FOOD FOR THOUGHT

GROWTH OF THE HUMAN BODY AND BRAIN IN EARLY LIFE

JORINE ROELANTS

STELLINGEN

1. Een kleiner embryonaal volume, gemeten met driedimensionale echoscopie-technieken en virtual reality, is al vanaf 9 weken zwangerschapsduur geassocieerd met een verhoogd risico op een ongunstige zwangerschapsuitkomst [**dit proefschrift**].

2. De corpus callosum - fastigium lengte is een nieuwe echografische marker waarmee variatie in hersengroei tussen gezonde foetussen en foetussen met groeivertraging in zowel de prenatale als de postnatale periode gemeten kan worden [**dit proefschrift**].

3. De hogere dosis aminozuren en het vroeger starten van vetten bij prematuur geboren kinderen heeft nog niet geleid tot het behalen van de beoogde groei tijdens de ziekenhuisopname, noch tot een betere ontwikkeling in de eerste levensjaren [**dit proefschrift**].

4. Een hogere gewichtstoename tijdens de ziekenhuisopname is bij prematuur geboren kinderen geassocieerd met een hogere relatieve vetmassa 6 weken na de uitgerekende datum [**dit proefschrift**].

5. Het is onmogelijk om 'optimale groei' te definiëren als de definitie alleen gebaseerd is op klassieke kwantitatieve groeimaten [**dit proefschrift**].

6. Er dient meer aandacht gegeven te worden aan de groei en ontwikkeling van de foetus bij het evalueren van groei en ontwikkeling van de pasgeborene.

7. Absence of evidence is not the same as evidence of absence.

8. "When science becomes a business, what matters is not the quality of the product, but whether it sells" [**Michele Pagano, Nature 27 July 2017**].

9. Wees zuinig op de aarde, het is de enige planeet met chocola [**Loesje**].

10. "Als je 10 problemen tegelijk wilt oplossen, heb je er 11. Dat lukt je nooit. Focus." [**Jan Schaefer**].

11. Je moet je eigen smaak aan het leven geven.

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Jorine Atalante Roelants

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FOOD FOR THOUGHT

growth of the human body and brain in early life

Stof tot nadenken

groei van het menselijk lichaam en het brein in het vroege leven

Proefschrift

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WHAT IS DONE IN LOVE IS DONE WELL

Vincent van Gogh

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1

GENERAL INTRODUCTION

"IT IS NOT THE STRONGEST OF THE SPECIES THAT SURVIVES, NOR THE MOST INTELLIGENT THAT SURVIVES. IT IS THE ONE THAT IS MOST ADAPTABLE TO CHANGE – CHARLES DARWIN"

Individual experiences in early life affect long–term health. In the early 1990s, Hales and Barker were the first to propose the 'thrifty phenotype' hypothesis.[1] The main concept of this hypothesis is that poor intra–uterine conditions result in adaptation of the fetus to its environment, to increase short–term survival chances. This adaptive process can also result in altered organ growth, structure, and function. On the long term, these early adaptions contribute to the risk of adverse health outcomes, such as adiposity and cardiometabolic diseases, throughout the life course. This increased risk is mainly induced by a mismatch between the intra–uterine and extra–uterine environment, with the fetal phenotype being unsuitable for the extra–uterine environment that is often much richer in oxygen and nutrients.[1] Later on this hypothesis was rewritten into the Developmental Origins of Health and Disease [DOHaD] paradigm, stating that not only poor conditions but also subtle variations in the intra–uterine environment can affect the offspring's phenotype with consequences for diseases in later life.[2] Nowadays, the first 1000 days of life, ranging from conception to approximately 2 years of age, are considered the period most sensitive to environmental conditions.

The DOHaD paradigm has mainly focused on pregnancy as the most important period of fetal programming. In preterm born infants this might be different, as the period in–utero has been shortened. It is believed that in those infants postnatal environmental conditions can also induce relevant programming. Which organ systems are affected, and the magnitude of the effect, does not only depend on the severity of the adverse environmental conditions, but also on its timing.[3] The proposed underlying mechanism of fetal programming are epigenetic changes, which are heritable and acquired variations in the genetic material that do not affect the underlying DNA sequence.[4]

DEVELOPMENT OF THE EMBRYO AND FETUS

From conception to birth, a fertilized ovum develops into a blastocyst, an embryo [up to 10 weeks of gestation], and then becomes a fetus. Embryonic development can be divided into different stages: fertilization, implantation of the fertilized ovum, and embryogenesis. During embryogenesis the embryo forms and organs

are developed. The placenta is also formed during the process of embryogenesis. The fetal period [from 10 weeks of gestation onward] is characterized by growth and maturation of the organs, processes that are less susceptible to environmental conditions.

In the first and second trimester, fetal growth mainly consists of lean mass. After this period, fat mass accumulation increases rapidly, with approximately 500 gram [~10 – 15%] of the fetal weight consisting of fat mass at the end of pregnancy.[5]

DOHAD PARADIGM AND THE DEVELOPMENT OF THE BODY AND BRAIN IN EARLY LIFE

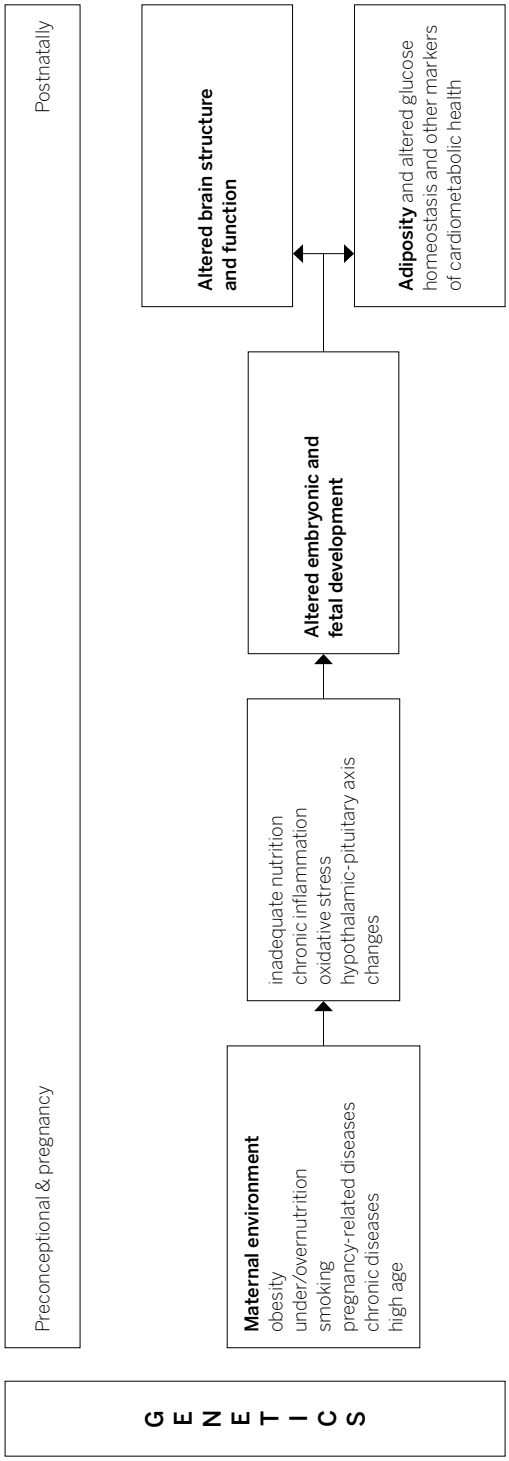
Adverse maternal health and lifestyle can have detrimental effects on the developing embryo and fetus, as prenatally, the mother is the fetal environment. These effects include increased risk of altered fetal growth, miscarriage, and fetal death, but also of preterm birth, fetal growth restriction, and congenital anomalies.[6–9] Besides these short–term effects, it can also influence postnatal health [DOHaD paradigm].[10] The predisposition for childhood obesity is, for example, thought to originate in the period around conception and gestation, with not only genetics but also environmental conditions playing an important role.[11] In infancy, BMI is not sensitive as marker of child– and adulthood obesity, while adiposity [fat mass relative to weight] is considered an early marker of childhood obesity and the development of metabolic syndrome.[12–14] Adiposity tracks across infancy into childhood and is associated with altered free fatty acid metabolism, which is associated with dyslipidemia and insulin sensitivity. It is also associated with higher levels of vasoactive peptides and cytokines, and hypertension, thereby increasing the risk of metabolic syndrome.[15–17] Fat distribution and adipose tissue function may be even more important for the individual cardiometabolic risk than absolute fat mass, as visceral fat is associated with a higher cardiometabolic risk than subcutaneous fat.[18] The accumulation of adipose tissue by either increasing adipocyte size [hypertrophy] or adipocyte number [hyperplasia] is also recognized as an important contributor to the risk of developing metabolic syndrome. Adipocyte hypertrophy is associated with higher levels of proinflammatory cytokines, reduced levels of insulin sensitivity related adiponectin and interleukin 10, and increased stress levels, all associated with metabolic syndrome. Why some individuals react with hypertrophy while others react with hyperplasia is not fully understood. It is believed that both environmental and genetic conditions play a role in the underlying mechanisms, which is in line with the DOHaD paradigm.[14] Several pathways are suggested to be

involved in the predisposition of childhood obesity and long–term cardiometabolic risk factors [**Figure 1**]. Environmental conditions such as maternal obesity, over– and undernutrition, smoking, and chronic diseases can increase oxidative stress and chronic inflammation in–utero, resulting in fetal reprogramming and thereby affecting adiposity in later life.[19,20] Structural and functional brain alterations of the hypothalamus and hippocampus, associated with maternal overnutrition, increased free radicals, and chronic inflammation can also influence adiposity by altering glucose homeostasis, as shown in animal studies.[21,22] Additionally, there might also be direct effects of maternal overnutrition and increased energy supply on the risk of adiposity.[23] Although altered brain development is often a consequence of brain injury postnatally, environmental conditions, that are associated with pathways leading to adiposity, also influence brain development and functioning. A clear example is the influence of folic acid on central nervous system development.[3] But also other environmental conditions are able to influence brain development in this very early stage. Chronic inflammation in pregnancy, associated with health and lifestyle conditions such as obesity and smoking, is for example associated with brain structure and function and with a higher risk for neurodevelopmental disorders such as autism and attention–deficit/hyperactivity disorder.[24,25] Proinflammatory cytokines are involved in fetal brain development, as facilitators of cellular survival, proliferation and differentiation, axonal growth, and synaptogenesis. Elevated levels can influence the onset of different stages of brain development, thereby influencing structure and functioning of the brain.[26]

MONITORING EMBRYONIC AND FETAL GROWTH

Traditionally, growth of the embryo and fetus is evaluated by using two–dimensional [2D] ultrasonography [US]. In clinical practice, the 2D measure crown–rump length [CRL] is used in the first trimester to evaluate embryonic growth.[27] In the second and third trimester, other measures are used to estimate fetal growth, including femur length, abdominal circumference, and head circumference.[28] In midgestation, a structural US for evaluation of fetal organ development is part of standard care.[28] Overall, evaluation of fetal growth mainly consists of estimates of quantitative size. Although some measures are available for monitoring of brain growth and quality of body growth [body composition and organ growth], those are not implemented in standard care yet.

FIGURE 1 | DEVELOPMENTAL ORIGINS OF ADIPOSITY AND BRAIN ALTERATIONS IN EARLY LIFE



Detailed visualization of the embryo and fetus has improved tremendously as a result of the development of three–dimensional [3D] US techniques. Using this technique, it is now possible to reliably measure embryonic size from around 8 – 9 weeks of gestation onward.[29] Also later in pregnancy, 3D US techniques are increasingly used in research for the detection of altered placentation, placental development, and fetal brain development, growth, and body composition.[30–33]

FOCUS OF RESEARCH

The prevalence of adverse birth outcomes such as preterm birth, small for gestational age, and congenital anomalies is still high worldwide, with relatively high morbidity rates in the Netherlands compared to other European countries.[34,35] Moreover, the last decades, the incidence of childhood overweight and obesity has started to increase exponentially worldwide.[36] The harmful consequences of adverse birth outcomes and chilhdood obesity for long–term neurodevelopmental and cardiometabolic health have been well established.[37,38] Many of these outcomes are believed to originate in the periconceptional period, with environmental conditions such as maternal health and lifestyle playing an important role in development of these adverse birth outcomes and adiposity [**Figure 1**].[39–41] To improve fetal and neonatal health outcomes, thereby influencing health throughout the life course, we need to identify embryos and fetuses at risk of an adverse outcome as early in pregnancy as possible.

PRETERM INFANTS

Preterm birth, defined as birth before 37 weeks of gestation, is the leading cause of perinatal morbidity and mortality in the developed world. Worldwide, approximately 15 million neonates are born preterm each year.[42] In the Netherlands, approximately 7% of the 175,000 infants born alive are born preterm.[43] Improvements in care of preterm infants, such as the introduction of artificial ventilation and antenatal steroids, have improved survival chances tremendously the last decades.[44] At this moment, mortality rates are around 30% at 24 weeks of gestation, and 1 – 4% at 32 weeks of gestation. Morbidity rates have, however, only slightly decreased.[38,45–47] With the increasing survival rates we now increasingly face the health burden and costs of these morbidities on the short– and long–term. Long–term follow–up of preterm infants is thus important for the individual patient, and should be focus of clinical research to evaluate perinatal care and to support important ethical discussions in early life.

FEEDING THE PRETERM INFANT

Due to the immaturity of the gastro–intestinal tract, very preterm infants are unable to tolerate full enteral feeding in the first days to weeks of life. They depend on parenteral [intravenous] nutrition consisting of glucose [mainly provision of energy], amino acids [precursors of proteins], and lipids [provision of energy and in particular important for brain development]. Routine use of parenteral nutrition in preterm infants was introduced in clinical practice in the early 1970s, with improvements in quality of the solutions resulting in a gradual increase of its use in neonatal practice. The latest guideline for parenteral nutrition in preterm infants of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition [ESPGHAN] was presented in 2005.[48] This guideline advocates the start of amino acids and lipids soon after birth. This more aggressive approach was implemented because of the high rates of postnatal growth retardation and the known association between growth retardation and impaired neurodevelopmental outcome.[49,50] Although randomized controlled trials demonstrated the short–term benefits of the recommendations, implementation of the guideline in clinical care proved to be difficult, resulting in persistent high rates of growth retardation.[51–54] The long–term benefits of these more aggressive practices need however further study.

GROWTH OF THE EARLY PRETERM BODY

Current opinion on optimal growth for preterm infants is that 'quantity and quality of growth should be equivalent to fetal growth, to enable normal organ growth'.[55,56] The most important feature of quantity of growth in neonatal care is weight gain. Length and head circumference, also measures that are frequently used in clinical care, are considered measures of quantity and quality of growth: length is a feature of muscle and bone growth [lean mass], and head circumference is a feature of brain growth and development. Other markers of quality of growth are body composition, and growth and development of organs such as the kidneys, lungs, heart, and the bones. These measures are not evaluated on a regular basis in clinical care.

Despite the general consensus to aim for growth rates comparable to fetal growth rates in the postnatal period, many infants show postnatal growth retardation, defined as growth less than expected based on birth weight.[57] Moreover, the feasibility of this target is under debate. First, because growth during neonatal intensive care unit [NICU] stay is influenced by morbidities affecting metabolic requirements, endocrine abnormalities, central nervous system damage, difficulties in suck– and swallow–coordination, and administration of drugs affecting nutrient metabolism. And second, because there are substantial differences between the intra–uterine and extra–uterine environment with regards to important regulators of growth, such as nutrient supply, environmental cues, and the endocrine system.

NUTRITION Prenatally, carbohydrates are the primary energy source, while postnatally lipids are the main suppliers.[58] Additionally, little is known on actual nutrient intake and circadian rhythm of nutrient supply during gestation, which likely differs from the continuous supply postnatally.

ENVIRONMENT Obviously, the intra–uterine and extra–uterine environment are completely different, especially in a NICU setting. Neonatal morbidities, regulation of vital processes, stress, and pain may all interfere with nutritional needs and the ability to achieve fetal growth targets postnatally.[59,60]

ENDOCRINE SYSTEM Growth is regulated by different hormones during pregnancy. In the embryonic period, insulin–like growth factor II [IGF II] is the primary factor, while later in gestation IGF I, secreted under control of the glucose/insulin axis, becomes increasingly important.[61] Postnatally, IGF I remains the main growth regulator, becoming now growth hormone [GH] dependent. IGF I levels are substantially lower in the

first weeks of life in preterm infants than observed in term born infants.[62,63] Possibly, mimicking of fetal growth rates is not feasible before IGF I levels are normalized, which partly depends on nutritional, and in particular protein, intake.[64]
Thus, many differences exist between important regulators of growth prenatally and postnatally. Possibly, it is not achievable to mimic fetal growth rates in the first weeks of life.

Prenatal and postnatal growth is a major contributor to short– and long–term health of preterm infants. Growth retardation is associated with, amongst others, broncho–pulmonary disease and retinopathy of prematurity on the short term, and with impaired neurocognitive outcome and increased occurrence of cerebral palsy on the long term.[65,66] Growth retardation often persists after the neonatal period, with recovery of initial weight, length, and head circumference Z score not before 6 months after term age, sometimes even tracking into childhood.[67] After the period of growth retardation many infants experience catch–up growth, which is defined as acceleration of growth after a period of starvation.[68] The benefits of catch–up growth, but also of growth acceleration without prior growth retardation, for neurodevelopmental outcome have been well established.[68] Several studies suggested however that growth acceleration and catch–up growth are also associated with risk factors of an adverse cardiometabolic health.[69,70] This has resulted in debate about the definition of 'optimal growth of preterm infants' and about what neonatologists should aim for when feeding a preterm infant: do the neurodevelopmental benefits of rapid growth favor promotion of rapid growth in the early period, despite growing evidence for adverse cardiometabolic consequences in childhood and adulthood?[68]

GROWTH AND DEVELOPMENT OF THE EARLY PRETERM BRAIN

The human brain grows rapidly in the first years of life, with the most impressive change during fetal life: between 20 and 40 weeks of gestation the volume of the brain increases 10–fold, and neural proliferation and migration, organization, and myelination take place.[3] In this period of rapid growth and development, the brain is particularly vulnerable to adverse conditions.[3,71–73] In very preterm born infants, this period corresponds with extra–uterine life on the NICU, a setting in which many adverse environmental conditions are present. Even in preterm infants without brain injury, brain development is altered:

preterm born infants have smaller brain volumes, particularly of the grey matter, and different subcortical structures than term born infants. Moreover, neural connectivity is decreased.[74,75] These alterations in early life partly explain the associations between preterm birth and impaired neurocognitive and psychomotor outcome and mental health problems. Although the occurrence of impairments varies based on gestational age at birth, overall, 10 – 20% of the very preterm infants have severe impairment, with studies reporting up to 50% having any impairment.[38] These impairments include neurosensory [deafness and blindness], but also neurocognitive [ranging from impaired executive functioning to severe mental retardation], motor [tone dysregulation to cerebral palsy], and behavioral problems [e.g., attention deficit disorder, autism, and depression].[38] Severe cases of neurodevelopmental impairment can usually be identified in the neonatal period when major brain injury is present. In those without major brain injury, we are still not able to adequately predict which infants will have [severe] impairments.

CARDIOMETABOLIC HEALTH

Cardiometabolic health concerns both heart disease and metabolic disorders such as diabetes and common cardiometabolic health risk factors including obesity, hypertension, dyslipidemia, and insulin resistance.[76] Already in early life preterm infants have a different cardiometabolic profile than term born infants, possibly reflecting their higher cardiometabolic risk in later life.[77] Body composition, representing the ratio between lean body mass and fat mass, is altered after preterm birth. Compared to those born healthy at term, preterm born infants have higher levels of fat mass relative to weight [adiposity] around term age, with increased visceral fat deposition.[77,78] Around 4 – 6 months after term age, adiposity levels of preterm infants normalize or even decrease to levels below those of term born infants.[79] Only a few studies tracked body composition into childhood, showing inconsistent findings. Some showed that children born preterm have similar body composition at preschool age as full–term born infants, while others observed altered body composition at preschool age in boys, but not in girls.[79–81] In adulthood, the adverse consequences of preterm birth are more evident. Preterm born infants have higher adiposity, higher blood pressure, and higher rates of metabolic syndrome than term born infants, with a dose–response relationship between shorter length of gestation and the occurrence of cardiometabolic risk factors.[82–84]

Relative or percentage fat mass is defined as absolute fat mass divided by total body weight [which is the sum of absolute fat and lean masses]. An increased relative fat mass can thus be the result of either a decreased absolute lean mass or an increased absolute fat mass relative to the overall weight. It has been suggested that, though there is increased visceral fat accumulation, the higher relative fat mass is mainly the result of decreased lean mass, and not of increased fat accumulation in preterm infants.[77,85] The effect of parenteral nutrition on adiposity in infancy is still unclear, as previous studies showed inconsistent results.[86–89] The influence of early postnatal growth on body composition development, despite being recognized as important factor of health outcome, has neither been established yet.[86,89,90]

MONITORING OF GROWTH AND DEVELOPMENT OF PRETERM INFANTS

STANDARD ANTHROPOMETRICS

Evaluation of growth provides an impression of the child's health, making monitoring of growth an important part of neonatal and pediatric care. In the NICU setting, growth is mainly evaluated by using standard anthropometrics [weight, head circumference, and length]. The quantitative increase in size of these measures is evaluated by using reference curves developed for preterm infants. Based on this growth evaluation, nutritional practices are adjusted. Although weight in combination with length and head circumference measurement indeed provides a better impression of the quality of growth than weight alone, it still does not provide a good impression of quality of growth.[91] Markers of quality of growth include body composition and growth and functioning of specific organs, such as the brain. These markers are however not part of standard care in most clinics.

EVALUATION OF BODY COMPOSITION

The human body consists of fat mass and lean mass. The latter can be divided into muscle, bone, water, and [organ] tissue.[92] In adults, body mass index [BMI] is often used as proxy of obesity and body composition. This marker provides no accurate estimation of obesity in infancy and childhood.[93] Other methods to measure body composition in infancy include Dual Energy X–ray Absorptiometry, double–labelled water, bio–impedance, and air–displacement plethysmography.[92] Air–displacement plethysmography [PEA POD®, Infant Body Composition System, COSMED] uses direct measurement of body volume and weight, based on the whole–body densitometry

principle.[94] Several studies showed that body composition measurement using air displacement plethysmography is accurate, precise, and safe in neonates from 30 weeks of gestation onward. Moreover, the measurement is easily performed and patient–friendly.[87,94–96] Still, this method is not yet part of routine follow–up.

EVALUATION OF PRETERM BRAIN DEVELOPMENT

Overall brain size and size of specific brain structures are important predictors of neurodevelopmental outcome.[97,98] On the NICU, brain size is usually monitored by manual measurement of head circumference. This method has a low interrater agreement and does not correspond well with actual brain growth: it not only measures size of the brain but also of the skull and of the subarachnoid spaces, the latter being frequently enlarged in preterm born infants.[99–101] The correlation between head circumference and neurodevelopmental outcome on the long term is also limited.[66] Other markers are therefore needed to provide better understanding of the developing preterm brain and to more adequately predict the risk of an adverse neurodevelopmental outcome during NICU stay. Two scanning techniques are available on most NICUs: magnetic resonance imaging [MRI] and cranial ultrasonography. Although MRI allows for more precise evaluation of structural brain development, brain injury, and connectivity, this technique is expensive, not bedside–available, and does not allow for serial measurement, essential for monitoring of growth and development.[102] Cranial ultrasonography is a bedside–available, clinically applicable method that allows for serial measurement. Furthermore, cranial ultrasonography is routinely performed for evaluation of brain injury in the neonatal period in many centers. Cranial ultrasonography therefore provides a better opportunity to monitor brain growth and development in clinical practice than MRI, but feasible and reliable ultrasound markers to monitor brain growth are lacking.

After the neonatal period, infants regularly visit outpatient clinics to keep track of their growth and neurodevelopment. The neurodevelopmental test most frequently used in follow–up is the Bayley Scales of Infant and Toddler Development, third edition [BSID III], which was released in 2006.[103] This test compromises three categories: cognition, language, and psychomotor functioning. Measures of specific brain functioning or executive functioning are less well integrated in standard follow–up programs.

FOCUS OF RESEARCH

In the past, researchers and clinicians advocated enhanced nutrition and rapid growth to improve brain development and neurodevelopmental outcome in preterm infants. The last years however accumulating evidence has become apparent showing an increased occurrence of features of an adverse cardiometabolic health in preterm born infants. Nutrition and growth have been identified as key factors in this increased risk. But despite a large number of studies focusing on growth and nutrition of preterm infants, we are still not able to define "optimal growth" for optimal long–term cardiometabolic and neurodevelopmental health.

AIMS OF THIS THESIS

In this thesis we aim to link the prenatal and postnatal life, with focus on the association between nutrition and growth in early life and the development of the body and the brain of fetuses and preterm infants.

The main objectives are:

1. To investigate the use of three–dimensional ultrasound measures in pregnancy as early markers of birth outcome and neonatal body composition.

2. To develop ultrasound measures that can be used to monitor brain growth in the prenatal and postnatal period.

3. To describe current nutritional practices in preterm infants and to study the influence of enhanced early nutrition on neurodevelopmental outcome of preterm infants.

4. To assess the association between in–hospital growth and body composition in infancy in preterm born infants.

SETTING

This thesis is based on studies conducted at the Department of Obstetrics and Gynecology and the Department of Pediatrics, division of Neonatology, of the Erasmus MC – Sophia Children's Hospital, Rotterdam, the Netherlands.

ROTTERDAM PERICONCEPTIONAL COHORT

The Rotterdam Periconceptional Cohort [Predict study] is a prospective cohort study conducted in a tertiary hospital that focuses on three research areas: [1] determinants of periconceptional health; [2] pregnancy course and outcome; and [3] underlying epigenetic mechanisms.[104] Women are included in the first trimester of pregnancy and followed until 1 year after delivery, by using transvaginal three–dimensional [3D] ultrasounds at 7, 9, and 11 weeks of gestation, questionnaires throughout gestation, and [umbilical cord] blood withdrawals.

DREAM STUDY

Embedded in the Predict study is the DREAM study. Between November 2013 and July 2015, women with an ongoing singleton pregnancy, enrolled in the Predict study, were invited to participate in the DREAM study. Participants underwent additional 3D ultrasounds at 22, 26, and 32 weeks of gestation. After birth, a neonatal cranial ultrasound was performed. Between September 2014 and September 2016, neonatal body composition was additionally measured during the postnatal visit.

BOND STUDY

The BOND study [BOdy composition and NeuroDevelopment in preterm infants] is an ongoing, observational cohort study conducted at the neonatal intensive care unit and the outpatient clinic. The main aim of this study is to study the complex interaction between nutrition, growth, body composition, and neurodevelopment in preterm infants. Between September 2014 and April 2017, preterm infants born before 30 weeks of gestation without congenital chromosomal anomalies or severe brain injury were included. From birth up to 2 years after term age, body composition and brain development were monitored at standardized moments. In **Table 1** an overview is given of the different study assessments. In this thesis, we focus on the short–term results of this study.

TABLE 1 | OVERVIEW OF STUDY ASSESSMENTS BOND STUDY

	NICU stay	1st visit	2nd visit
Nutrition data	•	•	•
Anthropometrics	•	•	•
Cranial ultrasound	•	•	
Body composition		•	•
Questionnaire [lifestyle]		•	

OUTLINE OF THIS THESIS

PART I comprises research on the development of the early human body, with focus on nutrition, growth, and body composition. **Chapter 2** addresses the association between embryonic size and growth and birth outcome. In **Chapter 3** we present a systematic overview of current literature on prenatal markers of neonatal body composition. In **Chapter 4** the use of fetal fractional thigh volume, a three–dimensional ultrasound measure, is evaluated as prenatal marker of neonatal body composition. Current nutritional practices for preterm infants on a large neonatal intensive care unit are presented in **Chapter 5**. In **Chapter 6** the association between in–hospital growth and body composition in infancy is investigated in preterm infants.

In **PART II** we study the human brain in pregnancy, early postnatal life, and childhood. In **Chapter 7 and 8**, a new ultrasound marker for measurement of brain growth is presented. **Chapter 9** describes the results of a randomized controlled trial on parenteral nutrition in preterm infants, focusing on growth and neurodevelopmental outcome. **Chapter 10** covers the general discussion of the main findings and provides suggestions for further research. In **Chapter 11** a summary of this thesis is provided in Dutch and in English.

PART I THE EARLY HUMAN BODY

2

EARLY FIRST TRIMESTER EMBRYONIC SIZE AND GROWTH PARAMETERS AND THE ASSOCIATION WITH ADVERSE BIRTH OUTCOMES: THE ROTTERDAM PERICONCEPTIONAL COHORT

Submitted

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ABSTRACT

OBJECTIVES To study associations between three–dimensional [3D] ultrasound parameters of embryonic size and growth in the early first trimester and the risk of adverse birth outcomes.

DESIGN Prospective periconceptional cohort study.

SETTING Tertiary hospital in the Netherlands.

PARTICIPANTS Pregnant women with a singleton pregnancy were included when they had one or more 3D ultrasounds examinations between 6 and 13 weeks of gestation. We excluded pregnancies conceived after oocyte donation, ending in a miscarriage before 16 weeks of gestation, and those with missing information on gestational age at birth, birth weight, congenital anomalies, and fetal and early neonatal mortality.

MAIN OUTCOME MEASURES Embryonic size, expressed in crown–rump length [CRL, mm] and embryonic volume [EV, cm^3], was measured by using 3D ultrasonography with virtual reality. Embryonic growth trajectories were longitudinally modeled between 6 and 13 weeks of gestation for CRL and EV. Birth outcomes were preterm birth [delivery <37 weeks of gestation] and gestational age, small for gestational age [SGA; <10th percentile] and birth weight, major congenital anomalies, and mortality [fetal death from 16 of gestation onward or mortality in the first week after birth]. The primary analyses included associations between embryonic size parameters measured at 7, 9, and 11 weeks of gestation or embryonic growth and the composite outcome of any of the adverse birth outcomes [yes/no]. The secondary analyses were on the association between embryonic size or growth and the outcomes of preterm birth, SGA, congenital anomaly [all logistic regression], gestational age and birth weight [both linear regression] separately.

RESULTS The primary analyses showed that only a larger EV at 11 weeks of gestation was negatively associated with adverse birth outcome: an increase of 1 cm^3 EV was associated with a 12% smaller odds of an adverse birth outcome [odds ratio [OR] 0.88 [95% confidence interval [95%CI] 0.79 to 0.98], p=0.017]. The secondary analyses showed that larger EV and CRL at 11 weeks of gestation were negatively associated with SGA [EV: OR 0.84 [95%CI 0.71 ; 0.99], p=0.036; CRL: OR 0.92 [95%CI 0.86 – 0.99], p=0.027], but no significant associations were observed between embryonic size parameters and the other outcomes. From 9 weeks of gestation onward, both EV and CRL size and growth were positively associated with birth weight. EV growth, but not CRL growth, was negatively associated with SGA.

CONCLUSIONS Already early in the first trimester, larger embryonic size and growth are associated with a smaller odds of an adverse birth outcome. Our data suggest that EV is a more sensitive marker for detection of growth deviations than CRL in early pregnancy. Early 3D ultrasound screening can extend the window of opportunity for prevention and interventions to improve embryonic, fetal, and neonatal health with consequences for health throughout the life course.

INTRODUCTION

During the first trimester of pregnancy, the embryo is very sensitive to maternal health and lifestyle conditions, such as smoking and obesity, because of the high growth rates and organ development.[41,105] In addition, these conditions can influence DNA methylation, thereby epigenetically changing gene function with subsequent consequences for not only organ growth, development, and function, but also for fetal and neonatal health [Developmental Origins of Health and Disease [DOHaD] paradigm].[41,106] Previous studies showed that impaired embryonic growth at the very end of the first trimester is associated with an increased risk of adverse birth outcomes such as preterm birth and low birth weight.[107,108] With the recent development of three–dimensional ultrasonography [3D US], it is now possible to monitor embryonic growth reliably and precisely already in earlier in the first trimester.[29,109] Using 3D US combined with virtual reality [VR] for volume measurements, we may be able to identify pregnancies at risk of an adverse birth outcome already early in the first trimester. This would extend the window for potential interventions and follow–up to improve embryonic and fetal health and birth outcomes, which may improve health on the long term.

