC were exposed to 0.25 mM palmitate and/or 0.1 mM oleate for 24 h, and then exposed to 100 nM insulin for the last 15 minutes. Expression of insulin receptor (IR) isoforms, A (IR-A) and B (IR-B), was evaluated by quantitative Real Time PCR. IR protein expression was assessed by immunoblotting. Akt and p44/p42 MAPK protein levels, as well as phosphorylation levels, were studied by immunoblotting.

Results: human CPC express IR. Specifically, in human CPC both IR isoforms, IR-A and IR-B, were identified, and IR-A was found to be more expressed than IR-B. Exposure of human CPC to 100 nM insulin for 15 minutes resulted in increased Akt and p44/p42 MAPK phosphorylation ($p<0.05$). Treatment of human CPC with 0.25 mM palmitate for 24 h impaired the ability of 100 nM insulin, added for the last 15 minutes, to phosphorylate both Akt and p44/p42 MAPK ($p<0.05$), with no effect on total Akt and p44/p42 MAPK protein levels. Interestingly, palmitate was able to increase both IR-A and IR-B mRNA levels ($p<0.05$), and to reduce IR protein expression ($p<0.05$). Co-incubation of palmitate with 0.1 mM oleate for 24 h prevented the ability of palmitate to reduce insulin-induced Akt phosphorylation ($p<0.05$), but did not rescue the impaired p44/p42 MAPK phosphorylation. In addition, co-treatment with oleate also prevented both palmitate-induced upregulation of IR-A and IR-B mRNA ($p<0.05$), and downregulation of IR protein levels ($p<0.05$).

Conclusion: oleate prevents some of the palmitate-induced impairments in insulin signaling in human CPC from control subjects. Hence, oleate supplementation might preserve human cardiac progenitors from lipotoxicity-induced metabolic abnormalities in human heart.

Disclosure: **R. D'Oria:** None.

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Cardiovascular autonomic neuropathy and risk of heart failure in participants with type 2 diabetes enrolled in the DEVOTE trial

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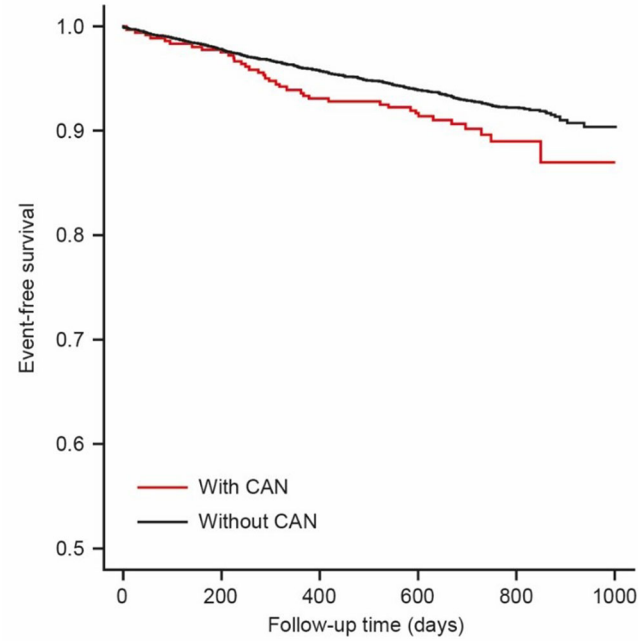
Background and aims: We assessed whether cardiovascular autonomic neuropathy (CAN), as assessed by heart rate variability (HRV), is associated with an increased risk of heart failure (HF) in 6347 patients with type 2 diabetes (T2D) enrolled in the DEVOTE trial with HRV data available.

Materials and methods: DEVOTE was a randomised, double-blind trial comparing the impact of insulin degludec to glargine 100 units/mL on cardiovascular outcomes. Indices of HRV were derived from 10-sec standard electrocardiogram at enrolment, with values below the 5th percentile of the cohort defined as abnormal. Time to first hospitalisation due to HF was analysed using Kaplan-Meier survival curves and the log-rank test.

Results: A total of 369 (5.8%) had CAN. Participants with and without CAN had significantly different [mean(standard deviation)] age [63.4(7.3) and 64.9(7.3) years], HbA_{1c} [8.9(1.9) and 8.4(1.6)%], BMI [33.0(7.4) and 33.6(6.8) kg/m^2] and number of subjects from a minority ethnicity [19.5 and 15.2%], respectively ($P<0.05$). In adjusted analyses, CAN at baseline was associated with a significantly higher risk of hospitalisation for HF [hazard ratio=1.47 (95% CI:1.04-2.06); $P=0.028$] over the follow-up period (**Figure**).

Conclusion: These data suggest that CAN may be used for risk stratifying in patients with T2D at risk for developing symptomatic HF.

Figure. Time to first hospitalisation due to HF for participants with and without CAN at baseline



CAN, cardiovascular autonomic neuropathy; HF, heart failure.

Clinical Trial Registration Number: NCT01959529

Supported by: Novo Nordisk

Disclosure: **R. Pop-Busui:** Employment/Consultancy; Averitas Pharma, Bayer, Boehringer Ingelheim, Novo Nordisk. Other; Regenacy.