We therefore aimed to investigate the association between early first trimester embryonic size parameters and growth rates and adverse birth outcomes, as well as gestational age at birth and birth weight, in a large periconceptional cohort study.

Common adverse birth outcomes are preterm birth, small for gestational age [SGA], congenital anomalies, and mortality.[35] We hypothesized that at 7, 9, and 11 weeks of gestation a larger embryo is associated with a smaller odds of preterm birth, SGA, major congenital anomalies, and fetal and early neonatal mortality. Moreover, we hypothesized that an increased embryonic growth rate between 6 and 13 weeks of gestation is associated with smaller odds of an adverse birth outcome.

METHODS

In this periconceptional prospective cohort study we assessed the association between embryonic size parameters measured using 3D US and VR at 7, 9, and 11 weeks of gestation and adverse birth outcomes, and between embryonic growth between 6 and 13 weeks of gestation and adverse birth outcomes. Additionally we assessed the association between embryonic size and growth and gestational age at birth and birth weight.

STUDY DESIGN

The Rotterdam Periconceptional Cohort [Predict study] is an ongoing hospital–based prospective periconceptional cohort study conducted in the Erasmus University Medical Centre, Rotterdam, the Netherlands.[104] For this study, we selected pregnant women [aged ≥ 18 years] enrolled between 2009 and 2015 with a singleton pregnancy, who underwent one or more 3D US examinations before the 14th week of gestation. The following exclusion criteria were applied: oocyte donation, miscarriage before 16 weeks of gestation, and missing information on pregnancy outcome [gestational age at birth, birth weight, congenital anomalies and fetal or early neonatal mortality]. Of the women who repeatedly participated in the Predict study, only the first pregnancy was used in the analyses.

ETHICAL CONSIDERATIONS

Written informed consent was obtained before enrollment from all participating women and their partners. The Central Committee on Research in The Hague and the local Medical Ethical Committee approved the study [MEC–2004–227].

EMBRYONIC ULTRASOUND EXAMINATIONS

Transvaginal 3D US scanning was performed between 6 to 13 weeks of gestation by using a 6 – 12 MHz transvaginal probe and GE Voluson E8 equipment combined with 4D View software [General Electrics Medical Systems, Zipf, Austria]. In the pilot phase [2009 – 2010], weekly 3D US were performed to assess the feasibility of first trimester growth trajectory monitoring. Based on these data, the schedule was changed to scanning only at 7, 9, and 11 weeks of gestation from 2013 onward.[104]
The obtained 3D datasets were transformed to rectangular volumes and transferred to the BARCO I–Space [Barco N.V., Kortrijk, Belgium] at the Department of Bioinformatics, Erasmus University Medical Center, Rotterdam, the Netherlands. The BARCO I–Space is a four–walled CAVE™–like virtual reality system which allows for depth perception and interaction with the projected scans [3D virtual reality].[110]

For evaluation of embryonic size and growth, crown–rump length [CRL] and embryonic volume [EV] were measured offline by trained researchers. Reliability, technique, and methods of these measurements are described in detail by Rousian et al. and Verwoerd–Dikkeboom et al, showing excellent inter– and intraobserver agreement and intraclass correlation coefficients of 0.98 for CRL and 0.99 for EV.[29,109,111] CRL measurements were performed in the I–Space three times by the same researcher and the average of these measurements was used in the analyses. The calipers were placed on the outer border of the crown and the caudal rump, thereby drawing a straight line. A semi–automated application, using grey–scale differences, was used to measure EV once.

Details on the study protocol and questionnaires have been reported previously.[104] In short, additional data were obtained via questionnaires completed at enrollment [first trimester], at 24 weeks of gestation, and shortly after delivery. These questionnaires contained questions on maternal health and lifestyle, pregnancy course, and neonatal outcome. All obstetrical and neonatal outcomes were crosschecked in the electronic medical file and delivery reports, which were considered the gold standard.

BASELINE DATA

We obtained the following maternal and neonatal characteristics: maternal age, geographical background [Western, non–Western], parity [nulliparous, multiparous], mode of conception [spontaneous, in vitro fertilization [IVF] with or without intra–

cytoplasmic sperm injection [ICSI]], periconceptional maternal smoking [yes/no], periconceptional vitamin and folic acid use [yes/no], date of delivery, neonatal sex, birth weight, occurrence of mortality between 16 – 42 weeks of gestation [fetal] or within 1 week after birth [early neonatal mortality], and the presence of major congenital anomalies according to the EUROCAT criteria.[112] Periconceptional BMI of the mother was calculated based on body weight before the last menstrual period and height obtained in the first questionnaire. If not available, weight and height measured at the first visit were used to calculate BMI. Birth weight percentiles adjusted for gestational age, parity, and sex were calculated using Dutch reference standards.[113] SGA was defined as birth weight below the 10th percentile.

Missing data were mainly due to incomplete questionnaires, which were completed based on patients' electronic medical file, if available.

PREGNANCY DATING

Data on mode of conception, first day of the last menstrual period [LMP], and regularity and duration of the menstrual cycle were obtained at the first visit by the research team and in the first questionnaire. Afterwards, these data were crosschecked with information in the electronic medical file.

In spontaneous pregnancies [including intra–uterine insemination and hormonal stimulation], dating was based on first day of the LMP if there was a regular [25 – 35 days] and known duration of the last menstrual cycle. If the menstrual cycle was prolonged [32 – 35 days], we adjusted for duration of the cycle. In spontaneous pregnancies with either an irregular menstrual cycle [<25 or >35 days] or an unknown date of the first day of the last menstrual period, dating was based on two–dimensional [2D] CRL measurement performed at [or close to] 9 weeks of gestation.

As previous studies showed a recall bias with regard to the LMP, we checked whether the calculated due date based on the menstrual cycle corresponded with dating on CRL.[114] If there was a difference of ≥ 7 days between the due date calculated by using LMP and CRL, we used the due date based on CRL measurement for the analyses.

In pregnancies conceived after IVF, with or without ICSI procedures, the due date

was calculated from the date of oocyte retrieval plus 14 days. The due date of pregnancies conceived after the transfer of cryopreserved embryos was calculated from the day of embryo transfer plus 18 days.

OUTCOME MEASURES

Our main outcome measure was an adverse birth outcome, defined as the combined outcome of preterm birth [birth before 37 completed weeks of gestation], SGA, major congenital anomalies, and fetal or early neonatal mortality.

The secondary outcome measures were preterm birth, gestational age at birth, SGA, birth weight, and major congenital anomalies. Because of the low mortality rates, mortality was not studied separately.

STATISTICAL ANALYSES

Data are presented as mean [standard deviation, SD], median [interquartile range, IQR] or n [%].

First, we assessed the association between mean embryonic size [CRL and EV] at 7, 9, and 11 weeks of gestation, and adverse birth outcomes, by using logistic [odds ratio [OR] and 95% confidence interval [CI]] regression analyses for dichotomous outcomes and linear regression [effect estimates and 95%CI] for continuous outcome measures.

All associations were explored in three models: a crude model, a basic model [model 1] and a fully adjusted model [model 2]. The crude model only adjusted for gestational age at 3D US. Model 1 additionally included maternal educational level and fetal sex, which were selected based on differences in baseline characteristics between those with [cases] and without [controls] an adverse birth outcome [**Table 1**]. Model 2 additionally included parity, maternal age, maternal periconceptional BMI, maternal geographical background, and mode of conception. Folic acid use and periconceptional smoking, important factors influencing embryonic growth and birth outcome, were not included in this model because of the respectively very high and low frequencies. All cases with an adverse birth outcome [n=156] were compared to all non–cases [controls, n=601]. For each of the secondary outcomes [preterm birth, SGA, and congenital anomalies], cases were compared with the controls of the primary analysis [having none of the adverse outcomes, n=601].

Second, individual CRL and EV growth trajectories were created to assess the association between embryonic growth and adverse birth outcomes. For optimal use of the available growth data, and taking into account the correlation between serial measurements per pregnancy, we used a 2–step approach. First, we modelled the individual growth trajectories in a linear mixed model. Approximately normal distributions and linear associations were obtained by square root transformation of CRL and cube root transformation of EV. The subject–specific random effects [intercept and slope], that summarize the individual growth trajectories, were then used as covariates in the second step of the analyses. In this step, again regression analyses including a crude, basic and fully adjusted model were applied, as described above.

SENSITIVITY ANALYSES One fourth of the pregnancies were dated based on CRL measured at 9 weeks of gestation, assuming uniform embryonic growth. This may have diluted the associations between embryonic size and growth and adverse birth outcome. We therefore performed sensitivity analyses in pregnancies with a strict regular cycle that were conceived spontaneously. We assume that there is limited variation in growth based on incorrect dating in those pregnancies, and no bias of the outcome because we do not use our exposure [CRL] to calculate the due date. In these analyses, the same models were applied as in the primary and secondary analyses, only mode of conception was deleted from the fully adjusted model [model 2].

The dataset was nearly complete, with only a few covariates missing for a small group of patients [n=18, 2.4%]. Therefore we chose not to impute missing data, but to report sample sizes in each analysis based on complete cases.

A two–tailed p value of <0.05 was considered statistically significant. Data were analyzed using SPSS [SPSS package 21.0, IBM, USA] and R [R: A language and Environment for Statistical Computing, version 3.4.1, R Core Team, Vienna, Austria].

RESULTS

The Predict study included 897 pregnant women with 3D US examination in the first trimester. We excluded pregnancies conceived after oocyte donation [n=14], miscarriages before the 16th week of gestation [n=9], and missing outcome data [n=70]. Exclusion of repeated participation [n=47], resulted in a study sample of 757 [84%] for analyses [**Figure 1**]. The baseline characteristics are shown in **Table 1**. Of the 757 pregnancies, 156 [21%] resulted in an adverse outcome.

Per patient, 1 – 8 3D US examinations were performed with successful measurements [median 3, interquartile range [IQR], 2 – 6]. In **Table 2**, the results of CRL and EV measurements at 7, 9, and 11 weeks of gestation are shown. CRL increased from median 12.9 [IQR 10.8 – 14.8] mm at 7 weeks to 50.2 [IQR 46.0 – 54.3] mm at 11 weeks of gestation. EV increased from median 0.28 [IQR 0.16 – 0.41] cm^3 at 7 weeks to 10.30 [IQR 8.31 – 12.25] cm^3 at 11 weeks of gestation.

PRIMARY ANALYSES

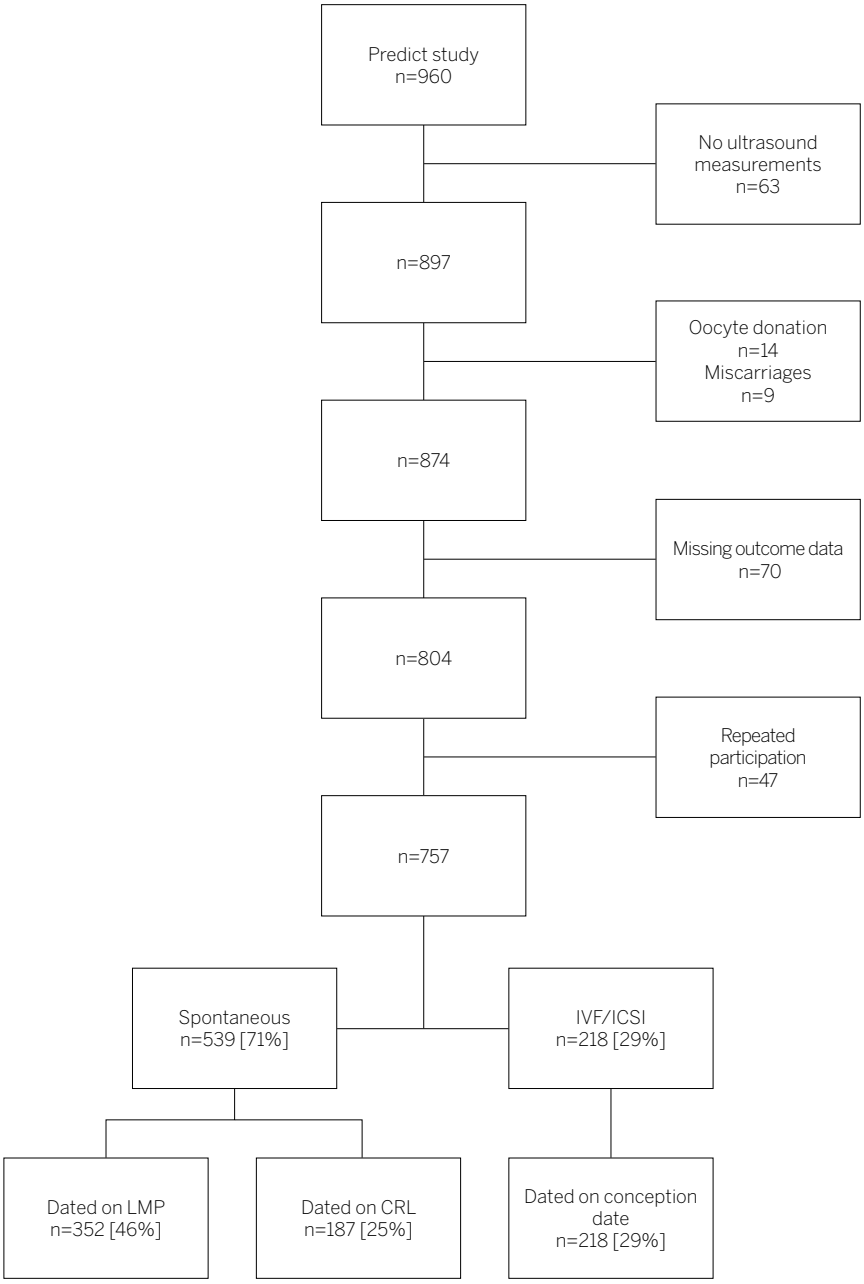
The association between CRL and adverse birth outcome was not statistically significant at any of the time points in any of the 3 models. Similar to CRL, the ORs for EV showed a lower odds at all time points, but were only statistically significant at 11 weeks of gestation [OR 0.88; 95% confidence interval [CI] 0.79 – 0.98]: a larger EV at 11 weeks of gestation is thus associated with smaller odds of an adverse birth outcome [**Table 3A**]. Studying the first trimester data longitudinally, we did not observe a statistically significant association between CRL or EV growth trajectories between 6 and 13 weeks of gestation and an adverse birth outcome [data not shown].

SECONDARY ANALYSES

We did not observe an association between CRL or EV and preterm birth or gestational age at birth at any of the time points [**Table 3B and 4A**].

The association between both embryonic size parameters and SGA was significant at 11 weeks of gestation, again showing a negative association [OR 0.92, 95%CI 0.86;0.99]. At 7 and 9 weeks, all ORs showed a negative association as well, but no statistically significant associations were found [**Table 3C**]. A positive association was found from 9 weeks of gestation onward between CRL and EV and birth weight:

FIGURE 1 | FLOWCHART



Abbreviations: n, number; LMP, last menstrual period; CRL, crown-rump length

TABLE 1 | BASELINE CHARACTERISTICS

		Total study population [n=757]	Cases [n=156]	Controls [n=601]	Missing data [n]
MATERNAL CHARACTERISTICS					
Age [years]		32.2 [29.1;35.6]	32.1 [28.8;35.5]	32.2 [29.1;35.6]	0
Geographical background	Western	635 [84%]	128 [82%]	507 [85%]	7
	Non-Western	115 [15%]	25 [16%]	90 [14%]	
Educational level	High	402 [53%]	69 [44%]	333 [55%]	21
	Middle	265 [35%]	62 [40%]	203 [34%]	
	Low	69 [9%]	18 [12%]	51 [9%]	
Periconceptional BMI [kg/m^2]		23.8 [21.3;26.9]	23.8 [21.0;27.8]	23.8 [21.4;26.8]	0
Folic acid use		740 [98%]	150 [96%]	590 [98%]	2
	Preconceptional initation	592 [78%]	121 [78%]	471 [78%]	9
Periconceptional smoking		125 [17%]	24 [15%]	101 [17%]	0
Nulliparous		399 [53%]	80 [51%]	319 [53%]	0
Conception mode	Spontaneous	539 [71%]	115 [74%]	424 [71%]	0
	IVF/ICSI	218 [29%]	41 [26%]	177 [29%]	

TABLE 1 | BASELINE CHARACTERISTICS [CONTINUED]

NEONATAL CHARACTERISTICS					
Gestational age at birth [weeks^{+days}]		39^{+2} [38^{+1};40^{+2}]	37^{+3} [36^{+0};39^{+4}]	39^{+2} [38^{+3};40^{+2}]	1
	Preterm birth	63 [9%]	63 [40%]	0	0
Birth weight [grams]		3340 [3004;3700]	2695 [2318;3020]	3425 [3158;3750]	0
Birth weight percentile		48.0 [27.4;74.6]	18.7 [5.5;60.7]	51.2 [32.3;76.3]	1
	SGA	64 [9%]	64 [41%]	0	1
Sex	Male	382 [50%]	75 [48%]	307 [51%]	0
Major congenital anomaly		28 [4%]	28 [18%]	0	8
Mortality	Fetal	14 [2%]	14 [9%]	0	0
	Early neonatal	3 [0.3%]	3 [2%]	0	0
Adverse birth outcome		156 [21%]	156 [100%]	0	0

Legend: Data are presented in median [interquartile range] or number [percentages].
Abbreviations: [k]g, [kilo]grams; BMI, body mass index; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; SGA, small for gestational age.

larger CRL and EV were associated with a higher birth weight [**Table 4B**].

Although nearly all ORs showed a negative association, no statistically significant associations were found between embryonic size parameters and congenital anomalies [**Supplemental table 1**].

We did not observe an association between CRL or EV growth trajectories and preterm birth or congenital anomalies. CRL and EV growth trajectories were significantly associated with SGA: a higher CRL and EV growth rate between 6 and 13 weeks of gestation were associated with an smaller odds of SGA [data not shown].

TABLE 2 | ULTRASOUND MEASUREMENTS

			GA [IQR]	N	Median	IQR	Min – Max
US at 7 weeks							
	CRL	[mm]	7^{+3} [7^{+2};7^{+5}]	415	12.9	10.8 – 14.8	5.4 – 20.3
	EV	[cm^3]	7^{+3} [7^{+2};7^{+5}]	346	0.28	0.16 – 0.41	0.01 – 0.95
US at 9 weeks							
	CRL	[mm]	9^{+3} [9^{+1};9^{+5}]	590	26.7	24.3 – 29.5	17.4 – 41.4
	EV	[cm^3]	9^{+3} [9^{+2};9^{+5}]	467	2.18	1.73 – 2.79	0.56 – 5.78
US at 11 weeks							
	CRL	[mm]	11^{+3} [11^{+1};11^{+5}]	620	50.2	46.0 – 54.3	34.3 – 67.8
	EV	[cm^3]	11^{+3} [11^{+1};11^{+5}]	451	10.30	8.31 – 12.25	3.69 – 21.11

Abbreviations: GA, gestational age; IQR, interquartile range; min, minimum; max; maximum; US, ultrasound; CRL, crown–rump length; EV, embryonic volume; mm, millimeter; cm^3, cubic centimeter.

SENSITIVITY ANALYSES

To explore whether potential bias introduced by dating based on CRL affected our findings, we performed sensitivity analyses in 352 spontaneously conceived pregnancies with a regular menstrual cycle. The baseline characteristics and embryonic size measurements of this subgroup are shown in **Supplemental table 2 and 3**. These analyses showed effect estimates comparable to those observed in the primary analyses, but only CRL at 9 weeks showed a statistically significant association with SGA in the fully adjusted model [**Supplemental table 4**].

No significant associations were observed between embryonic size or growth parameters at any of the time points and gestational age and birth weight [**Supplemental table 5**].

TABLE 3A | ASSOCIATIONS BETWEEN EMBRYONIC GROWTH PARAMETERS AND AN ADVERSE BIRTH OUTCOME

		Cases[a]				p	Cases[a]				p	Cases[a]				p
		N	[n]	OR	95% CI	value	N	[n]	OR	95% CI	value	N	[n]	OR	95%CI	value
		Crude					Model 1					Model 2				
7 weeks																
CRL	[mm]	415	82	0.91	0.81 – 1.03	0.123	411	79	0.91	0.81 – 1.03	0.147	409	78	0.91	0.81 – 1.03	0.138
EV	[cm^3]	346	69	0.25	0.03 – 1.87	0.178	342	66	0.28	0.04 – 2.12	0.218	341	65	0.20	0.02 – 1.69	0.140
9 weeks																
CRL	[mm]	557	102	0.92	0.85 – 1.00	0.053	545	99	0.94	0.87 – 1.02	0.155	543	98	0.94	0.86 – 1.02	0.124
EV	[cm^3]	467	80	0.73	0.48 – 1.09	0.118	459	78	0.79	0.53 – 1.18	0.253	457	77	0.78	0.52 – 1.17	0.235
11 weeks																
CRL	[mm]	620	115	0.96	0.92 – 1.00	0.076	605	110	0.97	0.93 – 1.02	0.211	602	109	0.97	0.92 – 1.01	0.155
EV	[cm^3]	451	86	0.88	0.79 – 0.97	**0.011**	440	81	0.89	0.80 – 0.99	**0.027**	437	80	0.88	0.79 – 0.98	**0.017**

Legend: Statistically significant findings [$p<0.05$] are in **bold**.
[a] Cases = preterm birth, small for gestational age, mortality, and congenital anomalies.
Crude model: adjusted for GA at US measurement;
Model 1: adjustment for GA at US measurement + educational level [high/not-high] and fetal sex;
Model 2: adjustment for Model 1 + parity [nulliparous/multiparous], maternal age, maternal periconceptional BMI, maternal geographical background [Western/non-Western], and mode of conception [spontaneous/IVF ICSI].
Abbreviations: n, number; OR, odds ratio; CI, confidence interval; CRL, crown-rump length; EV, embryonic volume; mm, millimeter; cm^3, cubic centimeter; GA, gestational age; US, ultrasound.

TABLE 3B | ASSOCIATIONS BETWEEN EMBRYONIC GROWTH PARAMETERS AND PRETERM BIRTH

		Cases[a]				p	Cases[a]				p	Cases[a]				p
		N	[n]	OR	95% CI	value	N	[n]	OR	95% CI	value	N	[n]	OR	95%CI	value
		Crude					Model 1					Model 2				
7 weeks																
CRL	[mm]	370	37	1.01	0.85 – 1.19	0.932	368	36	1.04	0.88 – 1.23	0.660	367	36	1.05	0.89 – 1.24	0.572
EV	[cm^3]	309	32	0.98	0.06 – 15.08	0.988	307	31	1.17	0.08 – 17.6	0.908	307	31	1.75	0.13 – 24.25	0.676
9 weeks																
CRL	[mm]	496	41	1.00	0.89 – 1.13	0.950	487	41	1.00	0.89 – 1.13	0.970	486	41	1.01	0.90 – 1.13	0.901
EV	[cm^3]	421	34	0.83	0.45 – 1.46	0.506	415	34	0.83	0.47 – 1.45	0.505	414	34	0.89	0.52 – 1.52	0.665
11 weeks																
CRL	[mm]	555	50	1.02	0.96 – 1.09	0.473	544	49	1.02	0.96 – 1.09	0.502	542	49	1.02	0.96 – 1.09	0.479
EV	[cm^3]	401	36	0.96	0.83 – 1.11	0.606	394	35	0.95	0.82 – 1.10	0.495	392	35	0.96	0.83 – 1.11	0.543

Legend: Statistically significant findings [$p<0.05$] are in **bold**.
[a] Cases = preterm birth.
Crude model: adjusted for GA at US measurement;
Model 1: adjustment for GA at US measurement + educational level [high/not-high] and fetal sex;
Model 2: adjustment for Model 1 + parity [nulliparous/multiparous], maternal age, maternal periconceptional BMI, maternal geographical background [Western/non-Western], and mode of conception [spontaneous/IVF ICSI].
Abbreviations: n, number; OR, odds ratio; CI, confidence interval; CRL, crown-rump length; EV, embryonic volume; mm, millimeter; cm^3, cubic centimeter; GA, gestational age; US, ultrasound.

TABLE 3C | ASSOCIATIONS BETWEEN EMBRYONIC GROWTH PARAMETERS AND SMALL FOR GESTATIONAL AGE

		Cases[a]				p	Cases[a]				p	Cases[a]				p
		N	[n]	OR	95% CI	value	N	[n]	OR	95% CI	value	N	[n]	OR	95%CI	value
		Crude					Model 1					Model 2				
7 weeks																
CRL	[mm]	366	33	0.85	0.71 – 1.01	0.060	365	33	0.85	0.72 – 1.01	0.068	363	32	0.85	0.71 – 1.01	0.070
EV	[cm^3]	304	27	0.08	0.00 – 1.85	0.115	303	27	0.08	0.00 – 1.89	0.118	302	26	0.02	0.00 – 0.77	**0.035**
9 weeks																
CRL	[mm]	499	44	0.89	0.79 – 1.00	0.051	489	43	0.91	0.80 – 1.02	0.099	487	42	0.89	0.79 – 1.01	0.069
EV	[cm^3]	418	31	0.58	0.30 – 1.10	0.093	412	31	0.58	0.31 – 1.11	0.099	410	30	0.54	0.28 – 1.05	0.070
11 weeks																
CRL	[mm]	553	48	0.92	0.86 – 0.99	**0.018**	541	46	0.93	0.87 – 1.00	**0.043**	538	45	0.92	0.86 – 0.99	**0.027**
EV	[cm^3]	400	35	0.85	0.73 – 0.99	**0.033**	392	33	0.86	0.74 – 1.01	0.064	389	32	0.84	0.71 – 0.99	**0.036**

Legend: Statistically significant findings [p<0.05] are in **bold**.
[a] Cases = small for gestational age.
Crude model: adjusted for GA at US measurement;
Model 1: adjustment for GA at US measurement + educational level [high/not-high] and fetal sex;
Model 2: adjustment for Model 1 + parity [nulliparous/multiparous], maternal age, maternal periconceptional BMI, maternal geographical background [Western/non-Western], and mode of conception [spontaneous/IVF ICSI].
Abbreviations: n, number; OR, odds ratio; CI, confidence interval; CRL, crown-rump length; EV, embryonic volume; mm, millimeter; cm^3, cubic centimeter; GA, gestational age; US, ultrasound.

TABLE 4A | ASSOCIATIONS BETWEEN EMBRYONIC GROWTH PARAMETERS AND GESTATIONAL AGE AT BIRTH

					p				p				p
		N	Effect size	95% CI	value	N	Effect size	95% CI	value	N	Effect size	95%CI	value
		Crude				Model 1				Model 2			
7 weeks													
CRL	[mm]	410	-0.05	-0.15 – 0.05	0.284	407	-0.06	-0.16 – 0.04	0.204	405	-0.07	-0.17 – 0.03	0.182
EV	[cm^3]	343	-0.59	-2.09 – 0.91	0.440	340	-0.68	-2.22 – 0.81	0.370	339	-0.97	-2.51 – 0.56	0.213
9 weeks													
CRL	[mm]	551	-0.03	-0.09 – 0.03	0.347	540	-0.03	-0.09 – 0.03	0.335	538	-0.03	-0.09 – 0.03	0.300
EV	[cm^3]	462	-0.03	-0.30 – 0.23	0.804	455	-0.05	-0.32 – 0.22	0.708	453	-0.08	-0.35 – 0.19	0.570
11 weeks													
CRL	[mm]	613	-0.02	-0.05 – 0.02	0.398	599	-0.02	-0.05 – 0.02	0.397	596	-0.02	-0.06 – 0.02	0.311
EV	[cm^3]	444	-0.04	-0.12 – 0.03	0.244	434	-0.04	-0.11 – 0.04	0.334	431	-0.04	-0.11 – 0.04	0.323

Legend: Statistically significant findings [$p<0.05$] are in **bold.**
Crude model: adjusted for GA at US measurement;
Model 1: adjustment for GA at US measurement + educational level [high/not-high] and fetal sex;
Model 2: adjustment for Model 1 + maternal periconceptional BMI, maternal age, maternal geographical background [Western/non-Western], mode of conception [spontaneous/IVF ICSI] and parity [nulliparous/multiparous].
Abbreviations: n, number; CI, confidence interval; CRL, crown-rump length; EV, embryonic volume; mm, millimeter; cm^3, cubic centimeter; GA, gestational age; US, ultrasound.

TABLE 4B | ASSOCIATIONS BETWEEN EMBRYONIC GROWTH PARAMETERS AND BIRTH WEIGHT

		N	Effect size	95% CI	p value	N	Effect size	95% CI	p value	N	Effect size	95%CI	p value
		Crude				Model 1				Model 2			
7 weeks													
CRL	[mm]	410	20	-8 – 48	0.157	407	19	-8 – 47	0.170	405	20	-9 – 48	0.172
EV	[cm^3]	343	373	-78 – 818	0.101	340	344	-99 – 786	0.127	339	369	-88 – 827	0.113
9 weeks													
CRL	[mm]	551	19	2 – 36	**0.030**	540	18	0 – 35	**0.046**	538	20	3 – 37	**0.023**
EV	[cm^3]	462	106	28 – 184	**0.008**	455	97	20 – 175	**0.014**	453	104	26 – 182	**0.009**
11 weeks													
CRL	[mm]	614	12	2 – 22	**0.021**	600	11	1 – 22	**0.032**	597	12	2 – 23	**0.020**
EV	[cm^3]	444	21	0 – 42	**0.046**	435	22	1 – 43	**0.041**	432	23	2 – 44	**0.030**

Legend: Statistically significant findings [$p<0.05$] are in **bold.**
Crude model: adjusted for GA at US measurement;
Model 1: adjustment for GA at US measurement + educational level [high/not-high] and fetal sex;
Model 2: adjustment for Model 1 + maternal periconceptional BMI, maternal age, maternal geographical background [Western/non-Western], mode of conception [spontaneous/IVF ICSI] and parity [nulliparous/multiparous].
Abbreviations: n, number; CI, confidence interval; CRL, crown-rump length; EV, embryonic volume; mm, millimeter; cm^3, cubic centimeter.

DISCUSSION

STATEMENT OF PRINCIPAL FINDINGS

In this periconceptional cohort of 757 pregnancies we observed that a larger embryonic size at 11 weeks of gestation was associated with smaller odds of an adverse birth outcome, in particular of SGA. Increased embryonic growth trajectories between 6 and 13 weeks of gestation were also associated with smaller odds of SGA. Moreover, already at 9 weeks of association, embryonic size was positively associated with birth weight. We did not observe statistically significant associations between embryonic size or growth and the occurrence of preterm birth and congenital anomalies.

COMPARISON WITH OTHER STUDIES

To the best of our knowledge, we are the first to assess the associations between embryonic growth parameters and adverse birth outcomes this early in the first trimester of pregnancy. Our findings show that association between first trimester size and birth outcome can already be detected before 13 weeks of gestation. A large study [n=1,631] of Mook–Kanamori et al., conducted in the general population, also observed that a smaller CRL measured on two–dimensional [2D] US before 14 weeks of gestation is associated with an increased risk of SGA.[115] We could however not reproduce the significant association between CRL and preterm birth. After adjustment for maternal health status and socioeconomic factors, it may, however, seem unlikely that common causes of preterm birth, such as maternal infection, are associated with first trimester growth. Several aspects may explain why we did not observe this association: the association may become visible after our observation period, is only detectable in very large cohorts, or is not observed in our study due to differences in methodology and statistical methods. Because of the large sample size of 1,631 participants, they were able to adjust for more health and social covariates then we were. We were not able to adjust for covariates such as diastolic blood pressure and hematocrit levels.[115] We did not adjust for folic acid use and smoking because of the respectively high and low frequency in our study population, and the equal distribution between the cases and controls.