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Meta-analysis of SGLT inhibitors on cardiovascular death or heart failure hospitalisation based on presence of type 2 diabetes, heart failure, or chronic kidney disease

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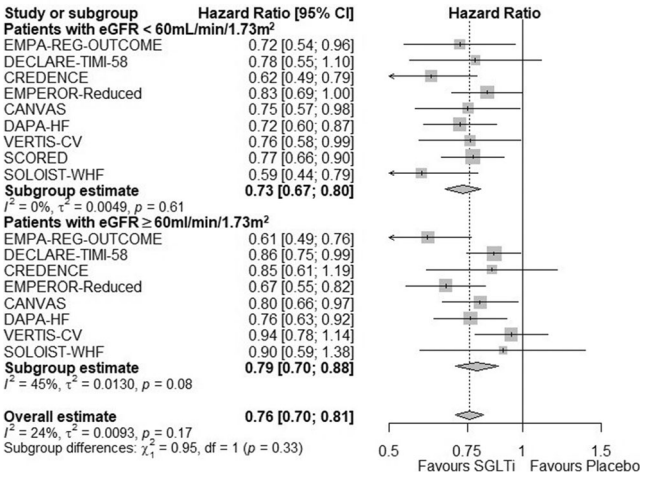
Background and aims: Individual cardiovascular outcomes trials have shown a favourable effect of SGLT inhibitors on cardiovascular and heart failure outcomes in patients with type 2 diabetes, heart failure, or chronic kidney disease. We assessed whether the magnitude of effect of SGLT inhibitors on cardiovascular death or hospitalisation for heart failure (CVDHHF) is affected by presence or absence of either type 2 diabetes, heart failure, or chronic kidney disease, by synthesising all data for relevant subgroup populations from cardiovascular outcomes trials.

Materials and methods: We searched Pubmed, Embase, and the Cochrane Library until March 2021 for reports of randomised, event-driven, cardiovascular outcomes trials comparing SGLT inhibitors with placebo. We performed random effects meta-analyses synthesising data in subgroup categories according to disease status at baseline, namely presence of type 2 diabetes, heart failure, and at least stage 3 chronic kidney disease (estimated glomerular filtration rate [eGFR] < 60 ml/min/1.73m^2). We calculated hazard ratios (HRs) and 95% confidence intervals (95% CIs) for the composite of CVDHHF.

Results: We analysed data from ten trials (75,533 participants). Median follow-up period ranged from 9 months to 4.2 years. In seven trials all patients had type 2 diabetes, three trials recruited exclusively patients with impaired kidney function, and three trials recruited exclusively patients with heart failure. SGLT2 inhibitors assessed were empagliflozin (two trials), dapagliflozin (three trials), canagliflozin (two trials), and ertugliflozin (one trial), while two trials assessed the dual SGLT1/2 inhibitor sotagliflozin. SGLT inhibitors reduced the incidence of CVDHHF both in patients with type 2 diabetes (HR 0.75, 95% CI 0.70 to 0.81) and in patients without type 2 diabetes (HR 0.75, 95% CI 0.66 to 0.87), and no difference was evident between the two subgroups (p for interaction = 0.99). This beneficial effect was also consistent in the analysis based on presence (HR 0.74, 95% CI 0.68 to 0.80) or absence (HR 0.77, 95% CI 0.67 to 0.88) of heart failure (p for interaction = 0.65). Similarly, SGLT inhibitors reduced CVDHHF both in patients with an eGFR < 60 ml/min/1.73m^2 (HR 0.73, 95% CI 0.67 to 0.80) and in patients with an eGFR ≥ 60 ml/min/1.73m^2 (HR 0.79, 95% CI 0.70 to 0.88, p for interaction = 0.33) (Figure).

Conclusion: SGLT inhibitors consistently reduced the composite of cardiovascular mortality or heart failure hospitalisation in all adults who had a history of either type 2 diabetes, heart failure, or chronic kidney disease.

Figure. Effects of SGLT inhibitors versus placebo on CVDHHF according to baseline kidney function



Disclosure: **K. Charalampidis:** None.

SO 63 COVID-19: from the known to unknown

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Characteristics and predictors of mortality in diabetes patients hospitalised with COVID-19: a single-centre cohort study from Poland

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Background and aims: By the end of March 2021, COVID-19 was diagnosed in more than 25 million people in the European Union, and more than 2 million in Poland. Based on the previous studies, it is estimated that 10-20% of these COVID patients also suffer from diabetes. In the course of COVID-19, diabetes has been recognized as a major risk factor for an admission to the intensive care unit, initiation of mechanical ventilation and mortality. To the best of our knowledge, this is the first attempt of a large-scale investigation of the course of COVID-19 in hospitalized patients with diabetes in Poland. In this study, we aimed to characterize the group of diabetic patients hospitalized due to the COVID-19 infection and identify potential factors associated with unfavorable outcomes.

Materials and methods: We retrospectively analyzed data from a cohort of patients admitted due to COVID-19 to the University Hospital in Cracow, Poland between March 6th and October 15th 2020. Data was collected from electronic patients' records concerning their basic clinical and biochemical parameters, treatments and outcomes. We assessed their basic characteristics and predictors of hospital mortality.

Results: We included 1729 patients from the studied period. Diabetic prevalence was seen in 23,3% (404 patients). Diabetic patients in comparison with the non-diabetic group were older (median age 71 years, p<0.001), with similar gender distribution (males: 53.8% vs. 50.5%, p=0.24). Patients with diabetes had longer hospital stay (medians: 18 vs. 16 days, p<0.001), required more frequently admission into an ICU (16.5% vs. 9.7%, p<0.001), mechanical ventilation (12.1% vs. 8%, p=0.011), and had higher mortality (19.3% vs. 11.2%, p<0.001). We performed a multivariable logistic model for predictors of mortality. The model included the following variables - age, sex, glycemia over 10 mmol/l, C-reactive-protein (CRP) level and white blood cells count on admission, history of hypertension, heart failure, ischemic heart disease, in-hospital use of dexaven, antiplatelet drugs, anticoagulation therapy, ACEI/sartan, statin, metformin and insulin. Three variables - older age, higher CRP and dexaven use - were associated with a higher death rate (OR 1.7 95%CI 1.03-1.11; 1,005 95%CI 1.001-1.01 and 2.32 95%CI 1.013-5.33 respectively), whereas metformin use was associated with lower mortality (OR 0.22 95%CI 0.9-0.55).

Conclusion: Diabetes was present in about one fourth of hospitalized patients in our setting. Mortality was nearly twice as high in this group of patients with diabetes when compared to non-diabetics. Older age and markers of high inflammatory reactions were identified as risk factors for mortality.

Supported by: Polish National Centre for Research and Development

Disclosure: **K. Mazur:** Grants; Polish National Centre for Research and Development, num. SZPITALE-JEDNOIMIENNE/18/2020.

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The impact of acute-to-chronic glycaemic ratio as a predictor of COVID-19 severity and mortality

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Background and aims: Underlying diabetes mellitus (DM) has been considered a risk factor for increased COVID-19 severity and worse outcomes, including higher mortality. However, no previous studies have assessed whether the combined evaluation of acute and chronic glycaemic levels may have a better prognostic value than each component alone. The global aim of our study was to assess whether the acute-to-chronic glycaemic ratio (ACR) was associated with mortality and severity outcomes in subjects with DM hospitalized for COVID-19.

Materials and methods: An observational cohort study including all subjects with DM hospitalized for COVID-19 between March 2020 and May 2020 was realized. Demographic and clinical data were collected, including data on clinical management, analytical parameters, glycaemic control (glycaemia at admission, HbA1c) and inflammatory markers, severity scales (MEWS, CURB65) and severity outcomes (length of hospital stay, intensive care unit (ICU) admission, acute respiratory distress syndrome (ARDS), invasive mechanical ventilation (IMV) and mortality. To estimate the chronic glucose levels (CGL) we used the formula eCGL= (28.7xHbA1c(%))-46.7. The ACR (glycaemia at admission/eCGL) were calculated for all subjects.

Results: A total of 91 subjects were included. Baseline characteristics of the subjects included are shown in Table 1, stratified for ACR tertiles. Subjects in the 3rd tertile presented higher glucose levels upon admission and higher concentrations of inflammatory markers (Table 1). A glucose level at admission >200mg/dL was associated with ARDS (OR=3.8 (1.4-10.2); p=0.008), IMV (OR=8.6 (1.8-41.5); p=0.008) and ICU admission (OR=9.4 (1.9-45.1); p=0.005) but was not associated with the length of stay or mortality. By contrast, a U-shape curve association was found between the ACR tertiles and mortality. The 1st ACR tertile showed a trend towards higher mortality (OR=4.9 (0.9-25.2); p=0.059) and the 3rd ACR tertile was significantly associated with higher mortality (OR=6.0 (1.2-30.7); p=0.032) compared with the 2nd tertile.

Conclusion: In subjects with underlying diabetes, the ACR presented a U-shaped mortality curve. These results suggest that an unbalance between the acute glycaemia at admission and the chronic metabolic control results in higher mortality rates, adding a better prognostic value than each component alone. By contrast, hyperglycaemia at admission was associated with a higher inflammation status and poor severity outcomes.

Table 1. Baseline characteristics and outcomes of the subjects according to A/C glycaemic ratio tertiles

	Whole population n=91	1st tertile (<=0.82) n=31	2nd tertile (0.83-1.21) n=30	3rd tertile (>=1.22) n=30	*P for trend*
Age (years)	75 (63-85)	75 (62-85)	71 (61-82)	76 (66-85)	0.639
Gender (F) (n, %)	45 (49.5)	14(45.1)	14 (46.7)	17 (56.7)	0.623
BMI (Kg/m²)	28.7(25.1-31.3)	28.5(25.1-34.4)	29.1(25.9-31.4)	26.3(23.5-31.0)	0.389
Glucose at admission (mg/dL)	149(116-216)	109(94-142)	146(121-179)*	265(213-344)*,‡	<0.001
Estimated chronic glucose (mg/dL)	154 (140-194)	177 (154-197)	147 (131-171)*	151 (137-197)	0.032
HbA1c (%)	7.0(6.5-8.4)	7.8(7.0-8.5)	6.8(6.2-7.6)*	6.9(6.4-8.5)	0.031
C-reactive Protein (mg/dL)	7.9 (4-16.4)	5.4 (1.5-8.5)	8.9 (5.5 - 15.8)*	15.9(4.4 - 26.1)†	0.002
Interleukin (IL)-6 (pg/mL)	56.9(18.3-102.0)	56.0(37.8-103.0)	42.2(18.2-65.9)	83.0(21.4-131.8)	0.227
D-dimer (UI/l)	700(450-1490)	620 (350-970)	860(510-1555)*	865 (500-2710)†	0.045
MEWS	2 (1-3)	2 (1-3)	2 (1-3)	2 (2-3)	0.637
CURB-65	2 (1-2)	2 (1-2)	1.5 (1-2)	2 (2-3)*,‡	0.021

Data are given as percentages and median (interquartile range). BMI, body mass index. HbA1c, glycosylated hemoglobin. MEWS, modified early warning score. CURB-65, confusion-urea-respiratory rate-blood preassure-65 years. FiO2, fraction of inspired oxygen. PaO2, arterial partial pressure of oxygen. ARDS, acute respiratory distress syndrome, ICU intensive care unit. *p <0.05 for 2nd tertile compared with 1st tertile; † p <0.05 for 3rd tertile compared with 1st tertile; and ‡ p <0.05 for 3rd tertile compared with 2nd tertile.

Disclosure: **J. Ramon:** None.

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Bamlanivimab + etesevimab for the treatment of COVID-19 in high-risk ambulatory patients including those with diabetes

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Background and aims: Patients with underlying medical conditions, such as diabetes, are at greater risk of severe COVID-19. Neutralizing monoclonal antibodies provide immediate, passive humoral immunotherapy, with the potential to reduce disease progression, hospitalizations, and death.