Another study on embryonic size and birth weight also observed a positive association between CRL measured on 2D US scans before 14 weeks of gestation and birth weight,

and a positive association between CRL and the odds of delivering an SGA infant.[116] This study by Bukowksi et al. was conducted in an IVF/ICSI population. Although the known timing of conception in those patients facilitates proper study of this association, extrapolation to spontaneously conceived pregnancies may be limited. Though one third of our population consists of pregnancies conceived via IVF/ICSI, the absolute number was small, limiting the possibility to study all birth outcomes in this subgroup. We performed a post–hoc exploratory analysis on our primary outcome in this subgroup. This showed a stronger association than in the total study population, with already at 7 weeks of gestation a negative association between CRL or EV and adverse birth outcome [**Supplemental table 6**]. Possibly, the stronger associations than observed in the sensitivity analyses are caused by the exactly known timing of conception, which seems more reliably than in spontaneously conceived pregnancies with regular cycle.

We did not observe an association between embryonic size and congenital anomalies, although the direction of the effect estimates [OR<1.0] were similar on all time points. A previous study, with patients derived from the same source population showed that EV, but not CRL, was smaller in the first trimester in fetuses with severe structural congenital anomalies.[117] The absence of a statistical significant association in our cohort may be the result of the small sample size. Moreover, probably not all congenital anomalies are accompanied by altered growth. The large variety of congenital anomalies in our study, ranging from mild anomalies such as polydactyly to complex anomalies such as severe heart defects, may therefore have diluted our results.

STRENGTHS AND LIMITATIONS

Main strengths of our study are the large sample size, the standardized 3D US examinations and VR measurements which are performed already early in the first trimester – providing more precise and reliable measurements.[111] Another strength is the evaluation of growth trajectories rather than only evaluation size.

Several limitations should be noted. This study was carried out in a selected population in a tertiary hospital setting. This resulted in a higher maternal age, mainly Western geographical background, higher educational level, and higher IVF/ICSI rate than expected in the general population, which may have decreased the external validity. Validation of our findings in a general population is therefore needed, but this is

complicated by difficulty in enrolling patients this early in gestation. Last, although our sample size was large, and the relative number of cases was comparable or even higher than observed in previous studies, the power was still limited to study the separate birth outcomes which may have reduced our ability to detect potentially clinically relevant differences.[107,116]

IMPLICATIONS FOR CLINICAL CARE AND RESEARCH

Our data confirm that embryonic growth is not uniform and that already in the first trimester a larger embryo has a smaller odds of an adverse birth outcome: an increase of 1 cm^3 at 11 weeks of gestation is associated with a 12% smaller odds of having an adverse birth outcome. Earlier in pregnancy, the effect estimate is even larger, which is the result of the small absolute EV [~0.3 cm^3] and the small absolute variance [IQR 0.16 – 0.41] at 7 weeks. The associations with birth weight and SGA emphasize the relevance of adequate embryonic growth, because birth weight is associated with various traits and diseases in adult life.[118]

Knowing that embryonic growth is not uniform and that poor growth has clinical consequences again raises debate on the different methods of pregnancy dating. Often dating is based on the menstrual cycle, but frequently reliable information is lacking on the menstrual cycle.[114] In those women, pregnancy dating is performed by using CRL. In our study population, approximately one fourth of the study population was dated based on CRL. The most important downside of CRL dating is that this method ignores all [patho]physiological variation in embryonic growth. Thus, from a scientific point of view, pregnancies dated on CRL are less suitable for studying early embryonic growth and are therefore often excluded for analyses.[115] When designing our study, we argued that misclassification may also occur in dating based on the menstrual cycle, due to recall bias and variation in timing of ovulation and implementation.[119,120]
Moreover, our clinical research question on the impact of early embryonic growth was relevant in all pregnancies, including those dated on CRL. Furthermore, we were able to date pregnancy already at 9 weeks of gestation, which is earlier than usual in clinical care.[28] We argued that the earlier in pregnancy the due date is determined, the smaller the bias of ignoring variation in growth. Therefore, the primary analyses were conducted in the total study sample. We additionally performed a sensitivity analyses in pregnancies dated on the menstrual cycle only. Compared to the main

analyses, although not statistically significant, the associations of embryonic size and growth and the outcome measures were comparable with regards to magnitude and direction of the effect estimates. This suggest that our main analyses are not importantly biased by dating on CRL. Lack of statistical significant findings is likely the result of lack of power in the smaller subgroup [n=352 versus n=757 in the full sample]. To increase the generalizability to clinical care, we suggest that in future studies CRL dating should not be considered an exclusion criteria, but that subgroup analyses should be performed to evaluate the effect in this subgroup.

CRL dating should be performed as early in gestation as possible, which is nowadays possible from 7 – 8 weeks of gestation onward by using 3D US techniques and VR. For implementation in clinical practice, the use of 3D US is limited by standard 2D evaluation of the scans. The recent development of a desktop version of the Barco I–Space is promising to enable application of 3D evaluation in clinical care, as it allows for real–time bedside evaluation of 3D scans.[121] Improvements in 3D techniques and development of new measures might also improve detection of growth differences even before 9 weeks of gestation.

In conclusion, our study shows that associations between embryonic growth and birth outcome can already be detected from 9 weeks of gestation onward. The results support the relevance of the preconceptional period for preparing pregnancy for embryonic, fetal, and neonatal health – thereby also influencing health on the long term. Future studies should establish if stricter monitoring of embryos with smaller than expected size or growth is beneficial for birth outcome, and whether those embryos may benefit from support for parents–to–be in optimizing their health and lifestyle.

SUPPLEMENTAL TABLE 1 | ASSOCIATIONS BETWEEN EMBRYONIC GROWTH PARAMETERS AND CONGENITAL ANOMALIES

		Cases[a]				p	Cases[a]				p	Cases[a]				p
		N	[n]	OR	95% CI	value	N	[n]	OR	95% CI	value	N	[n]	OR	95%CI	value
		Crude					Model 1					Model 2				
7 weeks																
CRL	[mm]	348	15	0.82	0.65 – 1.05	0.113	346	14	0.79	0.61 – 1.03	0.078	345	14	0.79	0.61 – 1.02	0.069
EV	[cm^3]	291	14	0.03	0.00 – 2.82	0.133	289	13	0.05	0.00 – 4.35	0.186	289	13	0.04	0.00 – 4.49	0.185
9 weeks																
CRL	[mm]	475	20	0.87	0.73 – 1.04	0.129	465	19	0.91	0.76 – 1.08	0.277	464	19	0.91	0.76 – 1.09	0.316
EV	[cm^3]	405	18	0.86	0.40 – 1.88	0.712	398	17	1.07	0.49 – 2.32	0.870	397	17	0.99	0.49 – 2.36	0.863
11 weeks																
CRL	[mm]	525	20	0.92	0.83 – 1.02	0.125	514	19	0.94	0.84 – 1.05	0.276	512	19	0.94	0.84 – 1.05	0.272
EV	[cm^3]	381	16	0.82	0.65 – 1.03	0.081	374	15	0.82	0.64 – 1.06	0.126	372	15	0.81	0.62 – 1.05	0.106

Legend: Statistically significant findings [p<0.05] are in **bold**.
[a] Cases = congenital anomalies.
Crude model: adjusted for GA at US measurement;
Model 1: adjustment for GA at US measurement + educational level [high/not-high] and fetal sex
Model 2: adjustment for Model 1 + parity [nulliparous/multiparous], maternal age, maternal periconceptional BMI, maternal geographical background [Western/non-Western], and mode of conception [spontaneous/IVF ICSI].
Abbreviations: n, number; OR, odds ratio; CI, confidence interval; CRL, crown-rump length; EV, embryonic volume; mm, millimeter; cm^3, cubic centimeter; GA, gestational age; US, ultrasound.

SUPPLEMENTAL TABLE 2 | BASELINE CHARACTERISTICS OF SPONTANEOUSLY CONCEIVED AND STRICTLY DATED PREGNANCIES

		Subgroup [n=352]	Missing data [n]
Maternal characteristics			
Age [years]		32.2 [29.1;35.8]	0
Geographical background	Western	294 [84%]	2
	Non-Western	56 [16%]	
Educational level	High	205 [58%]	7
	Middle	107 [30%]	
	Low	33 [9%]	
Periconceptional BMI [kg/m^2]		23.9 [21.5;27.6]	0
Folic acid use		343 [97%]	1
	Preconceptional initation	245 [71%]	5
Periconceptional smoking		66 [19%]	0
Nulliparous		145 [41%]	0
Conception mode	Spontaneous	352 [100%]	0
	IVF/ICSI	0	0
Neonatal characteristics			
Gestational age at birth [weeks^{+days}]		39^{+0} [38^{+0};40^{+1}]	0
	Preterm birth	32 [9%]	0
Birth weight [grams]		3342 [3011;3715]	0
Birth weight percentile		47.4 [26.4;75.3]	0
	SGA	30 [9%]	0
Sex	Male	179 [52%]	0
Major congenital anomaly		11 [3%]	5
Mortality	Fetal	6 [2%]	0
	Early neonatal	1 [0.3%]	0
Adverse birth outcome		71 [20%]	0

Legend: Data are presented in median [interquartile range] or number [percentages].
Abbreviations: [k]g, [kilo]grams; BMI, body mass index; IVF, in vitro fertilization; ICSI, intracytoplasmic sperm injection; SGA, small for gestational age.

SUPPLEMENTAL TABLE 3 | ULTRASOUND MEASUREMENTS OF SPONTANEOUSLY CONCEIVED AND STRICTLY DATED PREGNANCIES

			GA [IQR]	N	Median	IQR	Min - Max
Number of US					**4**	**2 – 6**	**1 – 8**
US at 7 weeks							
	CRL	[mm]	7^{+3} [7^{+1};7^{+5}]	180	12.9	10.3 – 14.9	5.5 – 20.3
	EV	[cm^3]	7^{+3} [7^{+1};7^{+5}]	159	0.24	0.13 – 0.39	0.01 – 0.95
US at 9 weeks							
	CRL	[mm]	9^{+3} [9^{+1};9^{+4}]	260	26.3	23.6 – 29.2	17.4 – 41.4
	EV	[cm^3]	9^{+3} [9^{+1};9^{+4}]	217	2.08	1.57 – 2.78	0.56 – 5.78
US at 11 weeks							
	CRL	[mm]	11^{+3} [11^{+1};11^{+5}]	291	49.8	45.3 – 54.1	34.3 – 67.8
	EV	[cm^3]	11^{+3} [11^{+1};11^{+4}]	236	10.22	8.02 – 12.09	3.69 – 21.11

Abbreviations: US, ultrasound; CRL, crown-rump length; EV, embryonic volume; mm, millimeter; cm^3, cubic centimeter; GA, gestational age; N, number; IQR, interquartile; range; min, minimum; max, maximum.

SUPPLEMENTAL TABLE 4A | ASSOCIATIONS BETWEEN EMBRYONIC GROWTH PARAMETERS AND ADVERSE OUTCOME IN SPONTANEOUSLY CONCEIVED AND STRICTLY DATED PREGNANCIES

		Cases[a]				p	Cases[a]				p	Cases[a]				p
		N	[n]	OR	95% CI	value	N	[n]	OR	95% CI	value	N	[n]	OR	95%CI	value
		Crude					Model 1					Model 2				
7 weeks																
CRL	[mm]	180	33	0.93	0.80 – 1.07	0.303	178	32	0.93	0.80 – 1.08	0.313	178	32	0.90	0.77 – 1.05	0.173
EV	[cm^3]	159	30	0.13	0.01 – 1.77	0.125	157	29	0.12	0.01 – 1.82	0.121	157	29	0.07	0.00 – 1.23	0.069
9 weeks																
CRL	[mm]	260	49	0.92	0.83 – 1.01	0.076	255	47	0.94	0.85 – 1.03	0.190	255	47	0.91	0.82 – 1.01	0.073
EV	[cm^3]	217	40	0.68	0.42 – 1.09	0.108	214	39	0.74	0.46 – 1.19	0.218	214	39	0.70	0.42 – 1.16	0.165
11 weeks																
CRL	[mm]	291	55	0.97	0.92 – 1.03	0.328	286	53	0.99	0.93 – 1.05	0.678	285	53	0.97	0.92 – 1.03	0.358
EV	[cm^3]	223	48	0.92	0.82 – 1.03	0.146	219	46	0.93	0.83 – 1.05	0.254	218	46	0.92	0.82 – 1.04	0.199

Legend: logistic regression analyses in subgroup of spontaneously conceived and strictly dated pregnancies. Statistically significant findings [p<0.05] are in **bold**.
[a] Cases = preterm birth, small for gestational age, mortality, and congenital anomalies.
Crude model: adjusted for GA at US measurement;
Model 1: adjustment for GA at US measurement + educational level [high/not-high] and fetal sex;
Model 2: adjustment for Model 1 + parity [nulliparous/multiparous], maternal age, maternal periconceptional BMI, maternal geographical background [Western/non-Western].
Abbreviations: n, number; OR, odds ratio; CI, confidence interval; CRL, crown-rump length; EV, embryonic volume; mm, millimeter; cm^3, cubic centimeter; GA, gestational age; US, ultrasound.

SUPPLEMENTAL TABLE 4B | ASSOCIATIONS BETWEEN EMBRYONIC GROWTH PARAMETERS AND PRETERM BIRTH IN SPONTANEOUSLY CONCEIVED AND STRICTLY DATED PREGNANCIES

		Cases[a]				p	Cases[a]				p	Cases[a]				p
		N	[n]	OR	95% CI	value	N	[n]	OR	95% CI	value	N	[n]	OR	95%CI	value
		Crude					Model 1					Model 2				
7 weeks																
CRL	[mm]	165	18	0.97	0.80 – 1.17	0.727	164	18	0.97	0.81 – 1.18	0.785	164	18	0.94	0.78 – 1.14	0.544
EV	[cm^3]	146	17	0.30	0.01 – 8.63	0.486	145	17	0.32	0.01 – 8.76	0.499	145	17	0.18	0.01 – 5.69	0.332
9 weeks																
CRL	[mm]	233	22	1.01	0.89 – 1.14	0.932	230	22	1.01	0.89 – 1.14	0.914	230	22	0.99	0.86 – 1.13	0.821
EV	[cm^3]	195	18	0.89	0.48 – 1.65	0.706	193	18	0.89	0.47 – 1.67	0.705	193	18	0.82	0.42 – 1.62	0.571
11 weeks																
CRL	[mm]	264	28	1.03	0.96 – 1.11	0.450	261	28	1.03	0.96 – 1.11	0.438	260	28	1.02	0.94 – 1.10	0.636
EV	[cm^3]	198	23	1.01	0.87 – 1.17	0.918	196	23	1.02	0.87 – 1.19	0.816	195	23	1.02	0.87 – 1.19	0.838

Legend: logistic regression analyses in subgroup of spontaneously conceived and strictly dated pregnancies. Statistically significant findings [$p<0.05$] are in **bold**.
[a] Cases = prematurity.
Crude model: adjusted for GA at US measurement;
Model 1: adjustment for GA at US measurement + educational level [high/not-high] and fetal sex;
Model 2: adjustment for Model 1 + parity [nulliparous/multiparous], maternal age, maternal periconceptional BMI, maternal geographical background [Western/non-Western].
Abbreviations: n, number; OR, odds ratio; CI, confidence interval; CRL, crown-rump length; EV, embryonic volume; mm, millimeter; cm^3, cubic centimeter; GA, gestational age; US, ultrasound.

SUPPLEMENTAL TABLE 4C | ASSOCIATIONS BETWEEN EMBRYONIC GROWTH PARAMETERS AND SMALL FOR GESTATIONAL AGE IN SPONTANEOUSLY CONCEIVED AND STRICTLY DATED PREGNANCIES

		Cases[a]				p	Cases[a]				p	Cases[a]				p
		N	[n]	OR	95% CI	value	N	[n]	OR	95% CI	value	N	[n]	OR	95%CI	value
		Crude					Model 1					Model 2				
7 weeks																
CRL	[mm]	161	14	0.87	0.70 – 1.07	0.171	160	14	0.87	0.70 – 1.07	0.183	160	14	0.87	0.70 – 1.08	0.192
EV	[cm^3]	141	12	0.01	0.00 – 1.29	0.064	140	12	0.01	0.00 – 1.31	0.065	140	12	0.01	0.00 – 1.25	0.061
9 weeks																
CRL	[mm]	232	21	0.85	0.73 – 0.98	**0.030**	228	20	0.87	0.75 – 1.01	0.068	228	20	0.84	0.71 – 0.99	**0.037**
EV	[cm^3]	193	16	0.45	0.19 – 1.04	0.061	191	16	0.46	0.20 – 1.06	0.069	191	16	0.43	0.18 – 1.06	0.068
11 weeks																
CRL	[mm]	258	22	0.91	0.84 – 1.00	**0.039**	254	21	0.93	0.85 – 1.01	0.092	253	21	0.92	0.84 – 1.01	0.065
EV	[cm^3]	194	19	0.81	0.67 – 0.99	**0.038**	191	18	0.84	0.69 – 1.02	0.079	190	18	0.83	0.68 – 1.02	0.073

Legend: logistic regression analyses in subgroup of spontaneously conceived and strictly dated pregnancies. Statistically significant findings [p<0.05] are in **bold**.
[a] Cases = small for gestational age.
Crude model: adjusted for GA at US measurement;
Model 1: adjustment for GA at US measurement + educational level [high/not-high] and fetal sex;
Model 2: adjustment for Model 1 + parity [nulliparous/multiparous], maternal age, maternal periconceptional BMI, maternal geographical background [Western/non-Western].
Abbreviations: n, number; OR, odds ratio; CI, confidence interval; CRL, crown-rump length; EV, embryonic volume; mm, millimeter; cm^3, cubic centimeter; GA, gestational age; US, ultrasound.

SUPPLEMENTAL TABLE 4D | ASSOCIATIONS BETWEEN EMBRYONIC GROWTH PARAMETERS AND CONGENITAL ANOMALIES IN SPONTANEOUSLY CONCEIVED AND STRICTLY DATED PREGNANCIES

		Cases[a]				p	Cases[a]				p	Cases[a]				p
		N	[n]	OR	95% CI	value	N	[n]	OR	95% CI	value	N	[n]	OR	95%CI	value
		Crude					Model 1					Model 2				
7 weeks																
CRL	[mm]	152	5	0.88	0.64 – 1.22	0.447	151	5	0.88	0.64 – 1.22	0.454	151	5	0.84	0.60 – 1.18	0.316
EV	[cm^3]	134	5	0.14	0.00 – 29.55	0.471	133	5	0.14	0.00 – 30.71	0.477	133	5	0.07	0.00 – 20.97	0.354
9 weeks																
CRL	[mm]	220	9	0.89	0.73 – 1.09	0.273	217	9	0.93	0.76 – 1.13	0.454	217	9	0.90	0.72 – 1.11	0.322
EV	[cm^3]	185	8	0.80	0.32 – 2.00	0.629	183	8	0.93	0.39 – 2.35	0.930	183	8	0.95	0.36 – 2.51	0.923
11 weeks																
CRL	[mm]	245	9	0.95	0.84 – 1.08	0.460	242	9	0.97	0.85 – 1.11	0.671	241	9	0.93	0.80 – 1.09	0.390
EV	[cm^3]	184	9	0.91	0.71 – 1.18	0.473	182	9	0.89	0.67 – 1.18	0.411	181	9	0.85	0.63 – 1.16	0.301

Legend: logistic regression analyses in subgroup of spontaneously conceived, strictly dated pregnancies. Statistically significant findings [p<0.05] are in **bold.**
[a] Cases = congenital anomalies
Crude model: adjusted for GA at US measurement;
Model 1: adjustment for GA at US measurement + educational level [high/not-high] and fetal sex;
Model 2: adjustment for Model 1 + parity [nulliparous/multiparous], maternal age, maternal periconceptional BMI, maternal geographical background [Western/non-Western].
Abbreviations: n, number; OR, odds ratio; CI, confidence interval; CRL, crown-rump length; EV, embryonic volume; mm, millimeter; cm^3, cubic centimeter; GA, gestational age; US, ultrasound.

SUPPLEMENTAL TABLE 5A | ASSOCIATIONS BETWEEN EMBRYONIC GROWTH PARAMETERS AND GESTATIONAL AGE IN SPONTANEOUSLY CONCEIVED AND STRICTLY DATED PREGNANCIES

					p				p				p
		N	Effect size	95% CI	value	N	Effect size	95% CI	value	N	Effect size	95%CI	value
		Crude				Model 1				Model 2			
7 weeks													
CRL	[mm]	179	-0.05	-0.16 – 0.07	0.457	178	-0.05	-0.17 – 0.07	0.392	178	-0.05	-0.17 – 0.08	0.469
EV	[cm^3]	158	-0.52	-2.38 – 1.34	0.582	157	-0.63	-2.50 – 1.25	0.510	157	-0.63	-2.56 – 1.29	0.517
9 weeks													
CRL	[mm]	257	-0.05	-0.13 – 0.02	0.176	253	-0.05	-0.13 – 0.03	0.191	253	-0.05	-0.13 – 0.03	0.178
EV	[cm^3]	214	-0.16	-0.52 – 0.20	0.386	214	-0.17	-0.53 – 0.20	0.365	212	-0.19	-0.57 – 0.19	0.327
11 weeks													
CRL	[mm]	288	-0.04	-0.08 – 0.01	0.132	284	-0.04	-0.08 – 0.01	0.145	283	-0.04	-0.09 – 0.01	0.155
EV	[cm^3]	220	-0.08	-0.18 – 0.03	0.142	220	-0.07	-0.17 – 0.03	0.189	216	-0.07	-0.17 – 0.03	0.184

Legend: Linear regression analyses in subgroup of spontaneously conceived and strictly dated pregnancies. Statistically significant findings [$p<0.05$] are in **bold**.
Crude model: adjusted for GA at US measurement;
Model 1: adjustment for GA at US measurement + educational level [high/not-high] and fetal sex;
Model 2: adjustment for Model 1 + parity [nulliparous/multiparous], maternal age, maternal periconceptional BMI, maternal geographical background [Western/non-Western].
Abbreviations: n, number; OR, odds ratio; CI, confidence interval; CRL, crown-rump length; EV, embryonic volume; mm, millimeter; cm^3, cubic centimeter; GA, gestational age; US, ultrasound.

SUPPLEMENTAL TABLE 5B | ASSOCIATIONS BETWEEN EMBRYONIC GROWTH PARAMETERS AND BIRTH WEIGHT IN SPONTANEOUSLY CONCEIVED AND STRICTLY DATED PREGNANCIES

					p				p				p
		N	Effect size	95% CI	value	N	Effect size	95% CI	value	N	Effect size	95%CI	value
		Crude				Model 1				Model 2			
7 weeks													
CRL	[mm]	179	20	-11 – 52	0.213	178	20	-12 – 52	0.227	178	23	-11 – 56	0.180
EV	[cm^3]	158	441	-83 – 966	0.098	157	419	-108 – 948	0.119	157	430	-112 – 972	0.119
9 weeks													
CRL	[mm]	257	10	-11 – 30	0.360	253	8	-12 – 29	0.430	253	12	-9 – 34	0.261
EV	[cm^3]	214	58	-40 – 156	0.243	212	53	-45 – 152	0.286	212	61	-40 – 161	0.236
11 weeks													
CRL	[mm]	288	5	-8 – 17	0.485	284	4	-9 – 17	0.569	283	6	-7 – 19	0.368
EV	[cm^3]	220	15	-12 – 41	0.282	217	17	-11 – 44	0.235	216	17	-10 – 44	0.218

Legend: Linear regression analyses in subgroup of spontaneously conceived and strictly dated pregnancies. Statistically significant findings [p<0.05] are in **bold**.
Crude model: adjusted for GA at US measurement;
Model 1: adjustment for GA at US measurement + educational level [high/not-high] and fetal sex;
Model 2: adjustment for Model 1 + parity [nulliparous/multiparous], maternal age, maternal periconceptional BMI, maternal geographical background [Western/non-Western].
Abbreviations: n, number; OR, odds ratio; CI, confidence interval; CRL, crown-rump length; EV, embryonic volume; mm, millimeter; cm^3, cubic centimeter; GA, gestational age; US, ultrasound.

SUPPLEMENTAL TABLE 6 | ASSOCIATIONS BETWEEN EMBRYONIC GROWTH PARAMETERS AND ADVERSE OUTCOME IN IVF/ICSI PREGNANCIES

		Cases				p	Cases				p	Cases				p
		N	[n]	OR	95% CI	value	N	[n]	OR	95% CI	value	N	[n]	OR	95%CI	value
		Crude					Model 1					Model 2				
7 weeks																
CRL	[mm]	144	26	0.68	0.51 – 0.89	**0.006**	142	24	0.67	0.50 – 0.90	**0.008**	141	23	0.58	0.42 – 0.80	**0.001**
EV	[cm^3]	111	20	0.04	0.00 – 4.45	0.177	109	18	0.06	0.00 – 7.19	0.244	108	17	0.00	0.00 – 0.06	**0.006**
9 weeks																
CRL	[mm]	169	23	0.80	0.63 – 1.00	0.055	167	22	0.80	0.63 – 1.01	0.063	166	21	0.77	0.60 – 0.99	**0.042**
EV	[cm^3]	141	16	0.75	0.23 – 2.44	0.634	139	15	0.89	0.26 – 3.01	0.846	138	14	0.73	0.19 – 2.75	0.637
11 weeks																
CRL	[mm]	188	28	0.92	0.82 – 1.04	0.184	183	26	0.90	0.79 – 1.02	0.088	182	25	0.86	0.75 – 0.99	**0.030**
EV	[cm^3]	127	19	0.81	0.64 – 1.04	0.095	124	17	0.76	0.58 – 1.00	**0.049**	123	17	0.72	0.54 – 0.96	**0.026**

Legend: logistic regression analyses in subgroup of IVF/ICSI pregnancies. Statistically significant findings [p<0.05] are in **bold**.
[a] Cases = preterm birth, small for gestational age, congenital anomalies, and mortality.
Crude model: adjusted for GA at US measurement;
Model 1: adjustment for GA at US measurement + educational level [high/not-high] and fetal sex;
Model 2: adjustment for Model 1 + parity [nulliparous/multiparous], maternal age, maternal periconceptional BMI, maternal geographical background [Western/non-Western].
Abbreviations: n, number; OR, odds ratio; CI, confidence interval; CRL, crown-rump length; EV, embryonic volume; mm, millimeter; cm^3, cubic centimeter; GA, gestational age; US, ultrasound.

3

PRENATAL MARKERS OF NEONATAL FAT MASS: A SYSTEMATIC REVIEW

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ABSTRACT

BACKGROUND Environmental influences during pregnancy are able to affect offspring phenotype with lifelong effects. Clinical applicable markers are needed to identify fetuses at risk for neonatal adiposity. This systematic review aims to [1] review the current literature on prenatal markers of neonatal fat mass, and [2] appraise the clinical applicability of the assessed markers.

METHODS A systematic literature search was conducted to identify studies meeting the following inclusion criteria: [1] original research papers in English; [2] research on dynamic and measurable prenatal markers of neonatal fat mass; [3] neonatal fat mass measurement within one month after birth, using the four–compartment model, magnetic resonance imaging, dual–energy X–ray absorptiometry, or air displacement plethysmography. Two reviewers independently performed study selection, assessment of methodological [QUADAS–II] and statistical quality, and appraisal of clinical applicability.

RESULTS Of 2333 studies primarily identified by the search strategy, 16 studies were included. Four of these were both methodologically and statistically of moderate or high quality. Prenatal markers investigated were ultrasound parameters, maternal biochemical markers, and maternal characteristics. Markers of predefined interest were maternal prepregnancy body mass index, fasting glucose, and HbA1c, showing varying results. A meta–analysis was not possible due to substantial methodological heterogeneity. Clinical applicability of all markers was rated poor.

CONCLUSIONS Although associations were found, no useful marker was identified, due to lack of methodological and statistical quality, inconsistent results, and poor clinical applicability. No markers were investigated in the periconceptional and embryonic period.

INTRODUCTION

The worldwide increasing prevalence of obesity and features of metabolic syndrome in children leads to increasing morbidity and mortality at young age.[122] Obesity is a multifactorial condition, caused by environmental, biological, and genetic factors, initiated in early life. The hypothesis of 'developmental origins of health and disease' [DOHaD] states that environmental influences during pregnancy are able to affect offspring phenotype with lifelong effects.[2,107,123] Epigenetic changes are thought to be pivotal in this long–term programming of fetal endocrine and cardio–metabolic functioning and consequently fat and lean mass development.[124–126] Both fetal over– and undernutrition have been related to obesity, and consequently increased susceptibility for non–communicable diseases later in life.[123] Birth weight is often used as a proxy of fetal growth. However, birth weight alone poorly predicts long–term risks as it does not fully reflect the effects of fetal growth and programming of fat mass.[127] Therefore, neonatal body composition, rather than birth weight, may be a more accurate predictor of risk for non–communicable diseases later in life.[128] If we would be able to predict neonatal fat mass in the prenatal period, infants at risk for adiposity could be identified earlier. This would allow for early interventions to optimize fetal and neonatal growth and metabolic development. Prevention of unfavorable programming might help reduce non–communicable diseases in children, adults, and their offspring.[129] The aims of this study are to: [1] provide a systematic review of the literature on prenatal markers or predictors of neonatal fat mass, and [2] appraise the clinical applicability of the assessed markers.

METHODS

LITERATURE SEARCH AND STUDY SELECTION

A systematic search was conducted using computerized bibliography databases until February 2015 to identify original articles on prenatal markers of neonatal fat mass. Medline, Google Scholar, Web of Science, Pubmed Publisher, Cochrane, and Embase were comprehensively searched using keywords as described in the **supplements**. Two authors [JR and MV] independently screened the titles and abstracts of all citations retrieved and excluded those clearly outside the scope of the study. Papers potentially eligible for inclusion were read in full text and included after consensus. Reference lists of all included papers were screened for potentially relevant publications not identified by our search. Studies were included for analysis if they met the following criteria, defined prior to the search; [1] Assessment of potential prenatal markers of neonatal fat mass in neonates; [2] Full report articles, written in English, with no limitation on publication date; [3] Use of measurable and dynamic prenatal parameters as markers, e.g., body mass index [BMI], laboratory diagnostics, or ultrasound parameters; [4] Fat mass as primary outcome measure, measured within one month after birth; [5] Use of accurate methods to measure body composition, comprising the four–compartment model, air displacement plethysmography [ADP], magnetic resonance imaging [MRI], and dual–energy X–ray absorptiometry [DXA].[130–132] Details of these methods are provided in **Table 1**. Studies using less accurate methods for measurement of body composition in neonates, such as double–labelled water, skinfold, and bioelectrical impedance analysis [BIA], were excluded. These methods are considered less accurate due to influences of fluid distribution and high inter–observer differences.[133,134] Secondly, studies using only static pregnancy characteristics [e.g., sex and parity] as prenatal markers were excluded because these cannot be influenced by interventions.

TABLE 1 | OVERVIEW OF INCLUDED METHODS FOR NEONATAL BODY COMPOSITION MEASUREMENT

	ADP	MRI	DXA	Four compartment model
Whole body or regional estimates?	Whole body	Whole body and regional estimates	Whole body and regional estimates	Whole body
Outcome measure	FM, FFM	FM	FM, FFM, BMC	FM, TBW, BMC, protein
Outcome unit	gram or %FM	ml or m^3	gram or %FM	gram or %FM
Pro's	No radiation exposure	Whole body vs regional fat	Whole body vs regional fat	Use of different methods to determine body composition
	High level of accuracy	No radiation exposure		
	Fast			
Contra's	Expensive device	Very sensitive to subject motion	Differences between manufacturers, hardware and software algorithms	Impractical
	Only whole body data	Expensive	Radiation exposure	Time consuming
			Very sensitive to subject motion	

Abbreviations: ADP, air displacement plethysmography; MRI, magnetic resonance imaging; DXA, dual energy X–ray absorptiometry; FFM, fat free mass; ml, milliliter; m^3, cubic meter; BMC, bone mineral content; FM, fat mass

QUALITY ASSESSMENT

Methodological quality of the included studies was assessed using the QUADAS–II checklist by two researchers [JR and MV] independently.[135] This tool is designed to assess the quality of primary diagnostic accuracy studies, with high interrater reliability, construct validity, and internal consistency.[136] Four main domains [patient selection, index test, reference test, and patient flow] are assessed in terms of risk of bias [low, high, or unclear]. The first three domains are also assessed on applicability of the study for the review question. On the basis of the degree of risk of bias of the different domains, methodological quality was rated as high, moderate, or poor. Quality of studies with an unclear risk of bias in two or more domains and with 'high risk of bias' in at least one domain was classified as poor in any case. Any disagreements were resolved via discussion and consensus with JR, MV, and RJ.