Materials and methods: In this phase 3 portion of the BLAZE-1 trial, a high-risk ambulatory cohort of 1035 patients with mild-to-moderate COVID-19 were randomly assigned 1:1 to a single intravenous infusion of a neutralizing monoclonal antibody combination treatment (2800mg bamlanivimab + 2800mg etesevimab together) or placebo, within 3 days of diagnosis. The primary outcome was overall patient clinical status, measured by the proportion of patients who experienced COVID-19-related hospitalization or death by any cause by Day 29.

Results: 1035 patients were randomized and infused (mean age [SD]; 53.8 years [16.8], female (52%)). There were 151 (29.2%) patients with diabetes in bamlanivimab + etesevimab treatment cohort and 134 (25.9%) on placebo. A 70% reduction in COVID-19-related hospitalization and death by any cause by Day 29 was observed in patients who received combination treatment (11/518 arm total) compared to placebo (36/517 arm total) (Δ[95% CI]=-4.8[-7.4,-2.3])(p=0.0004). No deaths were observed among patients who received the combination treatment, 10 deaths were reported in the placebo group, at least 8 designated COVID-19-related. For patients with diabetes, 4 (2.6%) receiving bamlanivimab + etesevimab combination treatment experienced a hospitalization or death from any cause event, while there were 13 (9.7%) patients with diabetes in the placebo group (Δ[95% CI]=-7.1[-12.7,-1.4])(p=0.0215). A significantly greater reduction in $\log_{10}$(viral load) from baseline at Day 7 was observed amongst patients who received bamlanivimab + etesevimab compared to placebo (Δ[95% CI]=-1.20[-1.46,-0.94])(p<0.00000001). The median time to sustained symptom resolution was shorter for combination treatment (days [95% CI]=8[7.0,8.0]) compared to placebo (days [95% CI]=9[8.0,10.0])(p=0.007). Similar rates of adverse events were observed between placebo (60/517, 11.6%) and combination treatment groups (69/518,13.3%).

Conclusion: 2800mg bamlanivimab + 2800mg etesevimab neutralizing monoclonal antibody combination therapy significantly reduced COVID-19-related hospitalizations and deaths amongst high-risk ambulatory patients and accelerated the decline in viral load and disease symptoms over time.

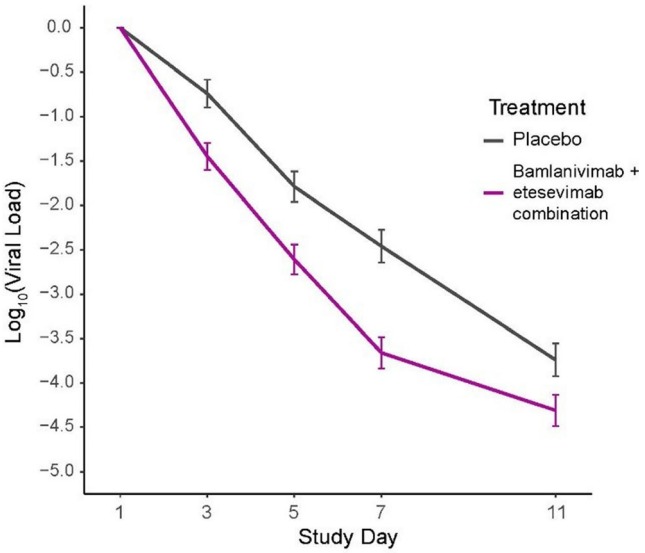

FIGURE: Bamlanivimab + etesevimab combination treatment effect on viral load (Days 1-11). Mean change in viral load ($\log_{10}$ scale) from baseline to Day 11 following bamlanivimab + etesevimab combination therapy versus placebo. Error bars represent 95% confidence intervals.

Clinical Trial Registration Number: NCT04427501

Supported by: Eli Lilly

Disclosure: **A. Tahbaz:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; awarded and holds Eli Lilly stock.

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The effectiveness of selected temporary testing protocols for gestational diabetes during the COVID-19 pandemic

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Background and aims: The oral glucose tolerance test (OGTT) is considered to be the "gold standard" in the diagnosis of gestational diabetes mellitus (GDM), despite multiple variations in criteria and diagnostic methods between countries. This approach was contested by some during the COVID-19 pandemic. Several organizations proposed new, temporary criteria for GDM diagnosis to limit the risk of a potential COVID-19 infection in pregnant women undergoing this multi-step diagnostic process. The evidence supporting such changes is lacking. "Diabetes Poland" guidelines did not change during the pandemic resulting in the utilization of traditional criteria for GDM testing. We aimed at assessing the effectiveness of selected temporary pandemic protocols (United Kingdom [UK] and Australia) for testing for gestational diabetes during the COVID-19 pandemic when applied to patients admitted to an outpatient clinic, University Hospital, Cracow, Poland in 2020.

Materials and methods: We performed a retrospective analysis of GDM patients admitted to the outpatient clinic at the University Hospital of

Cracow, Poland throughout 2020. This is a preliminary report of exploratory analysis of data from the time period between January and June. We compared the GDM frequency using the standard, pre-COVID-19 and COVID-19-specific diagnostic criteria from the UK and Australia. In the UK, risk-factor-based screening was recommended with testing HbA1c (GDM if ≥39 mmol/mol [5.7%]) or random plasma glucose (RPG, GDM if ≥9.0 mmol/L) during the first visit (usually up to 12 weeks of gestation), and when both are negative - a fasting plasma glucose (FPG) at 28 weeks of pregnancy (GDM if >5,6 mmol/l). In Australia, universal testing was proposed - in women with risk factors for GDM, the HbA1c should be assessed during the 1st trimester (GDM is diagnosed if >41 mmol/mol [5.9%]). When negative, a FPG at 24-28 weeks of gestation (GDM is diagnosed if ≥ 5.1 mmol/l, no GDM is diagnosed if <4.6 mmol/l, and the OGTT recommended if 4.7-5 mmol/l).