STATISTICAL QUALITY As the QUADAS–II checklist does not take into account statistical methods for interpretation of study quality, we rated the statistical quality separately. Statistical quality was considered high when prediction models were developed. Studies of moderate statistical quality used well described and performed prognostic models derived from multivariable analysis or well described and performed association models. Studies using poorly described and performed prognostic or association models, using only univariable analysis methods or correlation coefficients were considered to be poor of statistical quality.[137]

CLINICAL APPLICABILITY We considered only the results of studies with moderate or high methodological and statistical quality as evidence for potentially relevant markers and assessed clinical applicability only in studies meeting these criteria. Clinical applicability was assessed from the following items; logistic availability [possible to perform measurements in every setting], costs of measurement, level of difficulty to perform measurements, time to result after performance of measurement, and clinical relevance of the effect size.

META–ANALYSIS Meta–analysis was considered suitable provided [1] studies showed limited heterogeneity with respect to methodology and statistical methods, and [2] the same prenatal marker was used in two or more studies of moderate or high quality. Heterogeneity was assessed based on [1] patient inclusion criteria; [2] design of the studies [both methodological and statistical]; and [3] prenatal and postnatal outcome measures and timing and technique of measurements.

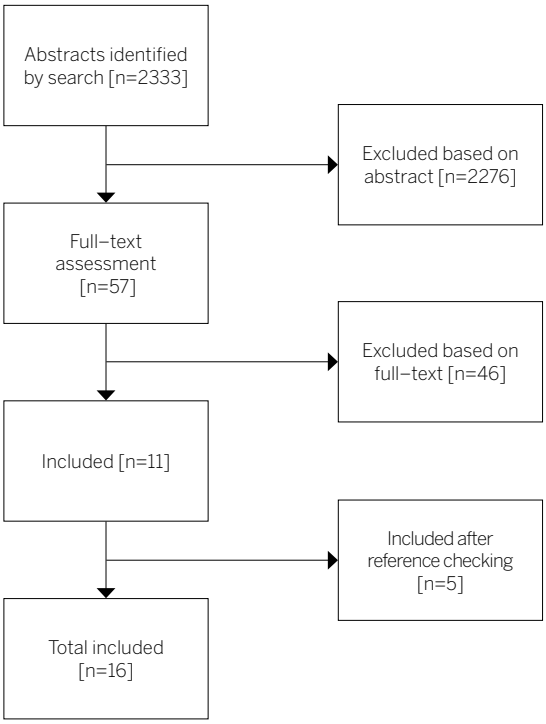
STUDY CHARACTERISTICS AND DATA COLLECTION The following data were extracted: [1] characteristics of study population [maternal, pregnancy related, and neonatal characteristics; [2] design of study, including prenatal markers and postnatal measurement of fat mass; [3] methods of analyses; and [4] results [including effect sizes] of the study.

RESULTS

SEARCH RESULTS

Figure 1 presents a flow chart of the study selection procedure. A search, performed on February 12th 2015, resulted in 2333 unique studies. After reference checking of the studies identified by the search, five more studies were included, leading to a final of 16 included studies.

FIGURE 1 | FLOWCHART OF INCLUDED STUDIES



STUDY CHARACTERISTICS

Table 2 provides details of these 16 studies. All studies were published between 2008 and 2015. Sample sizes varied between 23 and 948 infants. Inclusion and exclusion criteria differed between studies. Three studies specifically included obese pregnant

women.[138–140] One study investigated women with gestational diabetes mellitus [GDM].[141] The prenatal markers studied can be divided in 3 categories; ultrasound measures, biochemical markers from maternal blood, and maternal characteristics. Six studies investigated associations between ultrasound [2D and 3D] measures and neonatal fat mass. All ultrasound measures were assessed in the third trimester of pregnancy. Not only commonly used measures were assessed, such as expected fetal weight [EFW] and abdominal circumference [AC][142–144], but also newly developed techniques, including factional thigh volume [TVol], fractional arm volume [AVol], thigh fat [TF], fetal liver blood flow, and fetal abdominal subcutaneous tissue [FAST].[143,145–147] Six studies investigated the association between biochemical markers and neonatal fat mass. Maternal blood samples were obtained in the second and third trimester of pregnancy. Assessed were fasting glucose and postprandial glucose, HbA1c, insulin, leptin, total cholesterol, high–density lipoprotein cholesterol [HDL–c], triglycerides [TG], free fatty acids [FFA], C–peptide, insulin–like growth factor 1 [IGF 1], insulin–like growth factor binding protein 3 [IGFBP 3], and adiponectin.[140,141,144,148–150] In nine studies maternal characteristics were assessed, including prepregnancy BMI and gestational weight gain [GWG].[138,139,141,144,148,150–153] Eleven studies used ADP, four used DXA, and one used MRI scans for the assessment of neonatal fat mass [**Table 2**]. None of the studies used the four–compartment model.

QUALITY ASSESSMENT

Details of methodological quality assessment are presented in **Table 3**, for each study separately. Methodological quality of two studies was assessed as high, of seven studies as moderate, and of seven studies as poor. Two or three domains of twelve studies could not be assessed due to missing information. Assessment of different domains addressed by the QUADAS–II is discussed in more detail below.

GENERALIZABILITY Study populations ranged from very small [n=23] to large [n=948], and exceeded 100 subjects in eight studies.[138,145,148–153] Patient inclusion criteria differed widely between the studies. Most studies only included healthy women, singleton pregnancies, and healthy term born infants and excluded mothers with non–communicable diseases and medication use that could influence fetal growth and fat mass deposition. Two studies did not describe exclusion criteria.[145,152] In two studies only Caucasian women were included, while others only included women with diabetes mellitus or pregnancies at high risk for intra–uterine

growth retardation.[141,142,146,147] Some studies did not adequately describe the study characteristics.[144,145,148,151,153]

SELECTION BIAS Many studies did not adequately describe the methods used for patient selection; in most cases it was unclear whether a random sample of patients was selected. Three studies included patients from a larger study, but the selection process was not described in two of these studies.[138,145,150] None of the studies reported specifically whether the study population was derived from the general population or from a hospital population.

REPRODUCIBILITY One study did not report on the methodology of a newly developed ultrasound measurement.[145] One study mentioned the reproducibility of a new ultrasound parameter, TVol, but did not report the concrete values.[147] No other study reported on the reproducibility of the assessed marker.

BLINDING One study explicitly mentioned blinding of prenatal test results until the data analysis.[152] None of the other studies mentioned blinding for prenatal test results during body composition measurement or data collection.

QUALITY OF STATISTICAL APPROACH

Two studies reported on sample size calculation.[140,142] Statistical methods used in the different studies differed widely, as shown in **Table 4**. Four studies used correlation coefficients to evaluate the association between the prenatal marker and neonatal fat mass.[141,145,148,152] Six studies used association models[142,144,146,147,151,153] and ten prognostic models.[138–141,143,145,148–150,152] No prediction models were reported. The prognostic [regression] models were often of poor quality, due to inadequate description of the [pre]selection of variables into the model. No study scored 'high' on statistical quality.

APPRAISAL OF CLINICAL APPLICABILITY

None of the reports discussed the clinical value of the expected or observed findings. In total, four studies were both methodologically and statistically of moderate or high quality.[148,149,152,153] In these studies, three markers were assessed in more than one study including prepregnancy BMI [in three studies], maternal fasting glucose, and HbA1c [both in two studies]. All three were considered to be of poor clinical applicability because they predicted slight differences in fat mass.

TABLE 2 | OVERVIEW OF BASELINE CHARACTERISTICS OF INCLUDED STUDIES

Author, publication year	Comparison between groups	Inclusion criteria	Exclusion criteria	N	Prenatal measurement	Prenatal moment of measurement	Outcome measure	Timing of postnatal measurement
AIR DISPLACEMENT PLETHYSMOGRAPHY								
O'Connor et al. 2014 [29]	No	• Age>18 years • Caucasian women • Singleton pregnancies	• History of DM, hypertension, or PE • Chronic medical problems • Current drug abuse	23	US: TVol	At 28, 33, 38 weeks GA	FM [gram]	Day 2–5
O'Connor et al. 2014 [28]	No	• Age>18 years • Caucasian women	• History of hypertension or PE • Chronic medical problems	62	US: FAST, TF	At 28, 33 and 38 weeks GA	FM [gram]	Day 3
Law et al. 2011 [36]	Yes, EFW<p10 [n=26] vs AC <p5 [n=17] vs normal fetal growth [n=44]	• Singleton pregnancies • No major fetal or neonatal anomalies	• Delivery before 28 weeks of gestation	87	US: BPD, HC, AC, FL, EFW	< 3 weeks before birth	Reduced percentage body fat [%BF][1]	Within 7 days
Lee et al. 2009 [25]	No	• Singleton pregnancies • No fetal or neonatal anomalies	• Bad visualisation on ultrasound	78	US: TVol, AVol, BPD, HC, AC, EFW	< 4 days before birth	%BF	Within 48 h
Moyer–Mileur et al. 2009 [26]	No	• Singleton pregnancies	• Bad visualisation on ultrasound • Maternal drugs or alcohol use during pregnancy • Delivery before 34 weeks of gestation • Congenital anomalies • Neonate requiring intensive care treatment	47	US: EFW, BPD, AC, FL, HC Lab: insulin, IGF 1, IGFBP–3, leptin Maternal characteristics: prepregnancy BMI, GWG	Between 33 and 38 weeks GA	%BF	Within 24–72 h

TABLE 2 | OVERVIEW OF BASELINE CHARACTERISTICS OF INCLUDED STUDIES [CONTINUED]

Crume et al. 2015 [30]	No	• Singleton pregnancies	• History of premature delivery or still birth • Serious maternal chronic diseases	804	Lab: glucose, TG, total cholesterol, HDL–cholesterol, FFA, insulin, HbA1c	Early and mid/late–pregnancy	FM [gram] %BF	Within 48 h
Josefson et al. 2014 [22]	Yes, obese [n=38] vs non–obese [n=23] women	• Singleton pregnancies • Maternal age 18–40 years • GCT<130 mg/dl	• Mother who carried more than 3 pregnancies to term • Chronic medical conditions • Delivery before 37 weeks of gestation	61	Lab: glucose, TG, C–peptide, leptin, adiponectin	Between 36 and 38 weeks GA	%BF	Within 48 h
Au et al. 2013 [24]	No	• Term born infants • NICU stay < 2 days	• Maternal DM • Neonatal congenital anomalies	599	Lab: fasting and postprandial glucose, HbA1c Maternal characteristics: prepregnancy BMI	Third trimester Obtained from antenatal record	%BF	Within 48 h
Lingwood et al. 2011 [23]	No	• Gestational diabetes mellitus	• Multiple pregnancy • History of maternal illness other than GDM • Infant with congenital anomalies	84	Lab: mean fastening and mean 2–h postprandial BGL, insulin Maternal characteristics: prepregnancy BMI, GWG	Third trimester	%BF	Within 6 days

TABLE 2 | OVERVIEW OF BASELINE CHARACTERISTICS OF INCLUDED STUDIES [CONTINUED]

Starling et al. 2015 [34]	No	• Singleton pregnancies • Term born infants	• History of premature delivery or still birth • Pre–existing DM • Asthma managed with steroids • Cancer • Psychiatric illness	826	Maternal characteristics: prepregnancy BMI and GWG	Collected from hospital record	FM [gram] %BF	Within 3 days
Hull et al. 2008 [21]	Yes, obese [n=39] vs non–obese [n=33] women	• Healthy, term born infants • Maternal age 18–45 years	• Tobacco use • Alcohol consumption of >1 glass a week • Chromosomal or severe congenital anomalies • Mothers with [G]DM	72	Maternal characteristics: prepregnancy BMI	Self–reported after birth or self–reported in first trimester	%BF	Within 35 days
DUAL–ENERGY X–RAY ABSORPTIOMETRY								
Godfrey et al. 2012 [27]	No	• Singleton, uncomplicated pregnancies	• Not reported	152	US: fetal liver blood flow	At 36 weeks GA	FM [gram] %BF	Within 20 days
Friis et al. 2013 [31]	No	• Healthy pregnancies	• Multiple pregnancies • Pre–gestational DM • Severe maternal chronic diseases	207	Lab: glucose, insulin, total and HDL–cholesterol, TG Maternal characteristics: BMI	Lab at 30–32 weeks GA Maternal characteristics self–reported in first trimester	%BF	Within 4 days
Carlsen et al. 2014 [20]	Yes, obese [n=231] versus non–obese [n=80] women	• Maternal BMI ≥30 kg/m^2 [obese group] or BMI 18.5 – 24.9 kg/m^2 [non-obese group] • Singleton pregnancies • Healthy, term born infants	• Maternal chronic diseases • Infants with congenital anomalies • Infants requiring intensive care treatment	311	Maternal characteristics: GWG	Self–reported in first [obese women] or third [non–obese women] trimester and at 36–37 weeks GA	FM [gram] %BF	Within 2 days

TABLE 2 | OVERVIEW OF BASELINE CHARACTERISTICS OF INCLUDED STUDIES [CONTINUED]

Crozier et al. 2010 [32]	No	• Singleton pregnancies • Term born infants	• Congenital anomalies	948	Maternal characteristics: GWG	Before pregnancy and at 34 weeks GA	Internal SD score of FM [gram]	At birth
MAGNETIC RESONANCE IMAGING								
Modi et al. 2011 [33]	No	• Healthy infants • Term born infants	• Not reported	105	Maternal characteristics: BMI	Obtained from medical record	Total AT [ml]	Within 28 days

Legend:[1] Definition of 'reduced %BF' is not reported
Abbreviations: PE, pre–eclampsia; US, ultrasound; TVol, fractional thigh volume; FM, fat mass; %BF, percentage body fat; FAST, fetal abdominal subcutaneous tissue; TF, thigh fat; BPD, biparietal diameter; HC, head circumference; AC, abdominal circumference; FL, femur length; EFW, expected fetal weight; AVol, fractional arm volume; IGF 1, insulin–like growth factor I; IGFBP 3; insulin–like growth factor binding–protein 3; BMI, body mass index; GWG, gestational weight gain; TG, triglycerides; HDL–c, high density lipoprotein cholesterol; FFA, free fatty acids; GCT, glucose challenge test; BGL, blood glucose levels; [G]DM, [gestational] diabetes mellitus; GA, gestational age.

META–ANALYSIS

A meta–analysis was not performed because of substantial heterogeneity between studies. This judgement was based on differences in design [including inclusion and exclusion criteria, selection process, and timing and technique of measurements] and methodological and statistical quality. This is also illustrated in **Table 2**, in which the main characteristics of all studies are provided, and in **Table 3 and 4**, which respectively offer a quick overview of quality of the studies and of used statistical methods.

OUTCOMES

The results of the separate studies are presented in **Table 4**. Overall findings for each separate method of body composition measurement are described below.

AIR DISPLACEMENT PLETHYSMOGRAPHY Five ADP studies assessed the use of prenatal ultrasound parameters for predicting neonatal fat mass. Fractional thigh volume was the only parameter assessed in two studies, with conflicting results.[143,147] Studies assessing the association between common ultrasound parameters [e.g., EFW and AC] and fat mass found varying results, with explained variances ranging from 0.14 to 0.28.[142–144] Maternal blood glucose levels [BGLs] and HbA1c were assessed in respectively four and two studies, with conflicting results between the studies, and between boys and girls.[140,141,148,149] Assessment of leptin showed inconsistent results as well.[140,144] Insulin and triglycerides were not associated with neonatal fat mass in two studies. Maternal BMI and GWG were assessed in 5 studies using ADP.[139,141,144,148,153] Two studies did not find a significant association between fat mass and maternal BMI and GWG.[139,144] In contrast, three others found a significant increase in percentage body fat [%BF] with increasing prepregnancy BMI and GWG.[141,148,153]

DUAL–ENERGY X–RAY ABSORPTIOMETRY Positive relationships were found between fetal liver blood flow, fasting glucose, GWG, obesity and neonatal fat mass [gram and %] using DXA.[138,145,150,151] One study, assessing the association between GWG and neonatal fat mass, developed internal Z scores for fat mass, and expressed the found association using these Z scores. They did not report on their internal mean and Z score.[151]

MRI MRI was used in one study. A positive association was found between maternal BMI and total adipose tissue.[152]

TABLE 3 | ASSESSMENT OF METHODOLOGICAL QUALITY BASED ON QUADAS–II, SEPARATELY FOR EACH STUDY

Study	RISK OF BIAS				APPLICABILITY CONCERNS		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
O'Connor [28]	+	?	?	–	–	+	+
O'Connor [29]	–	?	?	+	–	+	+
Law [36]	–	+	?	+	–	+	+
Lee [25]	–	?	?	?	?	+	+
Moyer–Mileur [26]	+	?	?	+	–	+	+
Crume [30]	+	?	?	+	+	+	+
Josefson [22]	+	–	?	–	+	+	+
Au [24]	+	?	?	+	+	+	+
Lingwood [23]	+	?	?	+	–	+	+
Starling [34]	+	?	?	+	+	+	+
Hull [21]	+	+	?	+	+	+	+
Godfrey [27]	–	?	?	–	+	+	+
Friis [31]	+	?	?	?	+	+	+
Carlsen [20]	–	?	?	+	–	+	+
Crozier [32]	+	?	?	–	+	+	+
Modi [33]	–	+	+	+	+	+	+

+ Low Risk – High Risk ? Unclear Risk [not reported in paper]

TABLE 4 | OVERVIEW OF RESULTS OF INCLUDED STUDIES

Author, publication year	Quality of methods	Quality of statistics	Statistical model		Results						
	[High, moderate, poor]				Outcome measure			Outcome measure			Covariates adjusted for in multivariable model
					Effect size	Range	p value	Effect size	Range	p value	
AIR DISPLACEMENT PLETHYSMOGRAPHY											
O'Connor et al. 2014 [29]	Poor	Poor	ASSOCIATION MODEL		**FM**						
			Univariable	TVol [cm^3]	NS[1]						
O'Connor et al. 2014 [28]	Poor	Moderate	ASSOCIATION MODEL		**FM**	**95% CI**					
			Univariable	FAST [mm] at 28, 33 GA	p>0.1						
				At 38 GA	NA						
				TF [mm] at 28 GA	β=79	[11;146]	p=0.023				
				At 33 GA	p>0.1						
				At 38 GA	β=63	[22;103]	p=0.004				
			Multivariable	FAST [mm] at 28, 33 GA	NA						Smoking
				At 38 GA	β=64		p<0.001				
				TF [mm] at 28 GA	β=−146		p=0.026				Smoking
				At 33 GA	NA						
				At 38 GA	β=−108		p=0.071				
Law et al. 2011 [36]	Moderate	Poor	ASSOCIATION MODEL		**Reduced %BF[3]**						BW, GA
			Multivariable	EFW <p10	R^2=0.284[1]						
Lee et al. 2009 [25]	Poor	Moderate	PROGNOSTIC MODEL		**%BF**						
			Univariable	TVol [ml]	R^2=0.46		p<0.05				
				BPD, HC[cm]	R^2=0.04, 0.08		p>0.05				
				EFW [g]	R^2=0.28		p>0.05				
				AC [cm]	R^2=0.24		p>0.05				
				AVol [ml]	R^2=0.39		p>0.05				
			Multivariable	TVol [ml]	β=0.129		p<0.001				NA

TABLE 4 | OVERVIEW OF RESULTS OF INCLUDED STUDIES [CONTINUED]

Moyer-Mileur et al. 2009 [26]	Moderate	Poor	ASSOCIATION MODEL Univariable			**%BF**						
					AC [cm]	R^2=0.14		p<0.05				
					EFW [g]	R^2=0.11		p<0.05				
					BPD, HC, FL [cm]	NS[1]						
					Insulin [pmol/l], IGF1, IGFBP3, leptin [all ng/l]	p>0.2						
					Prepregnancy BMI, GWG	p>0.2						
Crume et al. 2015 [30]	Moderate	Moderate	PROGNOSTIC MODEL Multivariable	**<20 weeks of gestation**		**FM**	**SE**		**%BF**	**SE**		Infant sex, GA, maternal age, race, parity, postnatal age at time of PEA POD measurement, maternal smoking, total maternal energy intake and physical activity during pregnancy, GWG, maternal prepregnancy BMI
					Fasting glucose [mg/dl]	p>0.1			p>0.1			
					HOMA–IR	β=8.38	[± 3.78][4]	p=0.03	β=0.25	[± 0.1]	p=0.01	
					Total cholesterol [mg/dl], TG [mg/dl], FFA [mg/dl]	p>0.1			p>0.1			
					HDL–cholesterol [mg/dl]	β=−1.00	[± 0.47]	p=0.03	β=−0.2	[± 0.01]	p=0.05	
				>20 weeks of gestation								
					Fasting glucose [mg/dl]	β=1.97	[± 0.69]	p=0.004	β=0.05	[± 0.02]	p=0.004	
					HOMA–IR, FFA [mg/dl], TG [mg/dl]	p>0.1			p>0.1			
					HbA1c [%]	β=44.67	[± 19.74]	p=0.02	β=1.06	[± 0.52]	p=0.04	
					HDL–cholesterol [mg/dl]	β=−0.76	[± 0.44]	p=0.09	p>0.1			
Josefson et al. 2014 [22]	Moderate	Poor	PROGNOSTIC MODEL Multivariable			**%BF**						Ethnicity, education, glucose control test
					Leptin [ng/ml]	p=0.0012[2]						
					Adiponectin [µg/dl] C–peptide [ng/ml], glucose, TG [both mg/dl]	NS[1]						
Au et al. 2013 [24]	Moderate	Moderate	PROGNOSTIC MODEL Multivariable			**%BF**	**95%CI**					NA
				Neonatal sex [ref: female]	male	β=-2.11	[-2.84;-1.39]	p<0.001				
				Ethnicity [ref: Caucasian]	Asian	β=-0.76	[-1.59;0.61]	p<0.001				
					Other	β=-3.15	[-4.68;-1.62]					
				Weight gain		β=0.13	[0.07;0.19]	p<0.001				
				GA [weeks, ref: 39-39.9]	<38	β=-2.08	[-3.59;-0.57]	p=0.009				
					38-38.99 weeks	β=-0.34	[-1.41;0.74]					
					40-40.99 weeks	β=0.75	[-0.21;1.71]					
				Prepregnancy BMI [kg/m², ref: 18.5 -24.99]	<18.5	β=-0.16	[-1.52;1.2]	p=0.010				
					25-25.99	β=1.19	[0.25;2.14]					
					30-34.99	β=-0.91	[-2.8;0.98]					
					>35	β=3.09	[0.68;5.5]					
				Parity [ref: 0]	>1	β=1.34	[0.59;2.09]	p<0.001				
				Hypertension [ref: No]	Yes	β=-1.62	[-3.05;-0.18]	p=0.028				
				GDM status [ref: No]	Yes	p>0.1						
			CORRELATION			**%BF**						
					Fasting and postprandial glucose, HbA1c [4]	p>0.1						
Lingwood et al. 2011 [23]	Moderate	Poor	PROGNOSTIC MODEL Multivariable		**Male infants**	**%BF**						NA
					Fasting glucose [mmol/l]	β = 0.45[1]	R^2=0.34					
					Parity	β = 0.22[1]						
					Female infants							
					Prepregnancy BMI [kg/m²]	β = 0.24[1]	R^2=0.19					
					Parity	β = 0.25[1]						
					GA [weeks]	β = 0.20[1]						

TABLE 4 | OVERVIEW OF RESULTS OF INCLUDED STUDIES [CONTINUED]

Lingwoord et al. 2011 [23] [continued]			CORRELATION	**All infants**	**%BF**						
				Fasting glucose [mmol/l]	r=0.30		[sign[1]]				
				Postprandial glucose [mmol/l]	r=0.25		[sign[1]]				
				Prepregnancy BMI [kg/m²]	r=0.23		[sign[1]]				
				GWG [kg]	NA						
				Male infants							
				Fasting glucose [mmol/l]	r=0.54		p<0.001				
				Postprandial glucose [mmol/l]	r=0.39		p=0.01				
				Prepregnancy BMI [kg/m²]	p>0.1						
				GWG [kg]	p>0.1						
				Female infants							
				Fasting glucose [mmol/l]	p>0.1						
				Postprandial glucose [mmol/l]	p>0.1						
				Prepregnancy BMI [kg/m²]	r=0.32		p=0.04				
				GWG [kg]	p>0.1						
Starling et al. 2015 [34]	Moderate	Moderate	ASSOCIATION MODEL		**FM[1]**	**95%CI**		**%BF[1]**	**95%CI**		
			Univariable	Prepregnancy BMI [kg/m²]	β=4.12	[2.53;5.72]		β=0.10	[0.06;0.14]		
				GWG [0.1 kg/wk]	β=17.30	[10.68;23.91]		β=0.37	[0.19;0.54]		
			Multivariable	Prepregnancy BMI [kg/m²]	β=5.21	[3.54;6.89]		β=0.12	[0.08;0.16]		Prepregnancy BMI, GWG, maternal age, ethnicity, education, household income, gravidity, GA at birth, sex, infant age at measurement, maternal smoking
				GWG [0.1 kg/wk]	β=23.95	[17.37;30.53]		β=0.55	[0.37;0.72]		
Hull et al. 2008 [21]	High	Poor	PROGNOSTIC MODEL		**%BF**						
			Multivariable	Prepregnancy BMI [kg/m²]	NS[1]						GA, infant age at testing, maternal prepregnancy weight, BMI, maximum body weight, weight gain, gender, socio-economic status
DUAL-ENERGY X-RAY ABSORPTIOMETRY											
Godfrey et al. 2012 [27]	Poor	Poor	PROGNOSTIC MODEL		**FM**			**%BF**			Sex, GA at birth, maternal age, parity, reported general health, smoking, social class
			Multivariable	Fetal liver blood flow	NA			p<0.001[2]			
			CORRELATION	Fetal liver blood flow	r=0.43		p<0.001	r=0.40		p<0.0001	

TABLE 4 | OVERVIEW OF RESULTS OF INCLUDED STUDIES [CONTINUED]

Friis et al. 2013 [31]	Poor	Moderate	PROGNOSTIC MODEL		**%BF**	**95%CI**					
			Univariable	GA [weeks]	β=0.35	[0.12;0.58]	p=0.004				
				BMI [kg/m²]	β=0.13	[0.04;0.22]	p=0.0003				
				Fasting glucose [mmol/l]	β=1.2	[0.48;2.0]	p=0.002				
				Placental weight [g]	β=0.004	[0.002;0.006]	p<0.001				
				FFA [mmol/l]	β=2.4	[−0.04;4.9]	p=0.054				
				HDL−cholesterol [mmol/l]	β=−0.85	[−1.7;−0.002]	p=0.049				
				Insulin [pmol/l], TG, cholesterol [both mmol/l],GWG [kg], parity	NS						
			Multivariable	GA [weeks]	β=0.34	[0.12;0.20]	p=0.004				NA
				BMI [kg/m²]	β=0.098	[0.01;0.19]	p=0.036				
				Fasting glucose [mmol/l]	β=0.84	[0.08;1.67]	p=0.048				
				FFA, HDL−c [both mmol/l]	NS[1]						
Carlsen et al. 2014 [20]	Poor	Moderate	PROGNOSTIC MODEL		**FM**	**95%CI**		**%BF**	**95%CI**		Maternal age, education, smoking
			Multivariable	Prepregnancy obesity [yes]	β=135	[85;186]	p<0.001	β=3.1	[2.0;4.1]	p<0.001	
				GWG [kg]	β=11	[8;15]	p<0.001	β=0.2	[0.1;0.3]	p<0.001	
Crozier et al. 2010 [32]	Poor	Moderate	ASSOCIATION MODEL		**FM [SD]**	**95%CI**					Prepregnancy BMI, maternal smoking, age, height, parity, educational attainment, breastfeeding duration
			Univariable	GWG [kg]	β=0.06	[0.00;0.11]	p=0.04				
			Multivariable	GWG [kg]	β=0.10	[0.04;0.15]	p=0.0004				
MAGNETIC RESONANCE IMAGING											
Modi et al. 2011 [33]	High	Moderate	PROGNOSTIC MODEL		**AT [ml]**	**95%CI**					Sex, infant weight
			Multivariable	BMI [kg/m²]	β=8	[0.9;14]	p=0.027				
			CORRELATION	BMI [kg/m²]	r=0.22		p=0.02				

Legend: [1]P value not reported; [2]Effect size not reported;[3] definition of reduced %BF is not reported; [4] Units are not provided in paper. **Abbreviations**: NA, not applicable; NS, not significant; SD, standard deviaton; SE, standard error; 95%CI, 95% confindence interval; FFM, fat free mass [gram]; FM, fat mass [gram]; %BF, percentage body fat; TVol, fractional thigh volume; FAST, fetal abdominal subcutaneous tissue; TF, thigh fat; EFW, expected fetal weight; BPD, biparietal diameter; HC, head circumference; AC, abdominal circumference; AVol, fractional arm volume; FL, femur length; BMI, body mass index; GWG, gestational weight gain; HDL−c, high density lipoprotein cholesterol; FFA, free fatty acids; TG, triglycerides; GA, gestational age in weeks; GDM, gestational diabetes mellitus; BGL, blood glucose levels; AT, adipose tissue.

DISCUSSION

This review provides a complete overview and a systematic methodological quality assessment of 16 studies on prenatal dynamic markers associated with neonatal fat mass. We aimed to appraise the clinical applicability of the large number of assessed markers. Only four studies met our criteria, resulting in three markers of interest; maternal prepregnancy BMI, fasting glucose, and HbA1c. Yet, none of these appeared to be clinical applicable to identify fetuses at risk for neonatal adiposity.

The association between maternal prepregnancy BMI and neonatal fat mass appears to be complex. When analyzed as continuous variable, maternal prepregnancy BMI was positively associated with neonatal fat mass.[152,153] However when assessed categorical, results per category were conflicting.[148] This can partly be explained by the association between maternal over– and undernutrition and offspring obesity.[123] Moreover, one could argue that this artificial categorizing of BMI could, in theory, lead to conflicting results by itself. Nevertheless, the earlier reported impact of prepregnancy BMI on offspring cardiovascular health confirms the clinical importance of this marker.[154] The dynamic character of prepregnancy BMI can be questioned. As we defined in the inclusion criteria, we aimed to focus on dynamic markers, which may be influenced by [lifestyle] interventions. In general, BMI is a dynamic marker, however, when assessed at one time point it is rather static. The 'prepregnancy period' was not well defined and may have included a long time span before pregnancy. Still, we decided to consider maternal prepregnancy BMI a dynamic marker and to include it in the review. Several studies included in this review did investigate GWG, but all were of poor quality. None investigated the influence of the change in BMI or maternal body composition during pregnancy on neonatal fat mass.

Fasting glucose levels and HbA1c are the other markers of interest. Only one study found a positive association with neonatal fat mass, while others did not find a significant association.[148,149] Glucose homeostasis during pregnancy is altered by impaired insulin sensitivity and increased fetal glucose consumption. Both insulin resistance and fetal glucose consumption start in the second trimester, and peak in the third trimester of pregnancy.[155] Timing of measurements is therefore crucial, and differences in timing [either in the second or third trimester of pregnancy] limit the comparison and interpretation of these studies. The findings of O'Connor et al. stress the importance of timing of measurements as well; they showed inconsistent results of TF and FAST measurements at different gestational ages.[147]

Ultrasound measurements are performed routinely in most pregnancies. Unfortunately, all studies investigating ultrasound markers were of poor methodological or statistical quality. Standard ultrasound parameters do not seem to be associated with neonatal fat mass. However, whereas some of the newly developed ultrasound parameters were associated with neonatal fat mass [**Table 4**], the clinical applicability of these markers could not be judged due to poor methodological quality.

LIMITATIONS OF REVIEWED STUDIES

Substantial methodological heterogeneity and incomplete reporting complicated the interpretation of results and made it impossible to perform a meta–analysis. For example, incomplete reporting on selection criteria or selection of specific patient groups limited generalizability. Also, statistical methods differed widely. Some studies only performed univariable analyses or calculated correlation coefficients, with limited scientific value. Appropriate methods accounting for confounders are multivariable and prognostic models, as performed in the higher quality studies. None of these studies used prediction modeling, which is considered the optimal method for risk prediction. Only two studies reported on sample size calculation. Lack of statistical significance in many studies may well be due to lack of power. Due to wide ranges in normal distribution of fat mass in neonates[85], a relatively large sample size is required to determine a significant association. Furthermore, only one study mentioned blinding for the prenatal results, while blinding for prenatal and postnatal test results is essential to prevent risk of bias.[152] Computerized methods for measuring body composition, such as DEXA or ADP, may theoretically reduce the risk of selection bias, but still do not exclude it as the results can be interpreted in the light of knowledge of prenatal data. Timing of measurement needs to be addressed, from two perspectives. For practical reasons, almost all assessed markers were investigated in the third trimester of pregnancy. As the DOHaD hypothesis states that the embryonic and fetal periods are crucial phases in fetal programming, it may be relevant to look for markers at an earlier stage of pregnancy.[125] The earlier detection of fetuses at risk would probably result in better opportunities to improve environmental factors and alter long–term outcome. Timing of body composition measurement after birth is also relevant. This varied between within 24 hours to 35 days postpartum in the reviewed studies. Measurement soon after birth highly reflects fetal nutritional status and growth, while after days to weeks body composition is influenced by factors such as volume and type of feeding.[156] However, the

most sensitive moment for prediction of long–term outcome still needs to be determined. Moreover, we faced problems with the interpretation of clinical applicability as statistically significant associations are not always of clinical value. A small increase of 0.12% in %BF per unit increase in BMI may be statistically significant in large cohorts but not relevant in individual patients.[153] As mentioned above, we aimed to take several factors into account for assessment of clinical applicability. In practice, we noticed that all markers had such small effect sizes that we did not have to assess the other factors to decide on the applicability of the marker. Evidence is still lacking on the exact effect of neonatal fat mass on long–term health. Whether such small differences in fat mass are related to increased risk of childhood obesity later in life is not clear yet. Markedly, clinical applicability of findings was neither addressed nor discussed in any of the papers.

STRENGTHS OF THIS REVIEW

Strength of this review are the use of multiple electronic database searches and clearly defined inclusion criteria to identify all relevant studies on prenatal markers of neonatal fat mass. The overview of methodological quality and effect sizes provide an insight into studies on this subject. Methodological quality was systematically evaluated with the QUADAS–II, a widely used and validated tool, with high interrater reliability.[136] A good measure of reliability was further ensured by having the QUADAS–II scored by two authors independently and discussing discrepancies in consensus meetings. Some items were difficult to score and many items were rated "unclear", as also reported by others.[136] As the QUADAS–II does not provide assessment of statistical quality, we added rating of the statistical approaches, based on current expert opinion on studies assessing associations or predictors.

LIMITATIONS OF THIS REVIEW

Our search strategy was limited to full report papers, written in English. This may theoretically have led to missing of relevant studies. We did not provide a meta–analysis of the data due to heterogeneity of the results, which might facilitate an easier interpretation of the results. It should be noted that meta–analyses are very useful for randomized controlled trials [RCTs], but for cohort studies its value is often debated. In cohort studies, the risk for publication bias [studies with negative results not being published] is considerably higher compared to RCTs, which influences the results of a meta–analysis substantially.[157] Moreover, differences in

quality often lead to a too diverse collection of data to provide a meta–analysis. Therefore, a clear overview of the literature is often of more value than an artificially performed meta–analysis with high heterogeneity and studies of poor quality.[158] An extra complicating factor of comparison of the different studies is the inclusion of different methods for determination of neonatal fat mass, which are difficult to compare. For example, it is known that DXA systematically overestimates fat mass compared to ADP.[159] However, we aimed to provide an up–to–date overview of the literature on all validated and accurate methods of body composition measurement in neonates.[133] Only dynamic markers were included in this review, as the primary aim of this review was to detect markers that can be monitored [and possibly influenced] during gestation. In our opinion, the ultimate goal is to have a prediction model, which can serve as a clinical tool to determine the risk of adverse long–term health. For such a model to be accurate, static parameters, such as parity or ethnicity, likely need to be entered in the model as well.[160]

FUTURE PERSPECTIVES

Radiological techniques have evolved over the last decades and offer new opportunities to measure very small structures, even in early pregnancy. An interesting method, not yet examined, may be assessment of [abdominal] fat mass by fetal MRI. This method is not yet widely available, however, and is expensive. Ultrasonography might be a more promising method, as it is relatively cheap, patient friendly, repeatable, and can be performed in all clinical and outpatient settings. The development and implementation of 3D ultrasonography in clinical practice, allowing for measurement of specific structures, has high potential.[161] Moreover, individual longitudinal growth patterns presumably have more predictive value for long–term health than a single measurement.[162] Although some studies measured ultrasound markers repeatedly over time, none have analyzed growth data longitudinally yet. Moreover, as mentioned above, screening earlier in pregnancy would lengthen the window of opportunities for interventions to prevent adverse programming, and thereby adverse growth and development.[41,124] For future development of risk profiling, it may be helpful to combine earlier mentioned markers with complex factors assumed to be related to the underlying fetal programming. Promising in this respect are fetal and maternal epigenetic patterns and the fetal hippocampus–hypothalamo–pituitary–adrenal [HHPA] axis, which is known to be associated with the risk of non–communicable diseases in adulthood.[123]

CONCLUSION

We conclude that so far clinically validated and applicable markers for identification of fetuses at risk for neonatal adiposity are lacking. Ultrasound measures, biochemical markers and maternal characteristics were evaluated in studies with generally poor methodological and statistical quality and inconsistent results. Fat mass is determined by multiple factors and may only be predicted prenatally by complex prediction models. Further development of sophisticated fetal radiographic parameters, probably combined with laboratory measures and possibly with [epi]genetic risk profiling, is needed to identify fetuses at risk for altered neonatal fat mass.

SUPPLEMENTS

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['body composition'/exp OR 'lean body weight'/de OR [[body NEAR/3 [composit* OR distribut* OR water OR potassium OR fat OR lean]] OR [[fat OR lean OR adipose] NEAR/3 [distribut* OR percent* OR composit* OR mass OR tissue]]]:ab,ti] AND ['immature and premature labor'/exp OR 'low birth weight'/exp OR 'intrauterine growth retardation'/de OR 'prenatal growth'/exp OR macrosomia/de OR [[[prematur* OR 'pre mature' OR 'pre maturity' OR preterm* OR 'pre term'] NEAR/6 [infant* OR neonat* OR birth* OR child* OR newborn* OR baby OR babies OR born]] OR [low* NEAR/3 ['birth weight' OR birthweight]] OR LBW OR VLBW OR ELBW OR SGA OR [small NEAR/3 [age OR date]] OR IUGR OR [[intrauterine OR 'intra uterine'] NEAR/3 [retard* OR restrict*]] OR [[fetus OR fetal OR foetus OR foetal OR prenatal OR embryo*] NEAR/6 [growth* OR develop* OR lean OR fat]] OR macrosom*]:ab,ti] NOT [[animals]/lim NOT [humans]/lim] NOT [[adult/exp OR adulthood/de OR adolescent/exp OR adolescence/exp OR child/de OR 'preschool child'/de OR 'school child'/de] NOT newborn/exp]

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[exp "body composition"/ OR [[body ADJ3 [composit* OR distribut* OR water OR potassium OR fat OR lean]] OR [[fat OR lean OR adipose] ADJ3 [distribut* OR percent* OR composit* OR mass OR tissue]]].ab,ti.] AND ["Premature Birth"/ OR exp "Infant, Premature"/ OR exp "Infant, Low Birth Weight"/ OR "Fetal Growth Retardation"/ OR fetus/gd OR "Fetal Development"/ OR "Fetal Macrosomia"/ OR [[[prematur* OR "pre mature" OR "pre maturity" OR preterm* OR "pre term"] ADJ6 [infant* OR neonat* OR birth* OR child* OR newborn* OR baby OR babies OR born]] OR [low* ADJ3 ["birth weight" OR birthweight]] OR LBW OR VLBW OR ELBW OR SGA OR [small ADJ3 [age OR date]] OR IUGR OR [[intrauterine OR "intra uterine"] ADJ3 [retard* OR restrict*]] OR [[fetus OR fetal OR foetus OR foetal OR prenatal OR embryo*] ADJ6 [growth* OR develop* OR lean OR fat]] OR macrosom*].ab,ti.] NOT [exp animals/ NOT humans/] NOT [[exp adult/ OR adolescent/ OR exp child/ OR exp "infant"/] NOT "infant, newborn"/]

Cochrane

[[[body NEAR/3 [composit* OR distribut* OR water OR potassium OR fat OR lean]] OR [[fat OR lean OR adipose] NEAR/3 [distribut* OR percent* OR composit* OR mass OR tissue]]]:ab,ti] AND [[[[prematur* OR 'pre mature' OR 'pre maturity' OR preterm* OR 'pre term'] NEAR/6 [infant* OR neonat* OR birth* OR child* OR newborn* OR baby OR babies OR born]] OR [low* NEAR/3 ['birth weight' OR birthweight]] OR LBW OR VLBW OR ELBW OR SGA OR [small NEAR/3 [age OR date]] OR IUGR OR [[intrauterine OR 'intra uterine'] NEAR/3 [retard* OR restrict*]] OR [[fetus OR fetal OR foetus OR foetal OR prenatal OR embryo*] NEAR/6 [growth* OR develop* OR lean OR fat]] OR macrosom*]:ab,ti] NOT [[[mh ^adult] OR [mh ^adolescent] OR [mh ^child] OR [mh ^infant]] NOT [mh ^"infant, newborn"]]

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TS=[[[[body NEAR/3 [composit* OR distribut* OR water OR potassium OR fat OR lean]] OR [[fat OR lean OR adipose] NEAR/3 [distribut* OR percent* OR composit* OR mass OR tissue]]]] AND [[[[prematur* OR "pre mature" OR "pre maturity" OR preterm* OR "pre term"] NEAR/6 [infant* OR neonat* OR birth* OR child* OR newborn* OR baby OR babies OR born]] OR [low* NEAR/3 ["birth weight" OR birthweight]] OR LBW OR VLBW OR ELBW OR SGA OR [small NEAR/3 [age OR date]] OR IUGR OR [[intrauterine OR "intra uterine"] NEAR/3 [retard* OR restrict*]] OR [[fetus OR fetal OR foetus OR foetal OR prenatal OR embryo*] NEAR/6 [growth* OR develop* OR lean OR fat]] OR macrosom*]] NOT [[adult* OR adolescen* OR child*] NOT newborn*] NOT [[animal* OR rat OR rats OR mouse OR mice OR rodent* OR pig OR pigs OR swine* OR sheep OR babboon OR monkey OR primate*] NOT [human*]]]

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"body composition|distribution|water|potassium|fat"|"fat distribution|percentage|composition" prematurity|prematur|preterm|LBW|VLBW|ELBW|SGA|IUGR –adult –adulthood –adolescent –adolescence

FETAL FRACTIONAL THIGH VOLUME: AN EARLY 3D ULTRASOUND MARKER OF NEONATAL ADIPOSITY

Pediatr Obesity.
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4

ABSTRACT

BACKGROUND The predisposition for obesity is suggested to originate in the prenatal period. Prenatal markers are needed to identify fetuses at risk for neonatal adiposity, as early marker of childhood obesity.

OBJECTIVE The aim of this study is to assess the association between fetal fractional thigh volume [TVol] and neonatal percentage body fat from midgestation onward.

METHODS In this perinatal cohort study, singleton pregnancies with term born infants were included. Fetal TVol was measured on three–dimensional ultrasound scans [3D US] obtained at 22, 26, and 32 weeks of gestation. Neonatal body composition measurement [percentage body fat, [%BF]] was planned between 42^{+0} and 42^{+6} weeks postmenstrual age. Cross–sectional and longitudinal linear regression analyses were performed.

RESULTS Seventy–nine mother–child pairs were included. Median [interquartile range] TVol increased from 7.6 [7.1;8.5] cm^3 at 22 weeks to 36.5 [33.8;40.9] cm^3 at 32 weeks. Median neonatal %BF was 14.3% [11.7;17.0]. TVol at 22 weeks [β=–1.58, 95%CI 2.45; –0.70, explained variance 31%] was negatively associated with %BF, but no associations were found at 26 and 32 weeks of gestation. TVol growth between 22 and 32 weeks of gestation [explained variance 18%] was also statistically significantly negatively associated with %BF.

CONCLUSIONS Fetal TVol is a promising 3D US marker for prediction of neonatal adiposity from mid–gestation onward.

INTRODUCTION

The increasing prevalence of childhood obesity and its long–term health consequences lead to higher disease burden and healthcare costs already in early life.[163,164] Lifestyle programs and interventions focusing on secondary prevention of childhood obesity have limited effects as the predisposition of obesity is suggested to originate in the periconceptional and prenatal periods.[4,165] Prenatal markers of neonatal adiposity, considered a proxy of childhood obesity, are needed to predict the risk for neonatal adiposity and to monitor the effect of future prenatal prevention strategies for childhood obesity.

Previous studies have assessed the use of prenatal imaging as marker for neonatal adiposity.[166] Standard obstetrical two–dimensional [2D] biometric measures, such as estimated fetal weight, do not predict neonatal adiposity well.[144,167,168] A promising three–dimensional ultrasound [3D US] marker is fractional thigh volume [TVol].[167] In the third trimester of pregnancy, this soft–tissue marker is highly associated with neonatal percentage fat mass.[167,169] To the best of our knowledge, the usefulness of earlier TVol measurement or TVol measured serially has not yet been studied. As fetal fat accumulation increases exponentially in the third trimester, TVol may be most informative in late pregnancy.[31] On the other hand, both 3D US scanning and TVol measurements are technically easier to perform earlier in pregnancy, and early measurements provide a longer time frame for prevention strategies.

The primary aim of our study was to assess the association between TVol at different time points during the second half of pregnancy and neonatal adiposity. The secondary aim was to evaluate the association between TVol growth, based on serial measurements, and neonatal adiposity. We hypothesized that TVol is associated with neonatal percentage fat mass from midgestation onward, and that the association between TVol growth and neonatal percentage fat mass is stronger than the association between single TVol measurements and neonatal percentage fat mass.

METHODS

This study was embedded in the ongoing Rotterdam Periconceptional Cohort study.[104] Between November 2013 and July 2015, we invited women with an ongoing singleton pregnancy for prenatal 3D US examinations in the second and third trimesters of pregnancy and for cranial ultrasound in the neonatal period. Between September 2014 and September 2016, body composition [BC] measurement was added to the postnatal follow–up visit. For the present analysis, we selected infants who were term born [≥ 37 weeks of gestation at birth], without congenital anomalies [EUROCAT criteria] and who had a BC measurement at the postnatal visit.[170] All participating women and their partners gave written informed consent for both the Predict study and the follow–up study. The study was performed at the Erasmus MC – Sophia Children's Hospital, Rotterdam, the Netherlands. Both the Central Committee of Human Research in The Hague, the Netherlands and the regional Medical Ethical and Institutional Review Board of Erasmus MC, University Medical Center, Rotterdam, the Netherlands, approved the study [MEC 2004–227, amendment 08 Nov 2014].

PRENATAL SCANNING

Serial 3D US examinations were performed at 22, 26, and 32 weeks of gestation by a certified sonographer [IK] by using the Voluson E8 system [GE Medical Systems, Zipf, Australia] with a 1–7 MHz transabdominal transducer. Standard 2D biometric measurements were performed during the examinations. 3D volumes of the fetal thigh were obtained in the standard acquisition plane for femur diaphysis length measurement.[28] TVol [cm^3] was measured offline by one operator [JR] using validated commercially available 3D software [4DView, version 5.0 or higher, GE Medical Systems]. Volume measurement of the fetal thigh is based on 50% of the femoral diaphysis length. Five equidistant axial limb slices are shown by the software and are traced manually for each image slice. TVol is calculated automatically after tracing five slices [**Supplemental Figure 1**].[167] As there is no evidence for length or volume differences between the left and right femur, measurements were performed on the thigh closest to the transducer. TVol measurements were performed in triplicate on each scan. If the smallest and largest TVol measurement differed more than 10%, a fourth measurement was performed. Hereafter, the outlier of the four measurements was removed.

The mean of the three measurements was used in the analyses. 3D scans of very low quality, with severe movement artefacts or an inadequate sweep angle, were excluded. We performed reliability analyses of TVol in this cohort by having TVol measurements of 30 randomly selected scans repeated by a second operator [IK]. The analyses showed excellent intra– and interobserver reliability [intraclass correlation coefficients above 0.9].

OUTCOME MEASURE

The outcome measure was neonatal body fat as percentage of total body weight [%BF], obtained via air displacement plethysmography [PEA POD®, COSMED]. This validated method calculates the absolute fat mass and fat free mass by direct measurement of body volume and body mass, based on the whole–body densitometry principle.[95,96] BC measurements were planned in the early neonatal period, between 42^{+0} and 42^{+6} weeks postmenstrual age, independent of gestational age [GA] at birth. After having been trained by a dedicated research team, JR performed all BC measurements using a standardized protocol.

DATA COLLECTION

Maternal, obstetrical and neonatal outcome data were collected from questionnaires at enrolment, at 24 weeks of gestation, and after birth.[104] Postnatal data included sex, GA at birth, and birth weight. Birth weight Z scores corrected for GA and sex were calculated by using Fenton growth data.[171]

GA was calculated during the routine clinical intake visit, by using crown–rump length as dating measure for spontaneous pregnancies.[172] In pregnancies conceived through assisted reproductive technologies, dating was based on the date of oocyte retrieval, embryo transfer or insemination date, depending on the method of assisted reproductive technology. We defined fetal growth restriction [FGR] as estimated fetal weight or abdominal circumference below the fifth percentile.[173,174] Being small for gestational age at birth [SGA] was defined as birth weight below the fifth percentile for GA.

There were no missing covariates nor missing outcome data. As we assume that the missing fetal US data were missing at random, and linear mixed models make optimal use of all available data points, imputation was not needed.[175]

STATISTICAL ANALYSES

First, the association between TVol and neonatal fat mass was estimated for each measurement separately, i.e. 22, 26, and 32 weeks of gestation [cross–sectional analyses]. Second, individual TVol growth trajectories were created to assess the association between TVol growth and neonatal fat mass [longitudinal analyses]. All associations were explored in 2 models: a basic [model 1] and fully adjusted [model 2] model.

CROSS–SECTIONAL ANALYSES In model 1 we adjusted for GA at moment of fetal 3D US examination and GA at postnatal BC measurement. Model 2 included the covariates of model 1 plus a predefined set of covariates based on plausible biological effects and existing literature including sex, parity, maternal age, prepregnancy BMI, and smoking during pregnancy. The residuals of the linear regression analyses were approximately normally distributed.

LONGITUDINAL ANALYSES Regression analyses on growth trajectories of TVol between 22 and 32 weeks of gestation and neonatal %BF were performed in two steps. First, a linear mixed model was estimated with the cube root of TVol as the response and GA at 3D US examination as covariate. The cube root transformation was opted for as this made the residuals approximately homoscedastic and the relationship with GA approximately linear. The intercept and the GA were entered as random [i.e., subject specific] effects, providing individual intercepts and slopes for each fetus. The origin of the GA scale was placed at 160 days [baseline TVol size] and the TVol slope was measured as the increase in the cube root of TVol between 160 days and 220 days [Δ_{TVol}] to aid interpretation and break the strong correlation between the two random effects. The subject–specific random effects were used as covariates in the second step in a linear regression model with %BF as outcome. In the second step, a basic and a complex model were applied. For the longitudinal analyses, model 1 included only GA at postnatal BC measurement. Model 2 included the covariates of model 1 plus the covariates included in model 2 of the single measurements.

To compare the predictive ability of the longitudinal model with the models using single measurements we used leave–one–out cross validation. For this, we deleted a single subject from the dataset on which we estimated our model and used this model to

predict the outcome from the subject we deleted. This was carried out for all subjects. For the longitudinal model, this required computing the best linear unbiased predictions. The models were then compared using the root mean squared error [RMSE] to select the model with the highest predictive value [lowest RMSE].

SENSITIVITY ANALYSES In a tertiary hospital–based population, cases of pathologic fetal growth patterns are likely oversampled, which may influence the results. We therefore a priori planned sensitivity analyses with exclusion of those fetuses with FGR and infants who were SGA at birth, applying the same models as in the primary analyses.

The data are presented as number [percentage] or median [interquartile range]. A 2–tailed p value of <0.05 was considered statistically significant. Data were analyzed by using SPSS package 21.0 [IBM SPSS Statistics, Armonk, NY] and R [R: A language and Environment for Statistical Computing, version 3.1.3, 2015 for Windows, R Core Team, Vienna, Austria].

RESULTS

Postnatal BC measurement was available for 99 infants during the study period. BC measurement was not successful in eight infants because of logistic or child–related reasons [i.e., crying], and six parents gave no consent. Six infants were born prematurely, and therefore excluded. The baseline characteristics of the 79 mother–child pairs included are presented in **Table 1**.

TABLE 1 | BASELINE CHARACTERISTICS

	N=79
Maternal characteristics	
Age [years]	32.2 [28.2 – 34.2]
Geographic origin [Western]*	68 [86%]
Education [high]*	42 [53%]
Nulliparous	35 [44%]
Prepregnancy BMI [kg/m^2]	23.8 [20.7 – 27.4]
Mode of conception [IVF/ICSI]	27 [34%]
Periconceptional folic acid supplement use*	75 [93%]
Periconceptional smoking	15 [19%]
Neonatal characteristics	
Gender [male]	44 [56%]
Birth weight [grams]	3215 [3055 – 3530]
Gestational age at birth [weeks$^{+\ days}$]	39^{+0} [38^{+1} – 40^{+0}]

Legend: All data are presented in median [interquartile range] or n[%]. *2 missing values.
Abbreviations: IVF, in vitro insemination; ICSI, intracytoplasmic sperm injection.

In total, 229 3D US examinations were performed of which 37 scans, equally distributed over the three time points, were not usable for TVol measurement, resulting in 192 TVol measurements. Standard fetal biometry data are presented in **Table 2**. Median [interquartile range] TVol increased from 7.6 [7.1; 8.5] cm^3 at 22 weeks to 36.5 [33.8; 40.9] cm^3 at 32 weeks of gestation. Median neonatal %BF was 14.3% [11.7; 17.0].

TABLE 2 | FETAL BIOMETRY

N=79	22 weeks of gestation	26 weeks of gestation	32 weeks of gestation
GA at US examination [weeks]	22^{+3} [22^{+1};22^{+5}]	26^{+3} [26^{+2};26^{+5}]	32^{+3} [32^{+1};32^{+5}]
TVol [cm³]	7.6 [7.1;8.5]	16.7 [15.2;18.2]	36.5 [33.8;40.9]
EFW [gram]	530 [489;556]	983 [909;1057]	2028 [1862;2177]
BPD [mm]	55.5 [54.6;57.4]	69.1 [67.2;71.0]	84.8 [82.8;87.3]
HC [mm]	201.1 [196.2;206.4]	247.0 [242.8;253.7]	296.8 [291.3;302.7]
AC [mm]	182.4 [176.8;188.0]	225.8 [218.7;233.6]	290.1 [276.7;301.3]
FL [mm]	38.2 [37.0;39.5]	48.6 [47.2;49.6]	60.6 [59.2;62.3]

Abbreviations: GA, gestational age; US, ultrasound; TVol, fractional thigh volume; EFW, estimated fetal weight; BPD, biparietal diameter; HC, head circumference; AC, abdominal circumference; FL, femur length.

CROSS–SECTIONAL ANALYSIS

Both models showed a statistically significant negative association between TVol at 22 weeks of gestation and neonatal %BF. The explained variance was 21% in model 1 and 31% in model 2. None of the models showed a significant association between TVol measured at 26 or 32 weeks of gestation and neonatal %BF [**Table 3**].

LONGITUDINAL ANALYSIS

Both models showed a statistically significant negative association between TVol growth and neonatal %BF, explained by baseline TVol size and not by increase in TVol between 22 and 32 weeks of gestation [Δ_{TVol}]. The explained variance was 11% in Model 1, and 18% in model 2 [**Table 3**].

ROOT MEAN SQUARE ERROR

Comparison of the predictive ability of the different models showed the lowest [most optimal] RMSE for the model at 22 weeks of gestation [RMSE 4.11]. The RMSEs of the models at 26 and 32 weeks of gestation were respectively 4.48 and 4.38, and of the longitudinal model 4.29.

SENSITIVITY ANALYSIS

The sensitivity analyses were performed in infants with normal fetal growth [n=73], by excluding those with FGR [n=1], SGA [n=3], or both [n=2]. Again, both models showed

a statistically significant negative association between TVol at 22 weeks of gestation and neonatal %BF. TVols at 26 and 32 weeks were not significantly associated with %BF. The sensitivity analyses for TVol growth also showed a negative association, again explained by baseline TVol size and not by Δ_{TVol} [**Supplemental table 1**].

TABLE 3 | ASSOCIATIONS BETWEEN FETAL THIGH VOLUME AND NEONATAL PERCENTAGE BODY FAT

		model 1†				model 2‡			
	Unit	β	95%CI	p value	R^2	β	95%CI	p value	R^2
Cross–sectional analysis:									
TVol 22w GA [n=60]	cm^3	**–1.53**	**–2.38;–0.68**	**0.001**	**21%**	**–1.58**	**–2.45;–0.70**	**0.001**	**31%**
TVol 26w GA [n=68]	cm^3	–0.26	–0.78;0.27	0.331	4%	–0.29	–0.82;0.25	0.285	14%
TVol 32w GA [n=64]	cm^3	–0.02	–0.23;0.18	0.828	5%	–0.024	–0.23;0.18	0.810	21%
Longitudinal analysis:	**TVol growth [n=79]**				**11%**				**18%**
Baseline TVol size	$\sqrt[3]{cm^3}$	**–31**	**–53;–10**	**0.004**		**–28**	**–52;–4**	**0.02**	
Δ_{TVol}	$\sqrt[3]{cm^3}$/week	0.175	–0.76;1.12	0.71		0.53	–0.56;1.62	0.33	

Legend: Significant results are depicted in **bold** [p<0.05].
† model 1: adjusted for gestational age at moment of 3D–scanning and gestational age at body composition measurement. In the TVol growth analyses we did not adjust for gestational age at moment of 3D scanning.
‡ model 2: adjusted for covariates in Model 1 plus for maternal prepregnancy BMI, maternal age, maternal smoking during, parity, and sex.
Abbreviations: β, effect size; CI, confidence interval; GA, gestational age; n, number of scans available; R^2, explained variance for all covariates in the model; TVol, fractional thigh volume; w, weeks.

DISCUSSION

In this prospective perinatal cohort study, we evaluated the use of fetal TVol measured from midgestation onward as marker of neonatal adiposity. A statistically significant association between TVol and neonatal %BF was found at 22 weeks of gestation. This finding confirms our hypothesis that TVol in midgestation might be useful to predict neonatal adiposity. We did not observe a significant association at 26 and 32 weeks of gestation. The longitudinal analyses of TVol growth showed a less strong association with neonatal %BF than TVol at 22 weeks of gestation, negating our second hypothesis.

We are the first to report that TVol at 22 weeks is negatively associated with neonatal %BF [i.e., a lower TVol is associated with a higher neonatal %BF]. Future studies should confirm this finding. To understand this, at first glance on paradoxical finding, one should keep in mind the physiological growth pattern of a fetus. In early pregnancy, fetal growth only consists of lean mass, while from 28 weeks of gestation onward fetal growth increasingly consists of fat mass.[31] Thus, extrapolating overall fetal growth to the fetal thigh: TVol in midgestation solely measures lean mass, while at the end of pregnancy, it measures both fat and lean mass.

The used measure for adiposity, %FM, is defined as absolute fat mass divided by total body weight [sum of fat and lean mass]. An increased %FM can thus be the result of either a decreased absolute lean mass or an increased absolute fat mass; it is not necessarily contributed to fat mass alone.

In literature, it has been described that low birth weight and SGA infants carry a higher risk for obesity and cardiovascular risk factors.[176] Our findings confirm the results of these studies, but the underlying mechanisms are not fully unraveled yet. The validity of our finding is supported by the sensitivity analysis, showing an even stronger association in fetuses with normal growth. To gain more insight in the underlying mechanism, we performed a post–hoc analysis on the association between TVol at 22 weeks and the absolute amounts of neonatal fat and lean mass. We observed a negative association with absolute fat mass and no association with lean mass, indicating that a lower TVol is associated with a higher absolute fat mass in the neonatal period. This suggests that in fetuses with low lean mass in early pregnancy, it is fat accumulation that explains the higher neonatal %BF.

The clinical implications of this association, and of increased neonatal adiposity, are unclear. Although the effect size seems large [+1.6 %BF [≈ +1/2 SD %FM] per

cm^3 increase in TVol], the effect is limited due to the small absolute variance in TVol at 22 weeks [**Table 2**]. Altogether, our data suggest that effects of cardiometabolic programming are already present and detectable in–utero.

The potential of fetal thigh volume as predictor of neonatal adiposity in midgestation has not been described before as previous studies assessed the use of TVol after 27 weeks of gestation. They all used air displacement plethysmography for BC measurement, similar to our method. In 2009, Lee et al. were the first to report a positive association between TVol, measured shortly before delivery, and neonatal %BF.[167] Later, O'Connor et al. did not find any significant correlation between TVol measured at 28, 33, and 37 weeks of gestation and neonatal %BF, which corresponds with our findings.[147] The most recently published study by Moore et al. found no association at 36 weeks, but did find a strong positive association between TVol measured at 28 weeks of gestation and neonatal %BF.[169] Despite their sample size of only 32 mother–child pairs, they estimated an explained variance of almost 70% [effect size not reported]. Such a strong association at this time point has not been described before and is in contrast to our findings and those of O'Connor et al. As they do not speculate on the underlying absolute fat and lean masses, it is difficult to interpret their findings. Still, differences in study design and study population may explain the contrasting findings.

We consider the prospective design with serial standardized measurements and the longitudinal analyses of the serial data the main strengths of our study. Another strength is that both prenatal and postnatal measurements were performed around the same postconceptional age, allowing for better cross–sectional comparison. Of interest is the relatively high %BF in our population compared to data from previous studies.[144,177,178] This is likely explained by timing of BC measurement between 42 and 43 weeks of gestation that includes the period of physiological increase in body fat in the first weeks after term age.[94] Interpretation of fat mass measured sooner after birth is complicated by the initial physiological process of weight and fat loss, and regain of weight thereafter in the first days after birth.[168,179] In an additional post–hoc analysis, we studied the effect of variation in postnatal age at BC measurement [median 25, range 7 – 40 days] by adding this term to the final adjusted models. No improvement of model fit for all analyses suggests that differences in postnatal age did not importantly influence our results [data not shown]. Breastfeeding was not taken into account in the

analyses because misclassification is likely in our partially breastfed population.

Related to the design and the sample size of our study, we face some limitations. We did not perform 3D US examinations after 32 weeks of gestation, which would have given us a more complete overview of the development of TVol and its association with neonatal adiposity. Moreover, larger studies are needed to determine the long–term consequences of increased fat accumulation in the prenatal and early postnatal period. The external validity may be reduced as the women studied were relatively old, highly educated and mainly of Caucasian ethnicity. Moreover, our tertiary hospital population included high numbers of in vitro insemination/intracytoplasmic sperm injection pregnancies [**Table 1**].