Results: When applying the UK criteria to our cohort, 193 of 254 (76%) GDM cases would be missed. In the first step, 25 women without risk factors for GDM would be excluded from screening. Risk factors were prevalent in 229 women, of whom 22 were diagnosed with diabetes based on FPG and 60 underwent OGTT up to 12 weeks of gestation in our setting. If FPG or OGTT results from this group were treated as RPG, 28 cases of GDM would be identified and 54 missed, delaying the diagnosis. 201 women would be subject to next stage of screening at 28 weeks of gestation. In this group, only 33 women had FPG ≥ 5,6 mmol, 168 cases of GDM in this step would be missed. When applying the Australian criteria to our cohort, 53 of 254 (20,9%) GDM cases would be missed. GDM diagnosis in 135 women from our cohort, that were diagnosed before the 24th week of gestation, would be delayed to the 24-28 weeks of pregnancy. 53 GDM cases would be missed, 131 would be confirmed, and 70 women would require further OGTT.

Conclusion: Modifications proposed from the UK and Australia resulted in varying decreases and delays in GDM diagnoses. We acknowledge that these new criteria may be of probable benefit due to less potential exposure to COVID-19, however, we lack specific evidence to support this hypothesis. More studies are required to investigate the impact of the simplification of GDM diagnosis on pregnancy outcomes and the incidence of COVID-19 in this group.

Disclosure: **M. Kania:** None.

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How COVID-19 has impacted diabetes management in the United States (iNPHORM study)

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Background and aims: Most diabetes-related COVID-19 studies—while focusing on the epidemiology of hospitalized cases—have failed to consider how community diabetes care has suffered due to the pandemic. Disruptions to services, resources, and self-management forebode important population-based consequences to diabetes-related morbidities, especially in the United States (US) where COVID-19 and diabetes eminently collide. Using data from the real-world iNPHORM study, we measured how and to what extent the pandemic has affected glycemic management in diabetic Americans, nearly one year after the first US case was detected.

Materials and methods: A generalized cohort of Americans (≥18 years old) with type 1 or type 2 diabetes mellitus (T1DM, T2DM) taking insulin and/or secretagogues was recruited into the iNPHORM study from a probability-based internet panel managed by Ipsos Interactive Services Ltd. Online self-reported surveys were disseminated monthly for one year. This cross-sectional evaluation summarizes data collected in January 2021. Statistical differences in responses by diabetes type were assessed using the Wilcoxin-Mann-Whitney test or two-sample test of proportions.

Results: A sample of 772 respondents was analyzed (T1DM: 18.9%; male: 49.5%; age: 52.1 [SD: 14.5]). The prevalence of medically confirmed COVID-19 was 9.3% (March 2020 to January 2021). Among all participants, nearly 25% (T1DM: 24.7%; T2DM: 23.1%, *P*-value = 0.53) reported the COVID-19 situation made affording rent/living expenses somewhat or much harder, while 18.8% (T1DM: 21.2%; T2DM: 18.3%, *P*-value = 0.14) struggled to ensure adequate food supply to avoid hypoglycemia. Many also experienced challenges paying for their diabetes medications (overall: 17.4%; T1DM: 19.9%; T2DM: 16.8%, *P*-value = 0.16) or test strips/sensors (overall: 16.2%; T1DM: 17.1%; T2DM: 16.0%, *P*-value = 0.40), as well as issues retrieving therapies (overall: 22.2%; T1DM: 24.0%; T2DM: 21.8%, *P*-value = 0.46). Over 10% rationed their diabetes medications to extend supplies (T1DM: 15.8%; T2DM: 10.7%, *P*-value = 0.09) or avoid hypoglycemia (T1DM: 17.1%; T2DM: 9.1%, *P*-value = 0.005). Participants also found it difficult to remember to take their diabetes medication(s) as prescribed (overall: 11.6%; T1DM: 8.2%; T2DM: 12.3%, *P*-value = 0.82), test their blood glucose (overall: 12.5%; T1DM: 9.6%; T2DM: 13.1%, *P*-value = 0.44), or monitor hypoglycemia risk (overall: 12.5%; T1DM: 9.6%; T2DM: 13.1%, *P*-value = 0.42). Several (11.4%) felt less in control of their hypoglycemia (T1DM: 15.8%; T2DM: 10.4%, *P*-value = 0.01) with 16.0% reporting insufficient social support to help manage events (T1DM: 15.8%; T2DM: 16.0%, *P*-value = 0.71). One quarter thought the pandemic impeded their ability to consult with diabetes care providers (T1DM: 26.0%; T2DM: 25.0%, *P*-value = 0.60). Over a third struggled to stay as physically active as usual (T1DM: 38.4%; T2DM: 36.5%, *P*-value = 0.69).

Conclusion: This is the most recent and comprehensive investigation to measure the impact of the COVID-19 situation on socio-economic, behavioural/clinical, and psychosocial aspects of diabetes management in the US. The pandemic was found to cause substantial deficiencies in routine diabetes care with only few significant differences observed by diabetes type. The results of this study will be instructive for handling diabetes management both during the current public health emergency and in future.

Clinical Trial Registration Number: NCT04219514

Supported by: Funding for the iNPHORM study was provided through an investigator-sponsored grant from Sanofi Global.

Disclosure: **A. Ratzki-Leewing:** Grants; Sanofi Global. Honorarium; Eli Lilly, Novo Nordisk, Sanofi Canada.

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Effects of COVID-19 lockdown on health care for persons with type 2 diabetes in Germany: results from an electronic medical record database

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Background and aims: In the COVID-19 lockdown, patients´ fear of infection in hospitals and practices, and possible reduction of routine care due to focus on COVID-19 emergencies may have compromised health care for people with type 2 diabetes. Moreover, stay at home policies may have led to an unhealthy lifestyle (less physical activity, more caloric intake) what might have had an impact on body weight and metabolic control of persons with type 2 diabetes. The first lockdown in Germany took place from March to May 2020. The aim was to identify the effects of the lockdown on glycaemic control, BMI and cardiovascular risk factors.

Materials and methods: The nationwide German Disease Analyzer database includes a representative and anonymized panel of physicians´

practices in Germany providing real-world data of patients. We calculated absolute changes (95% confidence intervals) of means from June to November 2020 to data of the same persons with type 2 diabetes from June to November 2019, and June to November 2018, respectively, for BMI, HbA1c, fasting glucose, cholesterol, triglycerides, systolic and diastolic blood pressure, and creatinine.

Results: Data of 139,508 (2018), 143,151 (2019), and 146,305 (2020) persons with type 2 diabetes were analysed. 32,339 patients had ≥ 1 HbA1c measurement from June to November in each year. HbA1c change (95%CI) between 2019 to 2020 was +0.04% (0.03%; 0.05%) compared to -0.02% (-0.03; -0.01) between 2018 and 2019. Mean blood pressure, BMI, fasting glucose, HDL and LDL cholesterol, triglycerides, and creatinine changed only slightly between June to November 2019 and June to November 2020. These alterations barely differed from the corresponding changes between 2018 and 2019 (Table 1). Furthermore, the proportions of patients with poor metabolic or renal outcomes were similar in the three time intervals. The proportions of patients with BMI ≥ 30 kg/m^2 were 56%, 55%, and 54% in June to November 2018, 2019, and 2020, respectively. The corresponding proportions for HbA1c > 7.0% were 39%, 39%, and 40% (fasting glucose ≥ 126 mg/dl: 57%, 58%, and 60%; systolic blood pressure ≥ 140 mm Hg: 44%, 44%, and 46%; diastolic blood pressure ≥ 90 mm Hg: 19%, 18%, and 19%).

Conclusion: There is no evidence that the COVID-19 lockdown in March to May 2020 had a short-term harmful influence on health care outcomes in people with type 2 diabetes in primary care in Germany. Most likely, the lockdown did not compromise established routines of diabetes care in patients and providers.

Table 1. Changes of outcomes in people with type 2 diabetes in primary care between 2019[a] and 2020[a], and 2018[a] and 2019[a] (Disease Analyzer, Germany)

	N	A Change between 2019 and 2020 (95% CI)	B Change between 2018 and 2019 (95% CI)	A – B (95% CI)
BMI (kg/m^2)	3614	-0.11 (-0.17–0.06)	-0.19 (-0.26–0.13)	0.08 (-0.02-0.17)
HbA1c (%)	32339	0.04 (0.03-0.05)	-0.02 (-0.03–-0.01)	0.06 (0.04-0.07)
Fasting glucose (mg/dl)	19736	2.18 (1.5-2.85)	-0.2 (-0.89-0.49)	2.38 (1.23-3.53)
HDL cholesterol (mg/dl)	11459	0.3 (0.18-0.43)	0.48 (0.36-0.6)	-0.18 (-0.38-0.03)
LDL cholesterol (mg/dl)	13927	-3.22 (-3.65–2.79)	-4.06 (-4.5–-3.61)	0.84 (0.12-1.56)
Triglycerides (mg/dl)	12581	1.25 (-0.30-2.80)	-4.35 (-5.95–-2.76)	5.60 (3.00-8.20)
Systolic blood pressure (mm Hg)	12140	1.14 (0.83-1.46)	-0.19 (-0.51-0.13)	1.34 (0.8-1.87)
Diastolic blood pressure (mm Hg)	12134	0.19 (0.01-0.36)	-0.15 (-0.33-0.03)	0.34 (0.04-0.63)
Creatinine (mg/dl)	23108	0.02 (0.02-0.02)	0.02 (0.02-0.03)	-0.01 (-0.01-0)

[a] mean of measurements in June to November of the respective year

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Disclosure: **B. Kowall:** None.

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Obesity and decreased vibration perception associated with premature cardiovascular mortality in a single centre prospective of study of people with diabetes

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Background and aims: The aim of this prospective, single centre study was to define factors associated with early cardiovascular mortality in diabetes.

Materials and methods: 1345 patients under age 75 were included who were undergoing assessment of their diabetes between January 2008 and May 2010 as part of standard practice in a specilaist clinic at a regional teaching hospital in Serbia. Peripheral artery disease (PAD) was assessed by audible Doppler waveform and ABPI with cut-offs >1.4 and <0.9. Peripheral neuropathy was assessed by vibration perception threshold (VPT, using a semi-quantitative tuning fork: abnormal if ≤5), ankle reflexes (AR) and sudomotor function (Neuropad®) of the foot. Diabetic retinopathy (DR) was assessed by fundoscopy. Evidence of vascular disease: thrombendarterectomy and/or cerebrovascular insult (TEA/CVI), myocardial infarction (MI), heart failure (HF), diabetic foot ulcer (DFU), minor amputations (sAMP) and major amputations (mAMP) was also collected. Outcome was determined in 2021 and baseline characteristics were compared between those who had and had not suffered cardiovascular death under age 75 years within 10 years of review in two casually selected cohorts.