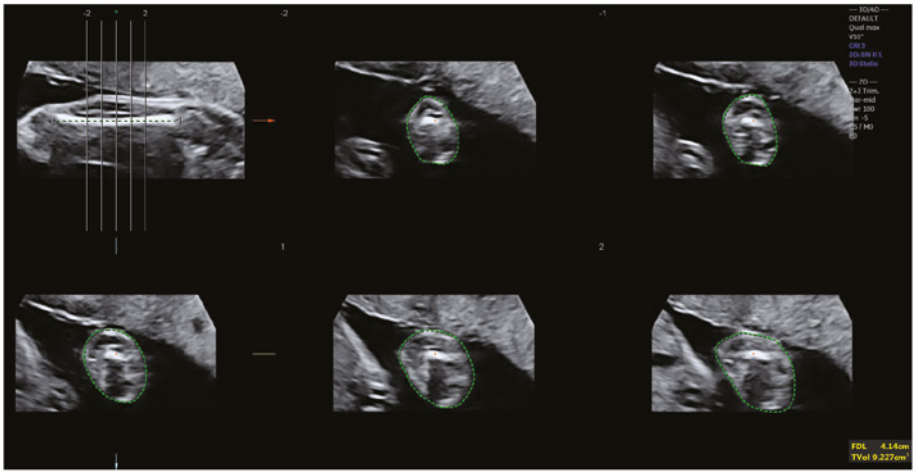
We believe that TVol measurement is easy to perform in 4DView, but we also experienced the technical limitations described earlier.[167] We expect that the ongoing development of automated image analysis tools with higher precision and faster calculations will increase the clinical feasibility of TVol measurement as an additional bedside–available technique for monitoring of fetal growth.[180]

In the future, TVol might be used in prediction models for neonatal adiposity, including other relevant predictors such as maternal BMI.[166] TVol might also be a useful tool to evaluate the effect of interventions and may help to unravel the complex interaction between environmental risk factors and recently identified genetic risk factors for childhood obesity.[181]

CONCLUSION

This prospective perinatal study showed that fetal TVol in midgestation is a promising 3D US soft tissue marker for prediction of neonatal adiposity and for monitoring of effects of prenatal prevention strategies for childhood obesity. Serial assessment for TVol growth between 22 and 32 weeks of gestation showed little additional value for prediction of neonatal adiposity when compared to single TVol measurement at 22 weeks.

SUPPLEMENTAL FIGURE 1 | TVOL MEASUREMENT IN 4DVIEW



SUPPLEMENTAL TABLE 1 | SENSITIVITY ANALYSES OF ASSOCIATION BETWEEN FETAL THIGH VOLUME AND NEONATAL PERCENTAGE BODY FAT IN INFANTS WITH NORMAL FETAL GROWTH

		model 1†				model 2‡			
	Unit	β	95%CI	p value	R^2	β	95%CI	p value	R^2
Cross–sectional analysis:									
TVol 22 w GA [n=57]	cm^3	**−1.72**	**−2.57;−0.88**	**0.000**	**26%**	**−1.76**	**−2.61;−0.90**	**0.000**	**38%**
TVol 26 w GA [n=64]	cm^3	−0.27	−0.88;0.35	0.395	7%	−0.37	−1.00;0.28	0.258	17%
TVol 32 w GA [n=58]	cm^3	0.02	−0.23;0.28	0.856	5%	−0.033	−0.28;0.21	0.786	25%
Longitudinal analysis:	**TVol growth [n=73]**			**0.007**	**15%**			**0.014**	**22%**
Baseline TVol size	$\sqrt[3]{cm^3}$	**−32**	**−55;−8**	**0.008**		**−33**	**−59;−7**	**0.01**	
Δ_{TVol}	$\sqrt[3]{cm^3}$/week	0.06	−1.23;1.35	0.926		−0.08	−1.5;1.35	0.915	

Legend: Normal fetal growth is defined as absence of FGR of SGA at birth. Significant results are depicted in **bold** [p<0.05].
† Model 1: Adjusted for gestational age at moment of 3D–scanning and gestational age at body composition measurement. In the TVol growth analyses we did not adjust for gestational age at moment of 3D–scanning.
‡ Model 2: Adjusted for covariates in Model 1 plus for maternal prepregnancy BMI, maternal age, maternal smoking during, parity, and fetal sex.
Abbrevations: β, effect size; CI, confidence interval; n, number of scans available; R^2, explained variance of all covariates in the model; TVol, fractional thigh volume; w, weeks.

6

FIRST WEEK WEIGHT DIP AND REACHING GROWTH TARGETS IN EARLY LIFE IN PRETERM INFANTS

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ABSTRACT

BACKGROUND & AIMS Aggressive parenteral nutritional practices were implemented in clinical practice over a decade ago to prevent early growth retardation in preterm infants. We aimed to study adherence to current nutritional recommendations in a population of very preterm infants, and to evaluate growth in early life.

METHODS Preterm infants [gestational age <30 weeks and birth weight <1500 grams] were included in a prospective observational cohort study. Data on parenteral and enteral intake were collected on days 1–7, 14, 21, and 28 [d28] of life. Growth data were collected at birth, at moment of maximal weight loss [dip], and either at discharge from the neonatal intensive care unit or at d28, whichever came first. Nutritional intakes were compared to recommendations of current guidelines. The target growth rate was 15 – 20 g/kg/d.

RESULTS Fifty–nine infants [63% male] were included. Median gestational age was 27^{+3} [interquartile range 25^{+6};28^{+4}], and birth weight was 920 gram [720;1200]. Median macronutrient intakes were within or above the targets on all study days, but energy targets were not met before day 5. Median growth rates were 9.5 and 18.1 g/kg/d, when calculated from respectively birth and dip to discharge/d28. Eight [14%] versus 46 [78%] infants met the growth targets, when evaluated from respectively birth and dip to discharge/d28.

CONCLUSIONS In this cohort, only energy intake up to day 5 was lower than recommended. Growth targets were achieved in the majority of the infants, but only when evaluated from dip onward, not from birth onward.

INTRODUCTION

Early nutrition and growth are pivotal for short– and long–term health of very preterm infants, with growth retardation being associated with mortality and poor neurodevelopmental outcome.[50,182] Adequate energy and protein intakes via the parenteral route can significantly increase postnatal growth in very preterm infants.[183–185]

In 1977, the American Academy of Pediatrics stated that postnatal growth of very preterm infants ideally should mimic fetal growth.[56] Despite the implementation of parenteral nutrition in neonatal care in the early 1970s, growth retardation during hospital stay remained very common in the following decades.[186–188] In 2005 and 2002 the latest guidelines for parenteral nutrition in preterm infants were released by respectively the European Society for Pediatric Gastroenterology, Hepatology and Nutrition [ESPGHAN] and the American Society for Parenteral and Enteral Nutrition [ASPEN].[48] Both guidelines recommended aggressive parenteral nutrition regimes for preterm infants, with the start of amino acids and lipids soon after birth. Randomized controlled trials showed that these recommendations were safe and improved growth on the short term.[53,189,190] However, soon after the release of the guidelines, concerns were expressed about the feasibility of the recommendations in clinical practice, and about the persistent high rates of in–hospital growth retardation.[191–193] This was supported by a survey in 2013 showing that parenteral nutrition practices are frequently not compliant with current recommendations, especially in the first days of life.[51]

The aggressive nutritional practices have been introduced in clinical neonatal practice over a decade ago. We presume that general improvements of perinatal and neonatal care over time affect nutritional practices and growth. We therefore aimed to evaluate adherence to current nutritional recommendations in a population of very preterm infants on a tertiary neonatal intensive care unit [NICU]. Additionally, we aimed to evaluate their growth in the first month of life.

METHODS

This prospective observational study was part of an ongoing cohort study at the level IV NICU of the Erasmus MC – Sophia Children's Hospital, Rotterdam, the Netherlands. Infants born before 30 weeks of gestational age [GA] and birth weight below 1500 grams were included between September 2014 and December 2015. Exclusion criteria included congenital anomalies [including chromosomal defects], severe brain injury [i.e., intraventricular hemorrhage grade III/IV and post–hemorrhagic ventricular dilatation], and admission to the NICU after 48 hours of life.[194] Informed consent was asked shortly before expected discharge [around 30 weeks GA]. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and approved by the local medical ethical review board [MEC–2014–379]. Written parental informed consent was obtained prior to enrollment in the study.

LOCAL NUTRITIONAL PROTOCOL

All infants were treated according to our local nutritional protocol, which is based on the parenteral [2005] and enteral [2009] ESPGHAN guidelines.[48,55] In short, parenteral glucose administration was started directly after birth, with a minimum of 4.0 mg/kg/min and a maximum of 12 mg/kg/min. Amino acid administration [Primene 10%, Baxter, Utrecht, the Netherlands] was also started directly after birth at 2.4 g/kg/d, and increased to 2.9 g/kg/d the next day. The target dose of amino acids was 3.5 – 4.0 g/kg/d. Lipids were started on the day after birth [Intralipid 20% or SMOFlipid 20%, both Fresenius Kabi, Bad Homburg, Germany] at 1.4 g/kg/d, and increased to 2.8 g/kg/d on the next day. The target dose for lipids was 2.5 – 3 g/kg/d. Parenteral intake was gradually increased with ~20 ml/kg/d to reach the target of 160 – 180 ml/kg/d. At an enteral intake of 130 ml/kg/d, parenteral nutrition was ceased. Triglyceride and urea levels were monitored on regular basis. Parenteral amino acid administration was temporarily lowered if plasma urea concentrations were above 10 mmol/l, and interrupted when above 14 mmol/l. Similarly, lipid administration was temporary lowered when triacylglycerol concentrations were above 3 mmol/l and interrupted when above 5 mmol/l. Enteral bolus feeding was started on day 1, and increased daily according to our local protocol.[195] Only if expressed breast milk was insufficiently available, preterm formula was supplemented [Nenatal start, Nutricia Advanced Medical Nutrition, Zoetermeer, the Netherlands]. Breast milk fortification was started

at an enteral intake of 100 ml/kg/d [Nenatal Breast Milk Fortifier, BMF, Nutricia, Zoetermeer, the Netherlands].

DATA COLLECTION

Maternal, obstetrical, and neonatal data were collected from electronic medical records. Intake data were collected from the bedside Patient Data Monitoring System on 10 study days: day [d] 1 to 7, 14, 21, and 28 of life. These intake data included [1] parenteral and enteral nutritional intake of fluids, macronutrients and energy, and [2] fluid, carbohydrate and energy intake via intravenous drug administration if either continuously administered or if intermittent boluses exceeded 2 ml/day.

Intakes are expressed in ml/kg/d for fluids, in mg/kg/min for carbohydrates, in g/kg/d for amino acids and lipids, and in kcal/kg/d for energy.

As infants are born around the clock, duration of the "first day of life" ranges form 0 – 24 hours. Locally, daily planned changes in [nutritional] therapy are carried out at 4pm. Therefore, in infants born before 4pm, day 1 was defined as day of birth. In those born after 4pm, day 1 was defined as the period from birth until 11:59:59pm of the day after birth. Duration of day 1 thus ranged from 8 to 32 hours. To adjust to an intake per 24 hours, the following formula was used: $\frac{\text{cummulative intake day 1}}{\text{duration of day 1 [in hours]} \,/\, \text{24 hours}}$

Intakes were adjusted for observation duration at the day of discharge similarly.

In the first week of life, birth weight was used to calculate intake per kg. From day 14 onward, actual weight was used, defined as weight measured closest to the study day. The macronutrient and energy composition of the parenteral nutrition solutions and of formula feeding were based on the manufacturer's product information. For breast milk, the composition was based on the meta–analysis of Gidrewicz and Fenton. This meta–analysis takes into account postnatal age after birth, but not GA at birth.[196]

GROWTH

GA and sex–corrected Z scores for weight and head circumference were calculated using international growth charts at the following time points: birth, moment of maximal postnatal weight loss [weight dip], and transfer from the NICU to a high care center

[NICU discharge at or before day 28 of life] or at day 28 [NICU discharge after 28 days of life].[197,198] Length data were not available. Calculated growth parameters included days until regain of birth weight, day of weight dip, maximal weight loss as percentage of birth weight, and weight Z score change. Short–term weight growth rate was calculated using the following formula [exponential method]:

$$\frac{\left[1000 \times \ln \frac{\text{Final weight [grams]}}{\text{Birth weight [grams]}}\right]}{\text{[Day of final weight – Day of birth]}}$$ [199,200]

Growth retardation was defined as a decrease in weight Z score of ≥0.67 between two time points. Weight Z score change, weight growth rate, and growth retardation were all calculated for both the period between birth and discharge/d28 and between weight dip and discharge/d28.

DATA ANALYSIS

Data are presented as number [percentage] or median [interquartile range [IQR]]. A sample size calculation was not deemed necessary because of the descriptive nature of the study.

Fluid, energy and macronutrient intakes were compared with recommendations of the ESPGHAN guidelines, on which our local protocol is based. In the first week we compared nutrient intake with the parenteral nutrition recommendations, and from day 14 onward with the enteral nutrition recommendations [**Table 1**].[48,55] For each patient, adherence to the guideline was scored per nutritional component at each study day as below, within, or above the target, using a margin of 5% on the lower and upper limits. The growth target was defined as growth rate between 15 to 20 g/kg/d.

Because of the heterogeneity in our NICU population and the use of two different time points, possibly influencing our growth analyses, we a priori planned a sensitivity analysis. Here, we distinguished between infants with a short NICU stay [≤28 days, growth evaluation at transfer to a high care center] and long NICU stay [>28 days, growth evaluation at day 28 of life]. The latter group represents the most immature infants and those with the most severe complications, who may have received different intakes and may have grown differently. As we did not perform a sample size calculation on this, and

to prevent type 1 errors due to multiple testing, we mostly provide a descriptive overview of the data and performed only a few statistical tests [Mann–Whitney U test for non–parametric data]. Data were analyzed using Excel 2010 and SPSS package 21.0 [IBM SPSS statistics, Armonk, NY].

TABLE 1 | SUMMARY OF RECOMMENDATIONS OF ESPGHAN GUIDELINES IN PRETERM INFANTS

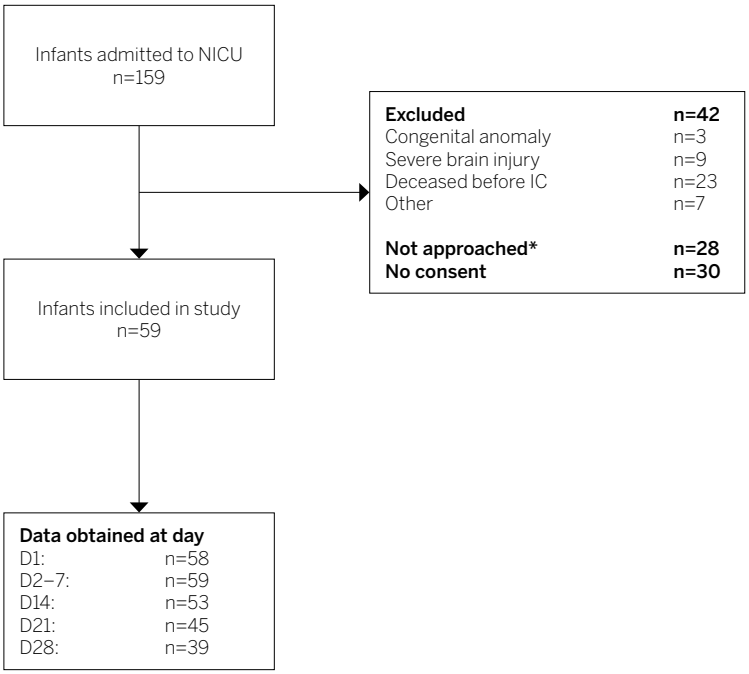
		Parenteral nutrition [2005] Day 1 – 7	**Enteral nutrition [2009] Day 14, 21, 28**
Fluid [ml/kg/d]	Day 1	80 – 90	135 – 200
	Day 2	100 – 110	
	Day 3	120 – 130	
	Day 4	130 – 150	
	Day 5	140 – 160	
	Day 6	160 – 180	
	Day 7	140 – 180	
Energy [kcal/kg/d]	Starting day	Day 1	110 – 135
	Dose	110 – 120, no increment scheme provided	
Carbohydrate [mg/kg/min]	Starting day	Day 1	8.05 – 9.20
	Dose	4 – 8, no increment scheme provided	
Amino acid [g/kg/d]	Starting day	Day 1	<1 kg: 4 – 4.5 1 – 1.8 kg: 3.5 – 4.0
	Dose	1.5 – 4, no increment scheme provided	
Lipids [g/kg/d]	Starting day	Start supply no later than 3rd day of life, but may start on day 1	4.8 – 6.6
	Dose	Maximum of 3–4, no starting dose, minimum dose, or increment scheme provided	

Abbrevations: ESPGHAN, European Society for Pediatric Gastroenterology Hepatology and Nutrition; ml, milliliter; d, day; [k]g, [kilo]gram; kcal, kilocalories; mg, milligram; min, minute.

RESULTS

Figure 1 illustrates the selection of the study population [n=59], which was not different from the target population [n=159] in sex, GA at birth, or birth weight [data not shown]. In **Table 2** the baseline and neonatal characteristics of the study population are shown.

FIGURE 1 | FLOW DIAGRAM OF ENROLLMENT IN STUDY



Legend: * Not approached due to language barrier or lost to follow–up.
Abbreviations: D, day; IC, informed consent; NICU; neonatal intensive care unit; N, number.

Actual intakes for all study days are presented in **Table 3**. In **Figure 2**, the intakes relative to the target ranges of the ESPGHAN guidelines are shown.

FLUID Median fluid intakes were above the target ranges on day 1 to 3, and within the target ranges thereafter. In the first days of life, over 50% had intakes outside the target ranges. At day 7, all intakes were within the target range, and nearly all [96 – 98%] from day 14 onward.

TABLE 2 | STUDY CHARACTERISTICS

	Study population [n=59]
Gestational age at birth [weeks^{+days}]	27^{+3} [25^{+6};28^{+4}]
Birth weight [grams]	920 [733;1193]
Sex [male]	36 [61%]
5' min APGAR score	7 [6;8]
Culture–proven sepsis	28 [47%]
Severe bronchopulmonary disease	11 [19%]
Surgical necrotizing enterocolitis	3 [5%]
Days on parenteral nutrition	11 [9;17]
Length of NICU stay [days][1]	43 [21;77]
Length of hospital stay [days][2]	89 [73;110]

Legend: Data are expressed in median [interquartile range] or n [%].
[1] 2 infants deceased before NICU discharge.
[2] 3 missing data.

CARBOHYDRATES Median carbohydrate intakes were within the target range during the entire first week, and above the target range thereafter. The carbohydrate intake of most infants was above the target from day 14 onward.

AMINO ACIDS Median amino acid intakes were within the target range on all study days. From day 14 onward, 9 – 34% of the infants had intakes below the target range. Of these infants, 45 – 65% still received parenteral nutrition.

LIPIDS Median lipid intakes were within or above the target ranges on all study days. From day 14 onward, 15 – 31% of the infants had intakes below the target range. Of these infants, 66 – 72% were still receiving parenteral nutrition.

ENERGY Median energy intakes were below the target range during the entire first week. None of the infants met the target intake before day 5. At day 28, 89% of the infants had intakes within or above the target range. All infants not meeting the recommendations after the first week still received parenteral nutrition, most as a consequence of surgical necrotizing enterocolitis [n=3].

TABLE 3 | COMBINED INTAKES OF PARENTERAL AND ENTERAL NUTRITION AND MEDICATION PER DAY

	Fluid [ml/kg/d]	Carbohydrates [mg/kg/min]	AA [g/kg/d]	Lipids [g/kg/d]	Energy [kcal/kg/d]
D1	96.4	4.9	2.3	0.09	38.9
[n=58][1]	[88.5;112.9]	[4.3;5.4]	[2.1;2.4]	[0.03;0.14]	[35.3;41.6]
D2	113.3	5.2	2.7	0.88	48.9
[n=59]	[95.7;122.8]	[4.4;6.0]	[2.5;2.9]	[0.66;1.11]	[44.9;55.5]
D3	133.1	5.6	3.2	2.4	67.4
[n =59]	[116.8;141.4]	[4.9;6.8]	[2.8;3.4]	[2.1;2.7]	[63.4;76.7]
D4	149.3	6.3	3.4	2.9	77.8
[n=59]	[134.3;158.2]	[5.3;6.8]	[3.1;3.8]	[2.4;3.8]	[71.3;86.1]
D5	156.8	6.7	3.6	3.0	82.8
[n=59]	[149.1;162.1]	[6.1;7.6]	[3.1;4.0]	[2.3;3.8]	[72.0;93.0]
D6	160.6	7.2	3.7	3.3	86.2
[n=59]	[153.8;167.5]	[6.4;8.0]	[3.3;4.1]	[2.7;3.9]	[79.1;98.2]
D7	160.2	7.5	3.9	3.4	90.3
[n=59]	[153.9;166.5]	[6.4;9.0]	[3.2;4.3]	[2.8;3.8]	[82.2;104.2]
D14	158.7	10.9	3.9	5.0	123.3
[n=53]	[148.2;167.7]	[8.7;12.2]	[3.4;4.1]	[4.2;5.6]	[106.6;137.0]
D21	155.0	13.0	3.8	5.3	137.9
[n=45]	[147.8;162.3]	[10.4;13.8]	[3.5;4.2]	[4.7;5.5]	[119.6;144.7]
D28	150.0	13.3	4.2	5.2	141.2
[n=39][2]	[144.0;160.5]	[11.3;13.7]	[3.8;4.7]	[4.9;5.4]	[128.6;146.5]

Legend: Data are presented in median [interquartile range].
[1] One missing case [outborn patient].
[2] One infant was readmitted to our NICU.
Abbreviations: Ml, milliliter; [k]g, [kilo]gram; D, day; mg, milligram; AA, amino acids; kcal, kilocalories.

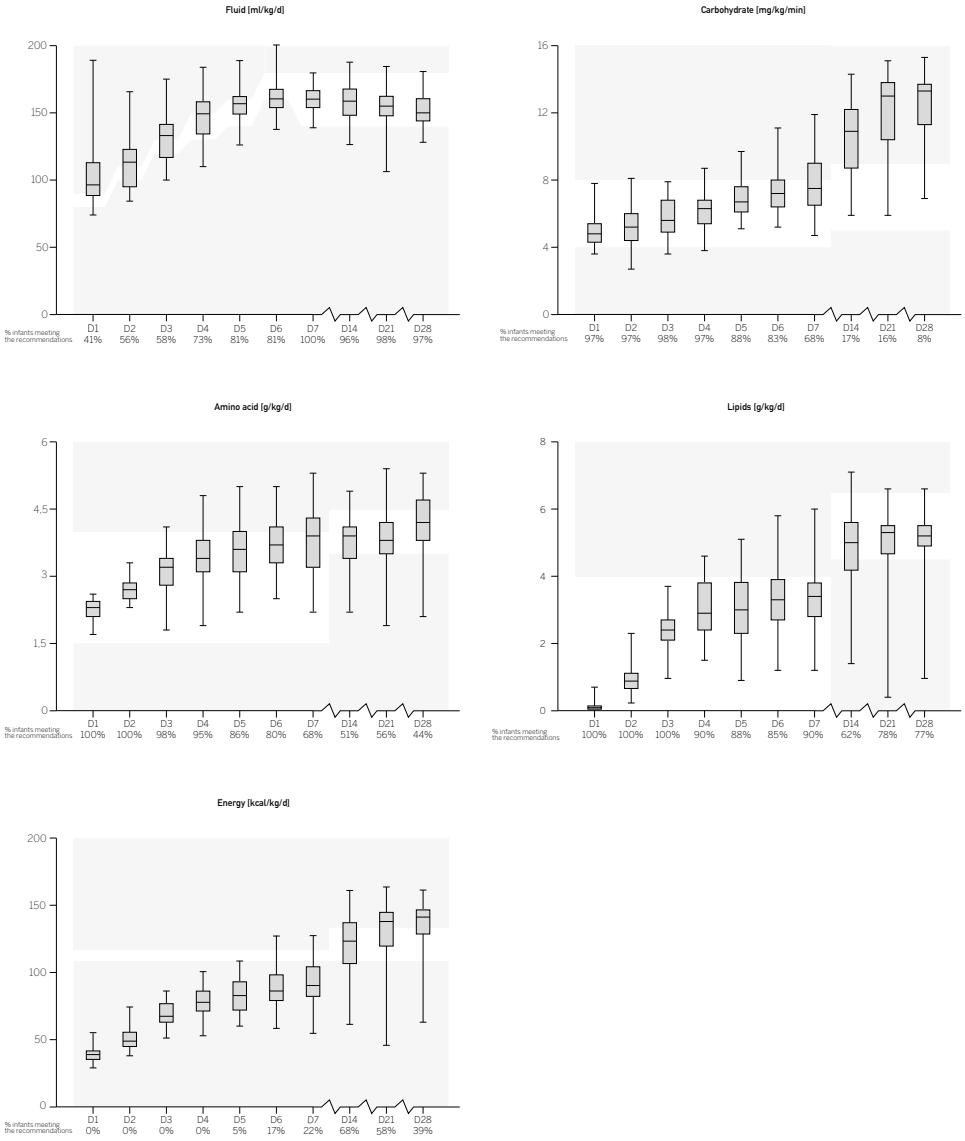
GROWTH Overall growth data are presented in **Table 4** and **Figure 3**. Both weight and head circumference Z score declined more than 0.67 Z score during NICU stay. Birth Z score was not regained at discharge home [**Figure 3A**]. Median weight dip relative to birth weight was 10.4%, and reached on median day 4. Weight at NICU discharge/d28 was 129% higher relative to birth weight, and 145% higher relative to dip weight [**Figure 3B**]. Median growth rate between birth and discharge/d28 was 9.5 g/kg/d [6.3; 13.3]. Growth retardation was present in 39 infants [66%]. Eight [14%] infants met the growth targets of 15 – 20 g/kg/d, when evaluated between birth and discharge/d28. Evaluation of growth between weight dip and discharge/d28 resulted in a higher median growth rate [18.1 g/kg/d [IQR 15.5; 21.1]], and the majority of the infants [n=46, 78%] meeting the growth targets.

SENSITIVITY ANALYSIS

The infants with a short NICU stay [≤28 days] were more mature at birth [median GA 28^{+3} [IQR 27^{+5};29^{+0}] than the infants in the long-stay group [median GA 26^{+1} [IQR 24^{+6};27^{+5}]]. Small differences in amino acid and lipid intake [higher in the short–stay group], and in carbohydrate, energy, and fluid intake [higher in the long–stay group] were found in the first days of life [data not shown].

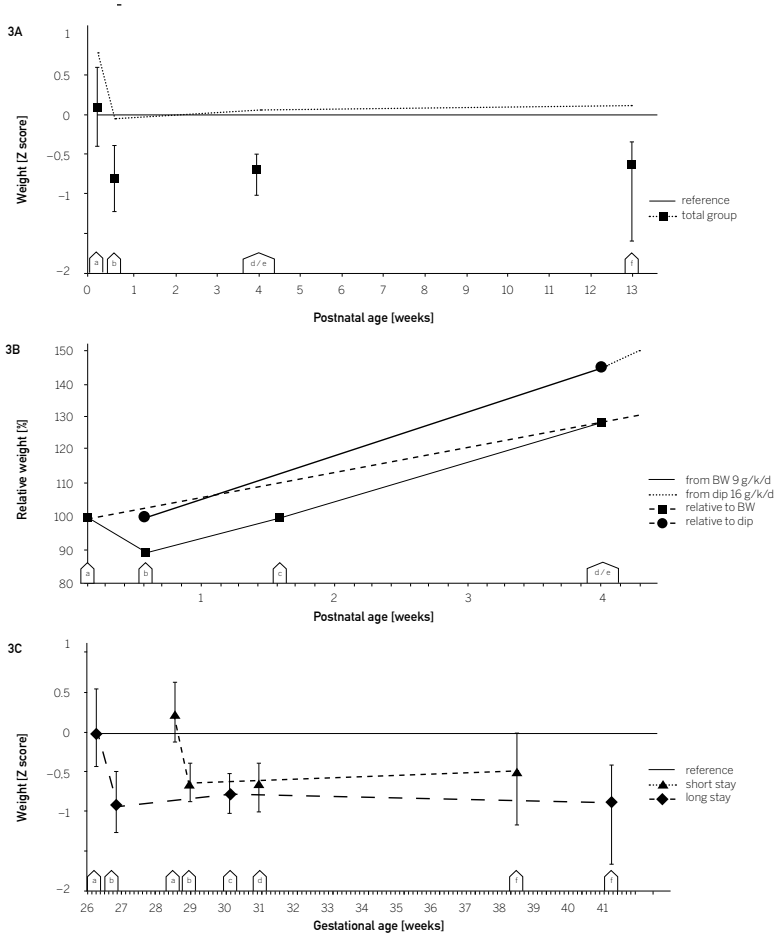
The groups showed a similar pattern of Z score decline between birth and dip, and hardly any regain in Z score between dip and discharge home [**Figure 3C**]. In the short–stay group, the dip was 1 day later than in the long–stay group [median day 5 versus day 4]. The weight dip was also deeper in the short–stay group, with a median weight loss of 12% relative to birth weight, compared to 10% in the long–stay group [p=0.033]. The growth rate calculated between birth and transfer was lower in the short–stay group [5.2 g/kg/d] than in the long–stay group [11.1 g/kg/d, p<0.00]. When evaluated from dip onward, median growth rates were similar in both groups, with 18.0 g/kg/d in the short–stay and 18.2 g/kg/d in the long–stay group [p=0.79].

FIGURE 2 | MACRONUTRIENT INTAKE



Legend: Boxplots of median [interquartile range and 95 percent interval] fluid, macronutrients, and energy intake on day 1 to day 7, day 14, 21, and 28, and percentages of infants meeting the recommendations per day. The white areas represent the recommendations of the ESPGHAN guidelines.
Abbreviations: D, day; [k]g, kilogram; min, minute; %, percentages.

FIGURE 3 | GROWTH OUTCOMES



Legend: Three figures showing growth measures of preterm infants at several time points during hospital stay: birth [a], weight dip [b], transfer from NICU to high care hospital [c], day 28 [e] and/or discharge home [f].

3A. Weight during hospital stay of the total study population [N=59].
On the x–axis is depicted the postnatal age in weeks with the time points and on the y–axis the weight Z score [sex and gestational age adjusted, Fenton growth charts[22]]. The vertical bars represent the interquartile ranges, the solid line the reference [Z score = zero].

3B. Two different calculations of growth velocity in the first month in the total study population.
On the x–axis is depicted the postnatal age in weeks with the time points, and on the y–axis the percentage of weight change, relative to starting weight [100%]. The squares depict the percentages weight relative to weight at birth, with the dashed line showing the growth velocity. The solid line with the circles starts at the weight dip and represents the weight gain relative to dip weight.

3C. Weight during hospital stay in patients with short [≤28 days] and long [>28 days] NICU stay.
On the x–axis are depicted gestational ages in weeks and the time points and on the y–axis the weight Z score is shown. The Z scores are depicted separately for the short–stay [n=22, triangles] and the long–stay group [n=39, diamonds] at the different time points. The vertical bars represent the interquartile ranges, the solid line the reference [Z score = zero].

TABLE 4 | GROWTH OUTCOMES

	N=59
Regain of birth weight [day]	11 [9;13][a]
Maximal weight loss [%]	10.4% [8.4;14.0]
Weight Z score at birth	0.09 [−0.40;0.62]
Weight Z score at discharge/d28	−0.7 [−1.0;−0.5]
Weight Z score change between birth and discharge/d28	−0.8 [−1.1;−0.5]
HC Z score at birth[b]	−0.08 [−0.69;0.48]
HC Z score at discharge/d28[b]	−0.9 [−1.6;−0.6]
Growth retardation during hospital stay[c]	39 [66%]
Growth rate [g/kg/d]	9.5 [6.3;13.3]

Legend: Data are presented in median [interquartile range] or n[%]. [a]3 missing cases, [b]2 missing cases, [c] growth retardation was defined as decrease of ≥0.67 in weight Z score from birth to discharge/d28.
Abbreviations: HC, head circumference; d, day.

DISCUSSION

In this study, we showed that in preterm infants admitted to a tertiary NICU adherence to macronutrient recommendations of current guidelines was good in the first month of life. Despite this, energy targets were not met before day 5 of life. Growth targets were achieved in the majority of the infants, but only when evaluated from postnatal weight dip onward. Overall, the first week [nearly] 1–point drop in Z score was not regained during hospital stay. These findings underline the importance of nutritional intake and growth, especially duration and amount of weight loss, in the first days of life for short–term growth outcomes after preterm birth.

The only nutritional component that was below the targets in the first days of life was energy. This resulted in a cumulative energy deficit of ±266 kcal/kg after the first week of life. This deficit may have influenced duration and amount of weight loss, and the poor growth outcomes, as each gram growth costs approximately 5 kcal. A deficit of 266 kcal/kg results in a potential growth loss of 266/5 = 53 g/week, or 7.5 g/day in an infant of 1000 g – equal to the difference between the actual and the recommended growth rates when evaluated from birth onward. Moreover, previous studies, using stable isotopes, showed that for adequate growth at least 100 kcal/kg/day are needed.[201]
Over the last 10 years whilst there has been more understanding of protein and other nutrient needs, optimal energy intakes for preterm infants have not been determined yet. A few studies attempted to determine the optimal protein:energy ratio, and only some have examined the effect of lipid compared to carbohydrate intake as energy source on growth.[202]
Prevention of energy deficits is difficult in clinical practice. First, because of the limitation in fluid intake, as excessive fluid intake may have serious side effects such as necrotizing enteral colitis and bronchopulmonary disease.[203] Second, higher concentrations of glucose solutions can be used in preterm born infants, but only with central venous catheters in place.[204] In those without central venous access, solutions above 1000 mOsm/L are generally regarded unsafe, limiting the possibility to increase energy intake while respecting fluid targets.[205,206] The third option is to start parenteral lipid administration immediately after birth, which is likely safe and effectively increases energy intake.[53,207] The ESPGHAN guideline states that lipid supply may be started immediately after birth, but should be initiated no later than on the third day of life. In

our study, parenteral lipid administration was started on the second day of life in the majority of the infants, partly explaining the energy deficit.

We observed that growth targets of 15 – 20 g/kg/d are feasible during the first month of life, but only when evaluated from weight dip onward. Our data show that this early weight loss greatly influences growth outcomes in early life, of which we know that they are important for long–term health and development.[208] The weight dip is mainly the result of the infants' adaptation to extra–uterine life by losing fluid. In term born infants, up to 10% weight loss in the first days of life is considered physiological. Studies on postnatal weight loss in preterm infants report losses of 5 to 15%.[209, 210] Based on current literature we do not know to what extend postnatal weight loss should be considered physiological in preterm infants. Early growth capacity may be limited by the low levels of insulin–like growth factor type 1 [IGF 1] during the stage of adaptation from placental nutrient supply to enteral nutrient uptake in preterm infants.[64] As early weight loss largely determines growth outcomes in the first month of life, it deserves more attention in research and in clinical practice.

The sensitivity analyses showed only small differences in nutritional intake between infants with a short and long NICU stay. The differences observed are largely explained by our local protocol, and criteria for central venous catheters insertion. Growth also differed slightly between the groups. The weight dip was deeper, and occurred later in infants with a short NICU stay. As a consequence, poorer growth outcomes were observed in the short–stay group, which is probably the result of the shorter observation period. Interestingly, both groups showed a similar growth pattern with a Z score decline between birth and weight dip, and stabilization thereafter until discharge home. These findings support more attention for nutritional practices in all preterm infants, regardless of their gestational age at birth.

Previous studies evaluating adherence to the guidelines obtained different growth results. We note that comparison of studies on growth and nutrition is complex. First, because different definitions are used to describe growth, as recently discussed by Fenton et al.[198] Second, varying inclusion criteria hamper the comparability of study populations. Third, growth partly depends on the level of care and on local policies, even when centers use the same guidelines. Last, current guidelines are based on

scarce evidence and on consensus of experts in the field, and leave ample room for interpretation and local differences in practice.

We have addressed the following strengths and limitations of our study.
First, our study population may not represent the general preterm NICU population on our ward, although baseline characteristics were not different from the target population.Second, we used the macronutrient and energy composition as provided by the manufacturers and did not correct for bioavailability of enteral and parenteral nutrients or for individual variability in breast milk content. Third, in this study we only describe in–hospital growth from a quantitative perspective, without answering the question which growth pattern is most beneficial for long–term health. Current opinion of optimal growth is that 'quantity and quality of growth should be equivalent to fetal growth'. Quality of growth is not defined, and a scientific basis for the long–term benefits of fetal growth rates is lacking. Features of quality of growth considered relevant are, amongst others, markers of cardiometabolic health including body composition, neurodevelopment, and bone health. Previous cohort studies irrefutable showed that growth retardation and nutritional deficits in early life have harmful effects on development of the brain, but long–term outcome data of nutritional intervention trials are scarce.[211] To prevent growth retardation, early enhanced feeding was recommended in the latest guidelines. However, concerns have been expressed about the effects of early high amino acid administration on morbidity rates and brain development.[212 – 214] Concerns have also been expressed on the effect of rapid growth on later cardiometabolic health, but the exact magnitude has not been unraveled yet.[215]

Whether we should consider the early weight loss as an essential part of adaptation to extra–uterine life in preterm infants, or whether we should interfere more aggressively in the first days of life, needs further study in nutrition intervention studies with long–term follow up. In this exploration, the results of a recent study in sick term infants and children may provide new insights. That study showed short–term benefits of withholding parenteral nutrition during periods of stress, especially in neonates.[216] Whether this also counts for preterm infants deserves further study.

CONCLUSION

Despite general improvement of neonatal care and drastic changes in nutritional practices, poor growth outcomes remain common after preterm birth. The first week weight dip seems to be most relevant for growth outcomes in the first month of life. Future research should define optimal nutritional intake and optimal growth in the first week of life, with focus on energy intake and long–term health benefits.

6

RAPID GROWTH DURING HOSPITAL STAY: IS THERE AN ASSOCIATION WITH LATER BODY COMPOSITION IN PRETERM INFANTS?

In preparation

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ABSTRACT

BACKGROUND Rapid postnatal weight gain is beneficial for neurodevelopment of preterm infants. Concerns are raised however on the influence of rapid weight gain on fat mass gain in early life and cardiometabolic health on the long term. In this study, we evaluated the association between postnatal weight gain and body composition in infancy in preterm infants.

METHODS In a prospective cohort study, we included preterm infants born before 30 weeks of gestation without major congenital anomalies or severe brain injury, admitted to the neonatal intensive care unit [NICU]. Growth trajectories of weight Z scores were estimated by using linear mixed models over four time periods: from moment of maximal weight loss postnatally [1] until transfer from the NICU to a secondary hospital [in–NICU growth]; [2] until discharge home [in–hospital growth]; [3] until 6 weeks corrected age [ca]; and [4] until 6 months ca. We evaluated the association between these growth trajectories and relative fat mass [%FM], absolute fat mass, and absolute lean mass measured at 6 weeks and 6 months ca.

RESULTS In 117 infants, 181 body composition measurements were performed; 101 at 6 weeks and 80 at 6 months ca. Median %FM was 21.9% at 6 weeks and 20.3% at 6 months ca. Growth after hospital stay was most strongly associated with body composition in infancy. In–NICU growth was significantly associated with increased absolute lean mass in infancy, explaining 4.7% of the variance in lean mas at 6 weeks ca. Higher in–hospital weight gain was positively associated with %FM, explaining 6% of the variance in %FM at 6 weeks ca.

CONCLUSIONS In–hospital growth of preterm infants was significantly associated with body composition in infancy. Although the overall effect was mainly on lean mass, faster weight gain after discharge to a secondary hospital was also associated with increased fat accumulation, resulting in increased %FM. The long–term relevance of these findings for cardiometabolic health needs further study.

INTRODUCTION

Preterm infants are born in a critical period for growth and development and they are at high risk of postnatal growth retardation.[50,182] Aggressive nutritional practices are recommended in early life, to accomplish rapid growth after the initial period of growth retardation.[48,56] The last years, however, evidence obtained in human and animal studies raised concerns on the influence of high nutritional intake and rapid growth on cardiometabolic health of preterm infants.[217]

The underlying theory is covered in the Developmental Origins of Health and Disease [DOHaD] paradigm, stating that after a period of nutritional deprivation, stress, or inflammation, an environment much richer in oxygen and nutrients can result in an increased risk of an adverse cardiometabolic health.[10] This had led to a debate on which growth pattern should be aimed for: do the neurodevelopmental benefits of rapid growth outweigh the potential risk of adverse cardiometabolic consequences in childhood and adulthood?[68]

To answer this question, more knowledge should be obtained on the period during which rapid growth is harmful for cardiometabolic health of preterm infants.[217] Although in term born infants the first three months of life have been identified as most critical, in preterm infants this is not clear yet. Some studies showed that rapid growth in the first years after preterm birth is beneficial for both neurodevelopment and cardiometabolic health, while others report harmful effects of rapid growth in the first years of life on markers of cardiometabolic health in childhood and adulthood.[79,218]

Body composition in early life is considered a marker of cardiometabolic health and can be measured reliably in the outpatient setting.[13] The human body consists of absolute lean and fat mass, which increase with increasing weight. Based on lean and fat mass, relative fat mass can be determined, which is the amount of absolute fat mass relative to weight.Physiologically, fat accumulation starts in the third trimester, increasing the relative fat mass until a plateau is reached around 3 – 6 months after term age.[5,219]

In this study, we aimed to gain more insights in the association between postnatal weight gain in the first months of life and body composition of preterm infants in infancy.

We expected that the later you evaluate growth, the stronger the association with body composition would be, but that also the early postnatal periods are important for body composition in infancy. Additionally, we hypothesized that increased weight gain during hospital stay is associated with higher relative fat mass due to decreased lean mass rather than to increased fat mass accumulation.

METHODS

In this prospective cohort study we assessed the association between weight gain in the first months of life and body composition measured at 6 weeks and 6 months corrected age [ca]. To identify during which time periods postnatal growth is most strongly associated with relative fat mass [fat mass relative to weight, often referred to as adiposity], we distinguished between the period of neonatal intensive care unit [NICU] stay, the period of secondary hospital stay, and the periods at home until the 6 weeks and 6 months visits. These time periods were chosen based on differences in settings [NICU, secondary hospital, home] and related nutritional practices [respectively parenteral nutrition and tube feeding, increasing bottle or breast feeding, and self–drinking with decreasing enrichment].

SETTING

This prospective observational cohort study was conducted at the level IV NICU and the outpatient clinic of the Erasmus MC – Sophia Children's Hospital, Rotterdam, the Netherlands, between September 2014 and April 2017. Infants born before 30 weeks of gestation who were admitted to our ward within 48 hours after birth were eligible for inclusion in the study. Exclusion criteria were congenital anomalies [including chromosomal defects] that may interfere with growth, severe brain injury [i.e., intraventricular hemorrhage grade III/IV and posthemorrhagic ventricular dilatation requiring lumbar punctures], congenital infections, and perinatal asphyxia [umbilical cord pH < 7.00 and APGAR score at 5'min < 5]. The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and was approved by the local ethical review board [MEC–2014–379]. Written parental informed consent was obtained before inclusion in the study.

LOCAL NUTRITION PROTOCOL

During NICU stay, all infants were treated according to our local protocol which is based on the parenteral and enteral ESPGHAN guidelines.[48,55] In short, parenteral glucose administration was started directly after birth, with a minimum of 4 and maximum of 12 mg/kg/min. Amino acid administration was also started directly after birth at 2.4 g/kg/d and gradually increased to a target dose of 3.5 – 4.0 g/kg/d. Lipids were started the day after birth at 1.4 g/kg/d and gradually increased to a target dose of 2.5 – 3 g/kg/d. Parenteral nutrition was ceased at an enteral intake of 130 ml/kg/d.

Enteral bolus feeding was started on the day of birth and increased daily according to our local protocol.[220] Expressed breast milk was the first choice of enteral feeding. If not [sufficiently] available, preterm formula was supplemented [Nenatal start, Nutricia Advanced Medical Nutrition, Zoetermeer, the Netherlands]. Breast milk fortification was started at an enteral intake of 100 ml/kg/d [Breast Milk Fortifier, Nutricia Advanced Medical Nutrition, Zoetermeer, the Netherlands].

CLINICAL DATA

Maternal characteristics and obstetrical and neonatal data were prospectively collected. Gestational age was calculated on the basis of the first day of the last menstrual period or the first trimester ultrasound. Postnatal age was defined as days after birth, with the day of birth corresponding with day 1. Corrected age was postnatal age adjusted for prematurity. At each visit, parents filled out a questionnaire on their infants' feeding practices to collect data on type of feeding, fortification, and complementary feeding. Socio–economic status was expressed in ZIP code based SD scores which are calculated based on local average income, low–income rate, low educational level rate, and unemployment rate.[221]

GROWTH

Body weight, head circumference, and length measurements were performed according to local protocols. Body weight was measured on an electronic scale to the nearest gram. Head circumference and length measurements were performed in a standardized way by using a tape measure and an Infantometer [Seca], respectively. Weight and head circumference were measured from birth onward as part of standard care in all infants, length measurements were only measured after transfer from the NICU to a secondary hospital.

Gestational age and sex–corrected Z scores for weight, head circumference, and length were calculated at the following time points: birth, initial weight dip [maximal weight loss postnatally], 30 weeks of gestation, NICU transfer, discharge home, and at both outpatient clinic visits [6 weeks and 6 months ca]. As recommended by Cormack et al., Z scores were based on the Fenton growth charts from birth until discharge home, and on the World Health Organization [WHO] growth charts thereafter.[171,197,222] The WHO Z scores were adjusted for prematurity.

Calculated growth parameters included day of maximum weight loss, maximum weight loss as percentage of birth weight, and days until regain of birth weight. We defined a decrease in Δ Z score of >0.67 Z score as growth retardation and an increase of >0.67 Z score as catch–up growth.[223]

OUTPATIENT CLINIC VISITS

As part of our standard follow–up program, preterm infants born before 30 weeks of gestation visited our outpatient clinic for medical and neurodevelopmental assessment at 6 weeks and 6 months ca. Body composition was measured at those visits by using the validated method of air displacement plethysmography [PEA POD®, Infant Body Composition System, COSMED].[96,130] By direct measurements of body volume and body mass, fat mass as percentage of total body weight [%FM] and absolute fat and lean mass are estimated.

Due to changes in national policies, the 6 weeks visit was shifted to 12 weeks ca in infants born after 1st of January 2017 [n=12] and visits of those born above 28 weeks of gestation could take place in the secondary hospital.

STATISTICAL ANALYSES

Descriptive data are expressed as median [interquartile range [IQR]] or number [percentage] of observations.

First, growth trajectories were estimated by using linear mixed models. Because infants experience weight loss in the first days of life, the growth trajectory was estimated from moment of maximum weight loss onward [median day 5]. We estimated four growth trajectories for weight Z score from the moment of weight dip until: [1] transfer to a secondary hospital [in–NICU growth]; [2] discharge home [in–hospital growth]; [3] first body composition measurement around 6 weeks ca [6 weeks growth]; and [4] second body composition measurement around 6 months ca [6 months growth]. The growth trajectories were estimated by using weight Z score [dependent] as the response over time with postnatal age at weight examination as covariate. No transformation was needed to make the residuals approximately homoscedastic and the relationship with postnatal age approximately linear. The random intercept and slopes were entered as subject–specific effects, reflecting individual growth trajectories for each infant. In the

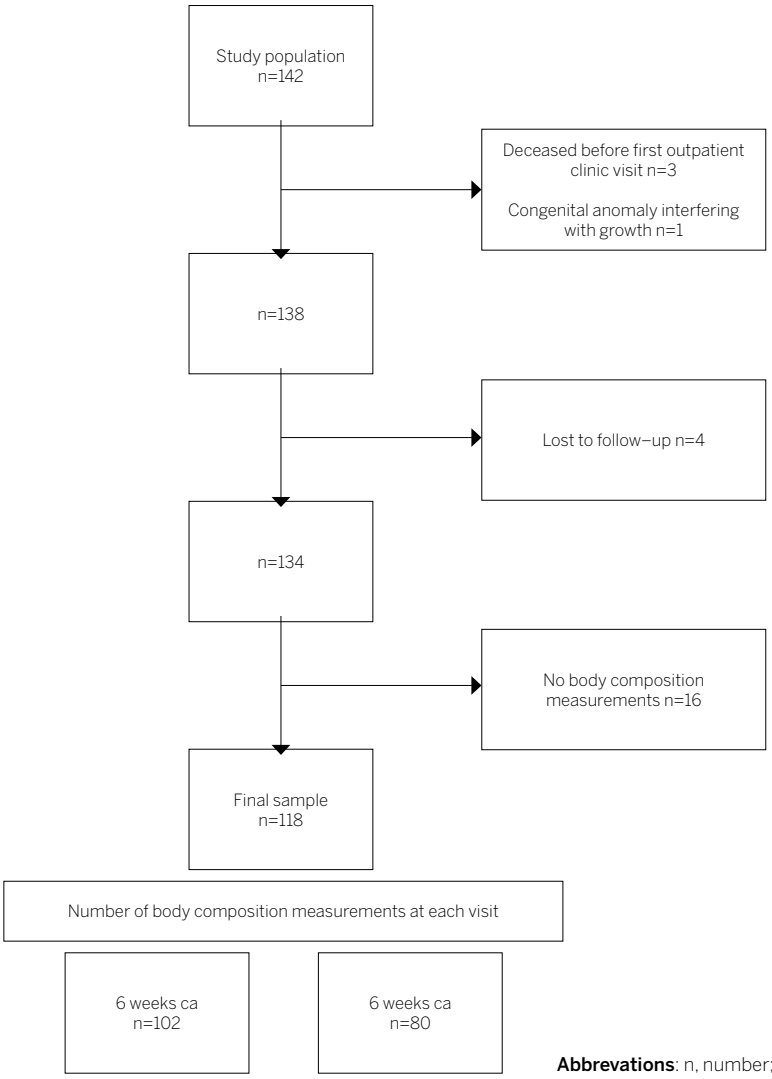
second step of the analysis, the subject–specific effects were used as covariates in the linear regression analyses with %FM and absolute fat and lean mass as outcome measures. The basic regression model included the subject–specific effects, sex, gestational age at birth, birth weight Z score, and corrected age at body composition measurement. The adjusted model included the covariates of the basic model and days on parenteral nutrition during NICU stay, days on mechanical ventilation, socio–economic status, and the use of any breast milk at the 6 weeks visit. To evaluate the contribution of the growth trajectories to all measures of body composition at 6 weeks and 6 months ca, we compared the explained variances of the model with and without including the subject specific–effects. The difference in explained variance can be contributed to the subject–specific effects of growth.

A 2–tailed p value <0.05 was considered statistically significant.
Analyses were performed using SPSS package 21.0 [IBM SPSS Statistics, Armonk, NY] and R [R: A language and environment for Statistical Computing, version 3.1.3, 2015 for Windows, R Core Team, Vienna, Austria].

RESULTS

In total, 142 infants were enrolled in the study. After excluding one infant with a congenital anomaly interfering with growth, three infants who deceased before discharge home, three with follow–up at another hospital, and 18 without body composition measurements, the study population consisted of 118 infants [**Figure 1**].

FIGURE 1 | FLOWCHART



Abbrevations: n, number; ca, corrected age.

BASELINE CHARACTERISTICS

The baseline characteristics of the study population are provided in **Table 1**. Median gestational age at birth was 27^{+5} weeks [interquartile range, 26^{+1};28^{+5}] with a birth weight of 1015 grams [800;1250]. Duration of parenteral nutrition [amino acid or lipid administration] during NICU stay was 10 days [8;16]. The majority of the infants [75%, n=88] received both own mothers milk and formula during NICU stay. The infants were transferred from the NICU to a secondary hospital at median postnatal age of 29 days [17;69], and were discharged home at a median postnatal age of 84 days [70;105].

GROWTH

Growth data during hospital stay are shown in **Supplemental Table 1**. Maximum weight loss of birth weight was 10.5% [7.8;13.6] which was reached on day 5 [3;6]. The majority of infants experienced growth retardation [weight and head circumference] during hospital stay, with catch–up rates starting to increase after discharge to a secondary hospital [**Figure 2 and Supplemental Table 2**]. Of the 117 infants, 102 [87%] had a body composition measurement at 6 weeks ca and 80 [81%] at 6 months ca [**Table 2**]. The %FM was within the same range at 6 weeks and 6 months ca.

ASSOCIATION BETWEEN GROWTH AND BODY COMPOSITION

The results of the regression analyses are presented in **Supplemental Table 3** [basic model] and **Table 3** [fully adjusted model]. In NICU–growth was positively associated with lean mass at 6 weeks of gestation in both the basic and fully adjusted model, and explains 4.7% of the variance in lean mass at 6 weeks ca. No association was observed between in–NICU growth and absolute fat mass and %FM. The association between in–NICU growth and absolute lean mass at 6 months ca was not statistically significant. In–hospital growth showed an association with all components of body composition at 6 weeks ca: a higher weight gain was associated with increased absolute lean mass, absolute fat mass, and %FM, with explained variances of 10.2, 10.8, and 6.3% respectively in the adjusted models. At 6 months ca, we only observed an association between in–hospital growth and absolute lean mass [explained variance 5.9%].

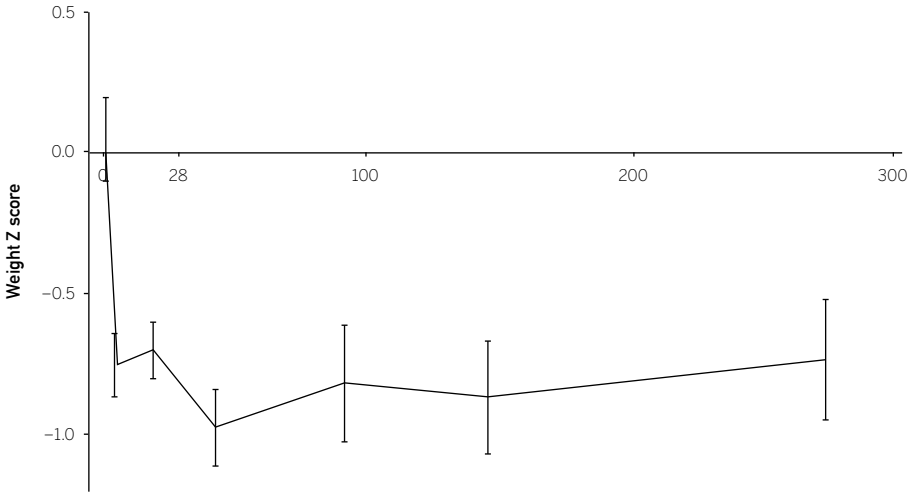
Growth up to 6 weeks ca was strongly associated with all measures of body composition at 6 weeks ca, but not at 6 months ca. At 6 months ca we observed an association with absolute lean and fat mass. Positive associations were also found between growth up to 6 months ca and all measures of body composition: a higher weight gain was associated with absolute lean mass, absolute fat mass, and %FM.

TABLE 1 | MATERNAL AND INFANT BASELINE CHARACTERISTICS

N=118		N=118	Missing
Maternal characteristics			
Age at delivery	[years]	30 [27;34]	0
Prepregnancy BMI	[kg/m^{2}]	24.8 [21.8;29.1]	13
Pregnancy complications	[G]DM	7 [6%]	0
	Hypertension[1]	8 [7%]	0
	PE/HELLP	20 [17%]	0
	IUGR[2]	22 [19%]	4
	PPROM	24 [20%]	0
Singleton		92 [78%]	0
Antenatal corticosteroids	0/1/2 doses	7/33/78 [6/28/66%]	0
Caesarean section		69 [59%]	0
	fetal distress	58 [49%]	0
Infant characteristics			
Sex	[male]	73 [62%]	0
GA at birth	[weeks^{+days}]	27^{+5} [26^{+1};28^{+5}]	0
Birth weight	[grams]	1015 [800;1250]	0
	[Z score]	0.1 [−0.4;0.7]	0
Apgar	[5' min]	8 [6;9]	1
pH umbilical cord		7.31 [7.26;7.36]	10
SNAPPE−II score		27 [14;39]	2
Culture−proven sepsis		41 [35%]	0
	early onset[3]	3 [3%]	0
	late onset	38 [32%]	0
NEC	Bell stage ≥2	5 [5%]	0
Treated PDA		38 [32%]	0
BPD[4]	mild	27 [23%]	0
	severe	17 [14%]	0
Postnatal steroid use		20 [17%]	4
Brain injury[5]		36 [31%]	0
Treated ROP[10]		6 [5%]	0
Mechanical ventilation[11]	[days]	2 [0;11]	0
NICU stay	[days]	29 [17;69]	0
GA at NICU transfer	[weeks^{+days}]	32^{+0} [30^{+3};36^{+2}]	0
Hospital stay	[days]	84 [70;105]	3
GA at discharge home	[weeks^{+days}]	39^{+5} [38^{+0};41^{+5}]	3

Legend: All data are expressed in median [interquartile range] or number [percentages]. [1] Either pre−existent or pregnancy induced; [2] Estimated fetal weight or abdominal circumference below 10th percentile on Robinson curve; [3] Positive blood culture within 72h after birth; [4] BPD: >28 days O2 + X−ray abnormalities, severe BPD: endotracheal or CPAP at 36 weeks of gestation or >30% fiO2 or >1L/min flow via nasal prongs; [5] Brain injury includes IVH gr I/II, cerebellar bleeding, arterial/venous stroke, periventricular leukomalacia and convulsions; [6] Endotracheal mechanical ventilation.
Abbreviations; n, number; IQR, interquartile range; BMI, body mass index; [G]DM, [gestational] diabetes mellitus; PE, pre−eclampsia; IUGR, intra−uterine growth retardation; PPROM, preterm premature rupture of membranes; GA, gestational age; CRIB, clinical risk index for babies; NEC, necrotizing enterocolitis; PDA, patent ductus arteriosus; BPD, bronchopulmonary disease; ROP, retinopathy of prematurity.

FIGURE 2 | WEIGHT Z SCORE COURSE FROM BIRTH TO 6 MONTHS CORRECTED AGE



Legend: on the x–axis are depicted postnatal age in days, on the y–axis the weight Z score.

TABLE 2 | GROWTH AND NUTRITIONAL PARAMETERS AT BODY COMPOSITION MEASUREMENTS

		6W – visit	6M – visit
N		**117 [99%]**	**99 [84%]**
Corrected age	[days]	52 [45;67]	185 [177;195]
Postnatal age	[days]	136 [128;161]	273 [260;287]
Weight	[kg]	4.68 [4.14;5.21]	7.08 [6.45;7.66]
	[Z score]	−0.9 [−1.6;−0.1]	−0.7 [−1.5;0.0]
Head circumference	[cm]	38.6 [37.5;40.0]	43.2 [42.0;44.2]
	[Z score]	0.2 [−0.7;0.9]	0.2 [−0.6;1.0]
Length	[cm]	55.2 [53.4;57.1]	66.4 [64.0;68.5]
	[Z score]	−1.1 [−1.9;−0.3]	−0.2 [−1.1;0.5]
Body composition measurement		102 [87%]	80 [81%]
Relative FM	[%]	21.9 [17.8;23.8]	20.3 [17.9;23.3]
Absolute FM	[kg]	0.98 [0.77;1.28]	1.43 [1.12;1.68]
Absolute FFM	[kg]	3.69 [3.33;4.02]	5.61 [5.10;6.06]
Feeding type[1]	Own mothers milk	19 [16%]	5 [4%]
	Formula feeding	76 [64%]	88 [75%]
	Mixed feeding	22 [19%]	3 [3%]
Enriched nutrition[2]		24 [20%]	5 [4%]
Tube feeding		16 [14%]	3 [3%]
Parenteral nutrition		2 [2%]	1 [1%]
Oxygen supply		9 [8%]	3 [3%]

Legend: [1] 1 missing feeding data at 6 weeks and 3 at 6 months ca; [2] Either preterm formula or fortified human milk enriched with extra protein, or with fat or carbohydrates [rare].
Abbreviations: kg, kilograms; cm, centimeters; %, percentage; FM, fat mass; FFM, fat free mass.

TABLE 3 | ASSOCIATION BETWEEN GROWTH [WEIGHT GAIN] AND BODY COMPOSITION AT 6 WEEKS AND 6 MONTHS CA [FULLY ADJUSTED]

	6 weeks [n=101]			6 months [n=79]		
	%FM	Absolute FM	Absolute LM	%FM	Absolute FM	Absolute LM
In–NICU	–	–	4.7%**	–	–	2.0%#
In–hospital	6.3%*	10.8%***	10.2%***	–	–	5.9%**
Up to 6 weeks ca	34.5%***	48.9%***	24.1%***	–	10.2%**	10.9%***
Up to 6 months ca	NA	NA	NA	27.9%***	47.9%***	27.3%***

Legend: The variance [%] in body composition explained by early growth trajectories is presented. In the regression analyses, we adjusted for gestational age at birth, birth weight Z score, sex, corrected age at body composition measurement, days on parenteral nutrition during NICU stay, days on invasive respiratory support, socio–economic status, and breast milk at 6 weeks corrected age [any/no].
Symbols: – indicates p value >0.1; # p value between 0.5 – 0.1; * p value between 0.5 – 0.01; ** p value between 0.01 and 0.001; *** p value <0.001.
Abbreviations: %FM, percentage fat mass relative to weight; FM, fat mass; LM, lean mass; NA, not applicable; ca, corrected age.

DISCUSSION

In this prospective cohort study in very preterm infants we studied the association between postnatal weight gain and body composition in infancy. As expected, we found that the effect of growth on later body composition was larger with smaller time intervals between the evaluated growth trajectory and body composition measurement. Still, we observed that in–NICU growth was associated with lean body mass at 6 weeks corrected age, which was median 15 weeks later. Evaluating the subsequent trajectories including growth in the secondary hospital and at home, we found an association with absolute and relative fat mass: higher in–hospital and home weight gains are associated with increased relative fat mass in infancy.

We focused on growth during hospital stay as this is likely easier to manipulate by changing intake in infants with parenteral and tube feeding, than in those who are self–drinking at home. Moreover, we hypothesized that programming mainly takes place in this early period, and may therefore be more important for later health.[10] We found that in–NICU growth was only positively associated with lean mass, and not with fat mass at 6 weeks ca. In–hospital growth including the secondary hospital stay, however, was not only associated with absolute lean mass, but higher weight gain was also associated with increased relative fat mass, explaining 6% of the variance in relative fat mass at 6 weeks ca. Unlike our hypothesis, this was the result of increased fat mass, and not of decreased lean mass.
At 6 months, the effects already seem more diluted: both in–NICU and in–hospital growth are only associated with absolute lean mass, and even growth up to 6 weeks ca shows little association with fat and lean mass. Growth up to 6 months ca again showed the strongest association with all components of body composition at 6 months ca.

Previous studies looking at the association between growth and body composition in preterm infants did not evaluate growth over different time periods, and nearly all focused on body composition at discharge, not on later effects. Simon et al. showed in 141 infants born before 35 weeks of gestation that weight Z score change between day 5 [moment of weight dip] and discharge was not associated with the risk of being in the upper %FM tertile at discharge home. This is in contrast to our findings with body composition measurement in infancy. This may be explained by differences in inclusion

criteria [<35w versus <30w gestational age], methodology [different growth charts], feeding practices, and timing of body composition measurement [37 weeks versus 47 weeks gestational age].[86] Other studies found results more similar to ours. Trembley et al. included infants more similar to our population [born <29 weeks, no severe brain injury or chromosomal abnormalities] and they also found a positive association between in–hospital weight gain and lean mass at discharge.[88] A study of Griffin and Cooke also observed a positive association with relative fat mass at discharge and in infancy, which is in accordance with our findings at 6 weeks ca.[79]

All associations found were positive, indicating that higher weight gain results in increased absolute lean and fat mass deposition. Only from around 32 weeks of gestation [after transfer from the NICU to a secondary hospital] higher weight gain resulted in a higher relative fat mass. Thus, in–NICU growth and relative fat mass, or adiposity, were not clearly related yet. An explanation might be found in the biological fetal development with in–utero fat accumulation starting in the third trimester.[5] This corresponds with the period of secondary hospital stay in our study. Our data indeed support the theory that preterm infants merely mimic this physiological pattern of growth in–utero; with in–NICU growth primarily relating to lean mass gain, and the period after NICU stay to both fat and lean mass growth.[215,224] Another explanation is that shortly after birth, preterm infants need all the energy they have for maturation of organs, breathing, and adaptation to the extra–uterine environment. Since providing adequate amounts of energy is challenging and may be insufficient in the first period of postnatal life in preterm infants, all calories will be used, and little will be stored as fat.