Results: Those who died (n_2=70) were more frequently male (60 vs. 45.3%, p=0.08), younger (66.4±7.4 vs. 79.9±3.4, p<0.000), had a shorter period of follow-up (3.6±2.3 vs. 11.2±1.7 years, p<0.000) when compared to those still alive (n_1=75). Those who died were also significantly (p<0.01) more likely to have had TEA/CVI (34.3 vs. 10.7%), HF (21.4 vs. 1.3%), MI (44.3 vs. 20%), PAD (48.6 vs. 9.3%), DFU (25.7 vs. 9.3%), mAMP (17.1 vs. 1.3%) at baseline. Minor amputations were significantly more likely (8.6 vs. 1.3%, p<0.04) but there were no differences in parameters of end-organ damage: proliferative DR (17.1 vs. 8%, p=0.10), laser photocoagulation (25.7 vs. 13.3%, p=0.06). Following multivariable logistic regression analysis significant differences between groups remained for only creatinine (123±45 vs. 88.9±16.9 mmol/L, p<0.003) and VPT ≤5 (7.8 [95% CI: 3.7-16.4)], p=0.008), cholesterol >6.2 mmol/l (2.0 [95% CI: 1.0-4.0)], p<0.05), estimated maximum lifetime BMI (3.4 [95% CI: 1.7-6.8)], p<0.000), alcohol usage (4.7 [95% CI: 1.5-14.7)], p=0.005), smoking habit (2.2 [95% CI: 1.1-4.3)], p<0.03) and earlier age of diabetes onset (43.4±12.5 vs. 49.2±9.9, p=0.0029). When the 72 patients with impaired vibration sense were compared with 73 with VPT≥6 and there were significant differences in TEA/CVI (4.2 [95% CI:1.6-10.9)], p=0.003) and PAD (3.9 [95% CI: 1.8-8.8)], p<0.001) and estimated maximum lifetime BMI (9.4 [95% CI: 3.4-25.7), p<0.000). Finally, when those who had had a previous MI at baseline (n=46) were compared with those who hadn't (n=99), MI was associated with increased death rate (3.2[95% CI: 1.5-6.6)], p=0.002), as was PAD (2.9[(1.3-6.1)], p=0.007).

Conclusion: Decreased VPT, the presence of PAD on clinical testing and higher maximum estimated lifetime BMI are strongly associated with premature cardiovascular death. These measures may be independent markers of greater risk of reduced life expectancy.

Disclosure: **D.S. Tesic:** None.

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Impact of prediabetes in heart failure with preserved ejection fraction: the NetDiamond cohort

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Background and aims: Heart Failure with Preserved Ejection Fraction (HFpEF) is a growing challenge due to the population aging and the rising prevalence of cardiovascular risk factors. Our group has previously described the influence of diabetes diagnosis on the signs and symptoms, comorbidities and echocardiographic and vascular evaluation of patients with HFpEF in a stable phase. Patients with diabetes have more comorbidities and morphologic cardiovascular changes, comparing to non-diabetic patients. However, the association of prediabetes with clinical and echocardiographic features in HFpEF is still unclear.We aimed to evaluate the influence of the diagnosis of prediabetes on the signs and symptoms, comorbidities and echocardiographic and vascular evaluation of patients with HFpEF in a stable phase.

Materials and methods: Cross-sectional study including 40 patients with HFpEF in stable phase, followed in our center. Signs, symptoms and comorbidities were obtained by anamnesis, physical examination and patient medical records. The cardiac function was evaluated by echocardiography performed by expert cardiologists. Endothelial function (Reactive Hyperemia Index) was evaluated with the EndoPAT^{TM}2000 device and carotid-femoral pulse wave velocity. The associations between prediabetes, defined as A1C 5.7-6.4% or fasting plasma glucose 100-125 mg/dL, and the previously described outcomes were assessed through linear and logistic regression models, adjusted for sex, age, systolic blood pressure (SBP) and body mass index (BMI).

Results: The included population (n=40) had an average age of 74.6±9.6 years and 60.0% were males. The average BMI was 28.1±5.1 kg/m^2 and 48.7% had prediabetes. Concerning signs and symptoms, no significant differences were recorded regarding oedema, orthopnea NYHA class or nocturnal paroxysmal dyspnea. About comorbidities, no differences were recorded concerning blood pressure, lipid profile, peripheral, cerebral and cardiovascular atherosclerotic diseases. The echocardiographic evaluation showed that patients with prediabetes have a thinner interventricular septum (ß=-1.23 [-2.2 to -0.24]; p=0.016) and a tendency to have a higher tricuspid annular plane systolic excursion (TAPSE).

Conclusion: Opposing to diabetes, in our cohort, prediabetes was not associated with deleterious changes in cardiac or endothelial functions. Those are continuous diseases, and our results highlight the importance of preventing progression of prediabetes to diabetes in HFpEF.

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Disclosure: **M. Borges-Canha:** None.

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Respective role of NT-proBNP and coronary artery calcium score for the detection of silent coronary disease in asymptomatic patients with diabetes

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Background and aims: BNP (B-type natriuretic peptide) is an indicator of pressure load, functional and structural left ventricle alterations, and severity of myocardial ischemia. Plasma NT-proBNP level has a good diagnostic value for heart failure and is a risk marker for cardio-vascular events. The present study aimed to analyse the predictive value of NT-proBNP and coronary artery calcification score (CACS) for the detection of silent coronary disease in asymptomatic patients with diabetes.

Materials and methods: We included 416 asymptomatic patients with diabetes at high or very high coronary risk but free of cardiac history and symptom. Silent coronary disease was assessed by measuring CACS (CT-scan), and performing a stress myocardial scintigraphy to detect silent myocardial ischemia (SMI), and a coronary angiography in the patients with SMI. NT-proBNP was measured in 243 of them.

Results: The prevalence of a high CACS (≥100 AU) was 44%, NT-proBNP ≥125 pg/mL 23% and SMI 9.6% (40 patients, including 15 with significant coronary stenoses (CS) on angiography). The prevalence of SMI and CS was higher among the patients with CACS ≥100 AU than among those with CACS <100 AU (15.4% *vs* 5.1% and 6.6% *vs* 1.3%; p<0.001 for both comparisons). The sensitivity and the specificity of CACS ≥100 AU for the detection of SMI were 70% and 59%, respectively. The proportion of patients with NT-proBNP ≥125 pg/mL was higher among the patients with CACS ≥100 AU than among those with CACS <100 AU (30% *vs* 16.5% ; p<0,05). This threshold of NT-proBNP offered sensitivity, specificity and positive predictive value of 30%, 84% and 61%, respectively, for the detection of a CACS ≥100 AU. NT-proBNP did not predict the presence of SMI nor CS.

Conclusion: In asymptomatic diabetic patients, a NT-proBNP level ≥125 pg/mL, cut-off point for heart failure diagnosis, may help making decision to measure CACS but, at variance with CACS, does not contribute to identify the patients with SMI or CS. Among the patients with high CACS, 30% are at risk of developing heart failure.

Disclosure: **S. Pinto:** None.

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Withdrawn

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To study the relationship between the mean platelet volume, immature platelet fraction, and glycaemic control in type 2 diabetes patients

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Background and aims: Mean platelet volume (MPV) and immature platelet fraction (IPF) have been shown to be surrogate markers of platelet size and activity, respectively. In patients with diabetes mellitus, altered platelet activity is one of the contributing factors for atherothrombosis. We studied the effect of chronic hyperglycemia on MPV and IPF in patients with type 2 diabetes mellitus (T2DM). The objective of the study was to find the correlation between MPV, IPF, and HbA1c in patients with T2DM and to study the impact of improvement in glycemic control on MPV and IPF.

Materials and methods: We prospectively evaluated 60 patients with T2DM and 38 nondiabetic healthy controls. Patients were divided into two groups based on their glycemic status (Group A [n = 23]: HbA1c

≤7% and Group B [n = 37]: HbA1c >7%). MPV, IPF, and HbA1c were measured in the study and control populations at baseline and only in Group B patients at 3 months.

Results: In diabetic patients, average MPV (Group A: 9.82 ± 0.62 fl, Group B: 10.14 ± 0.77 fl, vs. controls: 9.51 ± 1.35 fl, P = 0.02) and IPF (Group A: 1.78 ± 0.79, Group B: 2.47 ± 1.4 vs. controls: 1.59 ± 0.82, P = 0.001) values were significantly higher compared to controls. Seventeen of 37 patients (46%) in Group B achieved a target HbA1c level (<7%) on follow-up. MPV was significantly decreased in these 17 patients compared to their baseline MPV (9.72 ± 0.70 fl vs. 10.15 ± 0.72 fl, P = 0.00153); however, the reduction in mean IPF was not significant (2.42 ± 1.67 vs. 2.13 ± 1.11, P = 0.152).

Conclusion: Platelet activity, as measured by MPV and IPF, is significantly higher and correlates with glycemic control in T2DM patients when compared to healthy nondiabetic controls.

Disclosure: **S. Ahsan:** None.

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Type 2 diabetes significantly modulates the power of lipoprotein (a) to predict cardiovascular events and mortality in young coronary artery disease patients

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Background and aims: Lipoprotein(a) [Lp(a)] is an important cardiovascular risk factor especially in young individuals. The power of Lp(a) to predict cardiovascular events in young coronary artery disease (CAD) patients with type 2 diabetes (T2DM) however is unclear and is addressed in the present study.

Materials and methods: Lp(a) was measured in a cohort of 731 patients with angiographically proven CAD who were aged <65 years. Vascular events were recorded over a mean follow-up of 6.6±3.2 years.

Results: At baseline, 216 patients had T2DM, and 515 did not have diabetes. During follow-up, 30.2% of our patients suffered cardiovascular events. Lp(a) proved to be a strong and independent predictor of vascular events in the total study cohort (standardized adjusted HR=1.30 [1.07-1.56]; p=0.007). In subgroup analyses by diabetes status, Lp(a) significantly predicted vascular events in non-diabetic patients (standardized adjusted HR= 1.39 [1.12-1.74]; p=0.003) but not in diabetic patients (standardized adjusted HR=0.93 [0.63-1.38]; p=0.731). An interaction term Lp(a)xT2DM was significant (p=0.002), indicating that T2DM significantly modulated the power of Lp(a) to predict cardiovascular events.

Conclusion: We conclude that Lp(a) significantly modulates the power of Lp(a) to predict cardiovascular events in CAD patients <65 years.

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Remnant cholesterol in patients with established cardiovascular disease predicts cardiovascular events both among patients with type 2 diabetes and among non-diabetic subjects

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Background and aims: Remnant cholesterol, which is calculated as total cholesterol minus LDL cholesterol minus HDL cholesterol has attracted interest as a marker of cardiovascular event risk. The power of remnant cholesterol to predict cardiovascular events in patients with established cardiovascular disease is unclear and is addressed in the present study.

Materials and methods: We enrolled 1822 consecutive patients with established cardiovascular disease, including 1472 with angiographically proven stable CAD 350 with sonographically proven peripheral artery disease. Prospectively, cardiovascular events were recorded over a mean follow-up period of 6.2±3.2 years.

Results: At baseline, remnant cholesterol was significantly higher in patients with T2DM (n=608) than in non-diabetic subjects (27±25 vs. 21±21 mg/dl; p<0.001). During follow-up, 584 of our patients suffered cardiovascular events; the event rate was significantly higher in patients with T2DM than in non-diabetic subjects (45.4 vs. 32.2%; p<0.001). Remnant cholesterol in Cox regression models adjusting for age, sex, hypertension, smoking, body mass index and LDL cholesterol independently predicted cardiovascular events in the total study population (standardized adjusted HR 1.15 [1.07-1.23]; p<0.001), and in patients with T2DM as well as in non-diabetic subjects (standardized adjusted HRs 1.17 [1.03-1.34]; p=0.013 and 1.12 [1.01-1.23]; p=0.028, respectively).

Conclusion: From our data we conclude that remnant cholesterol in patients with established cardiovascular disease predicts cardiovascular events both among patients with T2DM and among non-diabetic subjects.

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