Cautiously extrapolating our findings to clinical practice, it suggests that rapid growth in the first week of life after the initial period of weight loss results in increased lean mass gain, which may be beneficial for both neurodevelopmental outcome and cardiometabolic health. This questions the increasing concerns on the potential harmful effects of influence of early enhanced nutrition on later body composition. In the period from circa 32 weeks to discharge, enteral nutrition becomes the main source of nutrition, usually provided via tube feeding, and enriched to promote rapid growth. Caution may however be required as high growth rates in this period were not only associated with lean mass, but also with fat mass accumulation, resulting in increased relative fat mass at follow–up. Also after discharge home, rapid weight gain

was associated with increased relative fat mass. In this period, many infants receive postdischarge formula, enriched in energy and proteins. Close monitoring of growth parameters and timely tempering of intake seem to be advisable in that stage. Studies on postdischarge formulas and body composition show, however, conflicting results, and have not yet elucidated the optimal ratio between energy and protein supply.[225–227]

Rapid growth in early life may be harmful, but still no clinical thresholds have been defined for relative fat mass or adiposity. This is complicated by most effects not being detectable until late childhood or even adulthood.[228] Follow–up into school age and adulthood is therefore warranted to provide a complete view of the influence of early postnatal growth on cardiometabolic health of preterm infants and to determine clinical relevant cut–offs for body composition in infants.

Both at group and at individual level, relative fat mass at 6 weeks and 6 months ca was generally within the same range, suggesting that fat mass accumulation remains relatively stable in this period, after an earlier phase of rapid increase. This corresponds with body composition data observed in other studies conducted in preterm and term born infants.[219,229,230] Body composition data earlier in life could have provided a better view on the exact timing of rapid fat accumulation in our patients, but this is hampered by the practical problem that body composition measurement is only possible without respiratory support.

Previous studies demonstrated that %FM is higher around term age in preterm infants, but normalizes or decreases even below levels of term born infant 3 – 4 months after term age.[77,231] We confirmed that at 6 weeks ca, %FM seems comparable or already lower than %FM of term born infants, with comparable lean mass but decreased fat mass. At 6 months ca, %FM was almost 1 SD lower than observed in term born infants, and also absolute fat and lean mas were lower than in term born infants.[219] The mechanisms and clinical consequences of this decreased adiposity are not clear.

The strengths of our study were the prospective design and longitudinal measurement, which enabled us to model individual growth trajectories. A few considerations should be taken into account when interpreting our study results. First, only 64 infants had both body composition measurements, which hampers the comparability between

the first and second measurement. Second, the PEA POD® was used which is a validated, feasible, and patient–friendly device to measure body composition in infancy. This method provides however no information on fat distribution within the body [e.g., subcutaneous versus visceral], which is considered an important risk factor of adverse cardiometabolic health.[18] It would have been interesting to study the effect on sex on the association between growth and body composition, but due to the small sample size it was not possible to do subgroup analysis. Furthermore, we did not take nutritional intake during hospital stay into account because all infants were treated according to the same protocol and macronutrient recommendations were expected to be within recommendations based on our earlier study.[232] To answer clinical questions regarding the association between nutritional intake and body composition, nutritional intervention trials are required.

Our findings show that rapid weight gain in early life of preterm infants is associated with increased relative fat mass up to 6 months corrected age. Although previous studies demonstrated that adiposity tracks into childhood, we have no data yet at older ages. Also, studying additional markers, such as fat distribution and vascular and endocrine parameters, are needed to detect other early changes in cardiometabolic health.

CONCLUSION

In this prospective cohort study in very preterm infants, we found that in–NICU growth was mainly associated with lean mass in the first months of life, while increased in–hospital growth was associated with increased adiposity. Although further conclusions should be taken with caution, this may suggest that in the early neonatal period high growth rates can be aimed for without adversely influencing body composition in early life.

SUPPLEMENTAL TABLE 1 | IN–HOSPITAL GROWTH PARAMETERS

		Birth	Dip	30W	Transfer to high care center	Discharge home
N		118	118	118	118	118
Gestational age	weeks^{+days}	27^{+4} [26^{+2};28^{+5}]	28^{+2} [26^{+5};29^{+1}]	30^{+0} [30^{+0};30^{+0}]	32^{+0} [30^{+3};36^{+2}]	39^{+5} [38^{+0};41^{+5}][4]
Postnatal age	days	1	5 [3;6]	18 [10;28]	29 [17;69]	84 [69;104][4]
Weight	grams	1015 [800;1250]	888 [725;1111]	1168 [1028;1273]	1450 [1250;2044]	3105 [2710;3590][5]
	Z score	0.1 [−0.4;0.7]	−0.7 [−1.2;−0.3]	−0.6 [−1.0;−0.4]	−0.9 [−1.5;−0.4]	−0.7 [−1.7;0.1][5]
Head circumference	cm	24.8 [23.6;26.6][1]	NA	26.0 [25.0;27.0][2]	28 [26.8;31.5][3]	34.5 [33.2;36.3][6]
	Z score	0.1 [−0.5;0.6][1]	NA	−0.9 [−1.6;−0.4][2]	−0.8 [−1.4;−0.3][3]	−0.1 [−1.1;0.6][6]

Legend: [1] 3 missing measurements; [2] 17 missing measurements; [3] 14 missing measurements; [4] 1 missing data; [5] 3 missing measurements; [6] 45 missing measurements.
Abbreviations: W, weeks; cm, centimeter.

SUPPLEMENTAL TABLE 2 | WEIGHT GROWTH PATTERNS BETWEEN BIRTH AND 6 MONTHS CORRECTED AGE

	Birth – Dip	Postnatal weight dip – NICU transfer	NICU transfer – discharge home	Discharge home – 6W ca	6W – 6M ca
Δ Z score	−0.8 [−1.1;−0.5]	−0.1 [−0.5;0.1]	0.1 [−0.3;0.6]	0.0 [−0.4;0.4]	0.2 [−0.3;0.7]
Appropriate growth	42 [36%]	94 [80%]	85 [72%]	78 [66%]	56 [48%]
Growth retardation	76 [64%]	20 [17%]	8 [7%]	19 [16%]	18 [15%]
Catch–up growth	0	4 [3%]	19 [16%]	14 [12%]	24 [21%]
Missing	0	0	6 [5%]	7 [6%]	20 [17%]

Legend: All data are presented in number [percentage] or median [interquartile range]. Appropriate growth is defined as a difference in Z score between time point X and Y of <0.67 and >−0.67 Z score. Growth retardation is defined as a difference in Z score between time point X and Y of >−0.67 Z score. Catch−up growth is defined as a difference in weight Z score between time points X and Y of > 0.67 Z score.
Abbreviations: Δ, delta; NICU, neonatal intensive care unit; ca, corrected age; W, weeks; M, months.

SUPPLEMENTAL TABLE 3 | ASSOCIATION BETWEEN GROWTH [WEIGHT GAIN] AND BODY COMPOSITION AT 6 WEEKS AND 6 MONTHS CA [BASIC MODEL]

	6 weeks [n=101]			6 months [n=80]		
	%FM	**Absolute FM**	**Absolute LM**	**%FM**	**Absolute FM**	**Absolute LM**
In–NICU	–	–	5.6%***	–	–	2.0%#
In–hospital	6.3%*	11.0%***	10.8%***	–	–	5.8%**
Up to 6 weeks ca	30.1%***	45.3%***	26.9%***	–	8.8%**	10.5%***
Up to 6 months ca	NA	NA	NA	27.0%***	46.5%***	27.3%***

Legend: The variance [%] in body composition explained by early growth trajectories is presented. In the regression analyses, we adjusted for gestational age at birth, birth weight Z score, sex, corrected age at body composition measurement.
Symbols: – indicates p value >0.1; # p value between 0.5 – 0.1; * p value between 0.5 – 0.01; ** p value between 0.01 and 0.001; *** p value <0.001.
Abbreviations: %FM, percentage fat mass relative to weight; FM, fat mass; LM, lean mass; NA, not applicable; ca, corrected age.

PART II THE EARLY HUMAN BRAIN

7

A NEW ULTRASOUND MARKER FOR BEDSIDE MONITORING OF PRETERM BRAIN GROWTH

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ABSTRACT

BACKGROUND AND PURPOSE Preterm infants are at risk for neurodevelopmental impairment, but reliable, bedside–available markers to monitor preterm brain growth during hospital stay are still lacking. The aim of this study was to assess the feasibility of corpus callosum – fastigium length as a new cranial ultrasonography marker for monitoring of preterm brain growth.

MATERIALS AND METHODS In this longitudinal prospective cohort study, cranial ultrasound was planned on the day of birth, days 1, 2, 3, and 7 of life; and then weekly until discharge in preterm infants born before 29 weeks of gestation. Reproducibility and associations between clinical variables and corpus callosum – fastigium growth trajectories were studied.

RESULTS Series of 1 – 8 cranial ultrasounds were performed in 140 infants [median gestational age at birth, 27^{+2} weeks [interquartile range, 26^{+1} – 28^{+1}]; 57.9% male infants]. Corpus callosum – fastigium measurements showed good–to–excellent agreement for inter– and intraobserver reproducibility [intraclass correlation coefficients > 0.89]. Growth charts for preterm infants between 24 and 32 weeks of gestation were developed. Male sex and birth weight SD score were positively associated with corpus callosum – fastigium growth rate.

CONCLUSION Corpus callosum – fastigium length measurement is a new reproducible marker that is applicable for bedside monitoring of preterm brain growth during neonatal intensive care stay.

INTRODUCTION

Brain growth is an important predictor of neurodevelopmental outcome in preterm infants.[98,233–235] In neonatal intensive care units [NICUs], brain growth is usually monitored by manual measurement of head circumference. However, head circumference measurement has a low interrater agreement and does not correspond well with actual brain development.[99,236] Therefore, there is a need for a new reliable bedside marker for monitoring preterm brain growth in clinical practice.

Brain structures measured by cranial ultrasound [CUS] could provide clinically applicable markers for brain growth. A few sonographic markers of brain growth have been used in the past, mainly measuring the corpus callosum [CC] or cerebellum, thereby reflecting growth of a small part of the brain only.[237–241] In addition to currently available markers of preterm brain development, we propose that the length between genu of the CC and the fastigium [roof of the fourth ventricle] could serve as a new marker for brain growth.

The aim of this study was to evaluate the usefulness of corpus callosum – fastigium [CCF] length and CC length, an existing marker, as markers for monitoring of brain growth in preterm infants during NICU stay. We assessed the reproducibility of CC and CCF length measurements, developed growth charts for preterm infants between 24 and 32 weeks of gestation, and evaluated prenatal and postnatal characteristics possibly associated with CC and CCF growth trajectories. We hypothesized that both measurements are highly reproducible. Furthermore, we hypothesized that CCF and CC growth trajectories are associated with prenatal and postnatal determinants of neurodevelopmental outcome in preterm infants.

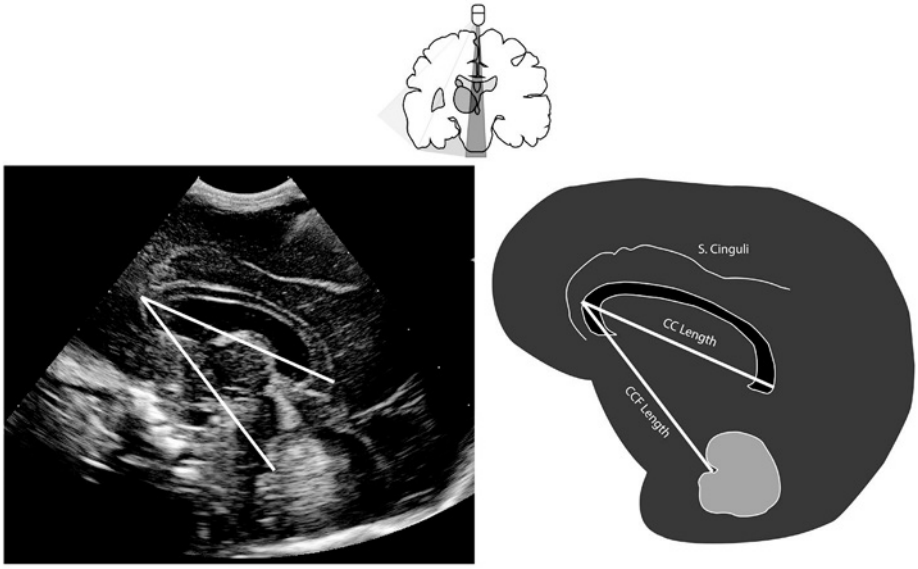
METHODS

This prospective observational cohort study was performed at the level III NICU of the Erasmus MC – Sophia Children's Hospital, Rotterdam, the Netherlands. The local medical ethics review board approved this study. Written parental consent was obtained before participation. Between 2010 and 2012, all newly admitted singleton, preterm infants born before 29 weeks gestational age [GA] were eligible for enrollment. We applied the following exclusion criteria: [1] unknown GA at birth; [2] major congenital abnormalities, and [3] extensive brain injury [including intraventricular hemorrhage grade III, post hemorrhagic ventricular dilatation, and venous infarction]. The latter complications are expected to influence the validity of the measurements due to possible midline shift and expected altered brain growth. GA at birth was calculated by using the first day of last menstrual period and was confirmed by first trimester crown rump length measurement on sonography. Postnatal age was expressed by postmenstrual age, calculated as GA at birth + weeks and days of postnatal age. Pregnancy and neonatal characteristics were collected prospectively. Maternal characteristics were retrospectively collected from medical records. Pregnancy complications, including intra–uterine growth retardation, pre–eclampsia and hemolysis, elevated liver enzymes, low platelet count [HELLP] syndrome were obtained from obstetrical records and were defined based on clinical definitions according to national guidelines.[242]

CRANIAL ULTRASOUND AND MEASUREMENTS

CUS was performed according to the standard local protocol on the day of birth, on days 1, 2, 3, and 7 of life and then weekly until discharge. The protocol was only disregarded on clinical grounds [e.g., hemodynamic instability]. One researcher [MR] performed all CUS by using a MyLab 70 scanner [Esaote, Genoa, Italy], with a convex neonatal probe [7.5 MHz]. Measurements were performed off line by using the Mylab software [Esaote]. Measurements of CC and CCF length were performed on a standard sagittal plane. In this plane a complete corpus callosum [genu to splenium] and distinct vermis of the cerebellum, including the fastigium, had to be visualized. CCF length was measured from the genu of the corpus callosum [outer border] to the fastigium. CC length was measured from outer to outer border [genu to splenium, **Figure 1**]. All measurements were performed by 1 investigator [MR]. To

FIGURE 1



Legend: In the upper part, we show the coronal view of the brain and the position of the sonography probe for assessment of the corresponding correct sagittal plane below. Measurements of the corpus callosum – fastigium length and corpus callosum length are displayed in the sagittal sonography view [left] and schematically [right]. S. Cinguli indicates sulcus cingula.

establish the reliability, a second investigator [JR], blinded to the previous results, measured 30 randomly selected scans of varying quality and of neonates with different GAs.

STATISTICAL METHODS

Data were analyzed by using SPSS [Release 21 for Windows; IBM, Armonk, New York] and R statistical and computing software [https://www.r–project.org]. P values <0.05 were statistically significant. Median value and interquartile range and mean and SDs were used as appropriate.

Intraobserver and interobserver agreements for CC and CCF lengths were evaluated by using the intraclass correlation coefficient [ICC] and Bland–Altman plots.[137] The ICC was analyzed by using a 2–way mixed model. Cut–off values were in accordance with Landis and Koch.[243] Growth charts were developed for CCF and CC growth as a function of postmenstrual age [weeks] and weight [grams]. To model the relation

between the measured CCF and CC lengths and a predefined list of covariates, linear mixed models were estimated using lme [in the R nlme package: https://www.inside-r.org/r-doc/nlme/lme].[244] To account for the within-subject correlation, we used a random intercept and random coefficient of GA as well as a power variance function to model the residual covariance. The predefined covariates were GA at birth, birth weight [BW] SD score, sex, intra-uterine growth retardation [defined as expected fetal weight below the 10th percentile], pre-eclampsia/HELLP, chorioamnionitis, death, sepsis, and days on mechanical ventilation. In all models both GA and GA2 [square of GA] were used as covariates. To this basic model the additional predictors were added separately [termed "univariable models" below] and also all at once [the "multivariable model"].

RESULTS

Of 336 infants admitted to our NICU during the study period, 152 were eligible for inclusion. Twelve infants were excluded because they met the exclusion criterion of extensive brain injury, resulting in a sample size of 140 infants. Baseline maternal and neonatal characteristics are listed in **Table 1**. The median gestational age at birth was 27^{+2} weeks [interquartile range, 26^{+1};28^{+1}]; the median birth weight was 955 grams [interquartile range, 780;1125]. The number of sonography scans per neonate ranged from 1 to 8.

TABLE 1 | BASELINE CHARACTERISTICS

	N=140	Missing[b]
Maternal characteristics		
Age [yr] [mean,[SD]]	30 [5.6]	0
Ethnicity		0
Dutch	74 [52.9%]	
Other Western	9 [6.4%]	
Non–Western	57 [40.7%]	
Smoking during pregnancy	26 [18.6%]	17
IVF/ICSI	9 [6.4%]	0
IUGR	42 [30%]	4
PE/HELLP syndrome	37 [26.4%]	0
Chorioamnionitis	37 [26.4%]	0
PPROM	32 [22.9%]	0
Neonatal characteristics		
GA at birth [weeks^{+days}]	27^{+2} [26^{+1} ;28^{+1}]	0
Male sex	81 [57.9%]	0
BW [grams]	955 [780; 1125]	0
Use of antenatal steroids	127 [90.7%]	2
Apgar score at fifth minute	8 [7;9]	0
CRIB score	3 [1;6]	1
Death	17 [12.1%]	0
Days on mechanical ventilation	5 [1;14]	3
Days to regain birth weight	9 [7;12]	14
Sepsis	67 [47.9%]	0
IVH grade I or II	32 [22.9%]	0
Severe BPD	15 [10.7%]	33

Legend: Baseline data of maternal and neonatal characteristics are presented as median [interquartile range] or n [%] unless otherwise specified. Missing data were mainly due to early transfer to a secondary hospital. Ethnicity was reported to provide insight in the generalizability of the study population.
Abbreviations: IVF/ICSI, in vitro fertilization with or without intra–cytoplasmic sperm injection; IUGR, intra–uterine growth retardation; PE, pre–eclampsia; PPROM, prolonged premature rupture of membranes; CRIB, clinical risk index for babies; IVH, intraventricular hemorrhage; BPD, bronchopulmonary disease.

REPRODUCIBILITY

The mean interobserver difference was −0.3207 ± 1.4527 mm for CCF [p=0.244] and0.4600 ± 1.8463 mm for CC length [p=0.183].

The ICCs for interobserver and intraobserver analysis showed excellent agreementfor both CCF and CC length [respectively, intraobserver: 0.958; 95%CI 0.912;0.980; interobserver: 0.885; 95%CI 0.770;0.944]; and intraobserver: 0.922; 95%CI 0.844;0.962; and interobserver: 0.893; 95%CI 0.783;0.948]. **Figure 2** shows Bland–Altman plots of interobserver and intraobserver agreement for both measurements.

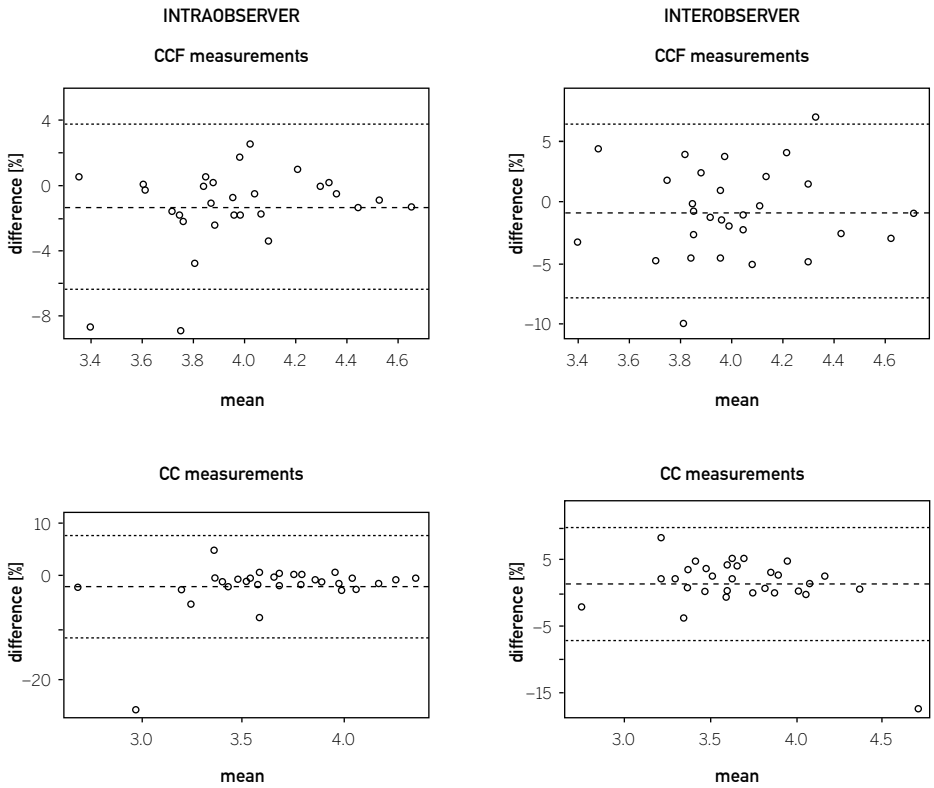
CC AND CCF LENGTH

The mean CCF length was 40.9 ± 2.97 mm, with a range from 34.0 to 54.3 mm. The mean CC length was 36.3 ± 3.33 mm, with a range from 26.6 to 48.8 mm. Growth charts of CCF and CC lengths by post menstrual age and by weight are shown in **Figure 3**.

LINEAR MIXED MODELS

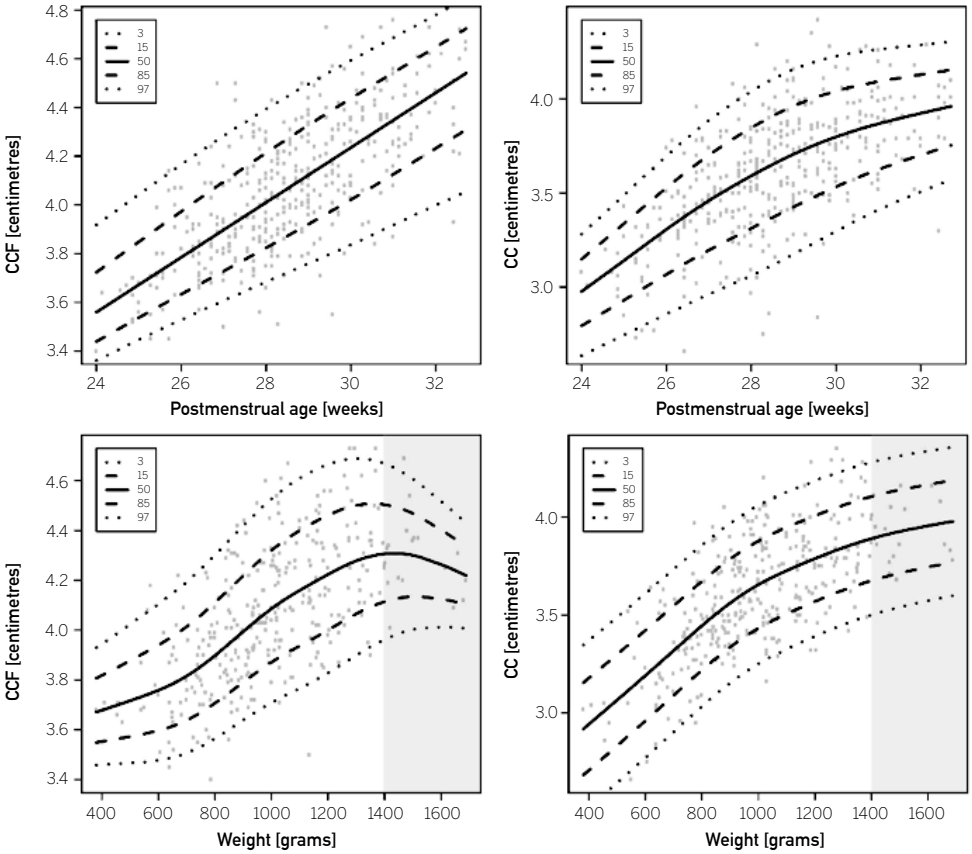
Results of univariable analyses are shown in **Table 2** for CC and CCF growth. The multivariable analysis confirmed a positive association between BW SD score and CCF growth rate and a negative association between female sex and CCF growth rate. For the CC growth rate, a positive association was found with BW SD score by using multivariable analysis.

FIGURE 2



Legend: Reproducibility of corpus callosum – fastigium and corpus callosum lengths by using Bland–Altman plots. The middle dashed lines depict the average measurement bias in percentage differences. The bold dashed horizontal lines represent the 95% limits of agreement for these percentage differences.

FIGURE 3



Legend: Growth charts of corpus callosum – fastigium [left] and corpus callosum [right] length for preterm neonates as a function of postmenstrual age [in weeks] and weight [in grams]. On the y–axis, corpus callosum – fastigium [left] and corpus callosum [right] lengths are presented in centimeters. The grey areas indicate the parts of the weight charts that should not be used as reference curves.

TABLE 2 | LINEAR MIXED MODELS

	CCF growth						CC growth					
	Univariable			Multivariable			Univariable			Multivariable		
	β	SE	p value	β	SE	p value	β	SE	p value	β	SE	p value
GA at birth	0.029	0.012	**0.022**	0.011	0.017	0.518	0.024	0.017	0.146	0.004	0.021	0.857
BW SD score	0.053	0.009	**<0.0001**	0.050	0.014	**<0.001**	0.094	0.011	**<0.001**	0.075	0.017	**<0.001**
Sex [female]	−0.109	0.030	**<0.001**	−0.070	0.029	**0.018**	−0.066	0.043	0.124	−0.003	0.035	0.938
IUGR [no]	0.094	0.033	**0.005**	−0.034	0.045	0.451	0.267	0.041	**<0.001**	0.046	0.054	0.390
PE/HELLP [yes]	−0.064	0.035	0.068	0.000	0.038	0.992	−0.200	0.045	**<0.001**	−0.052	0.046	0.260
Chorioamnionitis [yes]	0.030	0.035	0.397	0.031	0.035	0.370	0.136	0.047	**0.004**	0.069	0.042	0.106
Death [yes]	−0.103	0.048	**0.033**	−0.061	0.046	0.186	−0.200	0.064	**0.002**	−0.105	0.054	0.057
Sepsis [yes]	−0.034	0.031	0.272	−0.021	0.029	0.477	−0.050	0.042	0.239	−0.043	0.035	0.218
Days on mechanical ventilation	−0.001	0.002	0.432	0.002	0.002	0.340	−0.003	0.002	0.160	0.002	0.002	0.397

Legend: Presented are the effect estimates of maternal and neonatal characteristics on CF and CC growth in both univariable and multivariable linear mixed models. The effect estimates [β], standard errors [SE] and p values are given. Significant findings [p<0.05] are in **bold** font.
Abbreviations: GA indicates gestational age; BW, birth weight; SD score, standard deviation score; IUGR, intra−uterine growth retardation; PE, pre−eclampsia.

DISCUSSION

In this report, we demonstrated that CCF length, measured by using CUS, is a reproducible and feasible marker that could serve as a new bedside tool to monitor preterm infant brain growth during NICU stay. We provided growth charts of CCF and CC length for preterm infants from 24 to 32 weeks postmenstrual age. We found that a higher BW SD score results in increased CCF and CC growth rate during hospital stay, while female infants have a slower CCF growth compared with male infants.

Previous sonography studies have evaluated only a limited number of brain structures as potential markers for brain growth or predictors of neurodevelopmental outcome in preterm infants.[237–240] One explanation for this is that the brain has few easily recognizable and consistent landmarks for reliable measurements on CUS. The CC, a flat bundle of white matter that connects the left and right hemispheres, is one of the brain structures that is easily visualized and recognizable on CUS.[245] Prematurity is known to affect CC development, by the early transition from intra–uterine to extra–uterine life and by postnatal stress and injury,[246] leading to both structural and functional impairment.[247,248] Associations have been found between the length and thickness of the CC and brain volumes and neurodevelopmental outcome.[241,249,250] Further studies should elucidate whether CC length can be considered a proxy of telencephalon development, creating an impression of white matter development and brain maturation.

The advantages of using CCF length in the monitoring of brain growth rely on anatomic and practical issues. CCF length may be considered a marker of diencephalon and mesencephalon development and vermis growth. The diencephalon includes the thalamus, a neural relay center crucial for adequate cognitive function.[251] Altered development of the thalamus, and thus of the diencephalon, may lead to adverse neurodevelopmental outcome. Several studies showed impaired thalamus volume and extreme vulnerability of the thalamus to be risk factors after preterm birth.[252,253] Whether thalamic injury or growth impairment directly influences CCF length needs to be further studied.

One of the other advantages of CCF length measurement is the use of CUS instead of MR imaging or head circumference measurement. In **Table 3**, the pros and cons of every method are depicted. Although volumetric MR imaging is increasingly used

for growth assessment of the preterm brain, its use for serial assessment is still very limited.[233] Head circumference measurement has a low interrater agreement and limited association with long–term outcome and does not measure actual brain growth, but growth of the skull and the subarachnoid spaces, which are frequently enlarged in preterm infants.[99,236,254] Measurement of CCF length is not considered a burden compared with head circumference measurement as it can be performed on routine CUS, which are often recommended weekly in preterm infants.[255] Both CCF length and CC length can already be measured prenatally because the CC and the fastigium are already visible on sonography around 18 weeks of gestation; this feature allows the use of the same marker prenatally and postnatally for monitoring of brain growth.[256]

TABLE 3 | PROS AND CONS OF DIFFERENT METHODS FOR ASSESSMENT OF BRAIN GROWTH

	HC	CUS	MRI
Patient friendly	++	++	–
Bedside available	++	++	–
Serial measurements possible	++	++	–
Fast measurement	++	+	–
Reproducible	±	+	++
Reflecting actual brain growth	–	+	++
Low costs	++	+	–
Dimension	1D	2D	3D

Legend: ++ indicates very good, + indicates acceptable, – indicates bad agreement with the corresponding item.
Abbreviations: HC, head circumference; CUS, cranial ultrasound.

In accordance with previous studies, we showed satisfactory reproducibility for CC length.[237] CCF reproducibility was excellent too; this finding suggests that both measurements are feasible for longitudinal evaluation of brain growth. Increasing lengths with increasing ages and weights, as shown in the growth charts, support the use of these markers in clinical practice.

We observed a nonlinear growth pattern for CC and CCF length. Previous studies found an intra–uterine constant growth rate of 0.20 – 0.22 mm/day of the CC.[257,258] Also in preterm infants a constant–though–slower growth rate was observed.[237] In contrast to previous studies, we performed longitudinal measurements [1 – 8 scans per infant], allowing a more reliable estimation of CC growth. Other brain structures, such as the vermis of the cerebellum, show a nonlinear growth pattern as well.[238] Because we are the first to evaluate the use of CCF length, no literature is available for comparison. We did expect a nonlinear growth pattern based on current literature.

In **Figure 3**, parts of the weight charts are colored grey because we advise not to use these parts of the curves as a reference curve. We chose to analyze and present the complete original data of infants with a postmenstrual age between 24 and 32 weeks and not to select ideal reference cases. The drawback is seen in in the upper part of the weight charts; the curves appear to go down above 1400 gram and, despite the very small numbers, the confidence interval narrows. This finding, of course, does not reflect an incline of brain size, but rather selection and censoring. These data are not "first measurements" [reflecting intra–uterine accomplished growth] but are follow–up data of patients with prolonged NICU admission, representing the most complex cases [e.g., with severe chronic lung disease] not stable enough to be discharged early. In conclusion, the last part of this curve depicts valid data that you would expect in a NICU population, but we consider these not representative for normal growth in preterm infants.

The decreased growth rate of CCF length in female infants is in accordance with previous studies, which identified sex differences in brain structures and neurodevelopmental outcome.[259,260] The positive association between BW SD score and CCF and CC growth rate is also in accordance with current literature.[261]

One investigator who was trained in visualizing a standard sagittal plane performed all scans. This likely improved the quality of the scans and may have enhanced the reproducibility. We realize, therefore, that the clinical applicability is probably overestimated in our cohort. Reliable measurements and a correct sagittal plane using CUS depend on the experience of the observer but are easy to learn. Recently developed software to identify the sagittal plane automatically may further increase the reproducibility and clinical applicability.[262]

This study has some limitations. First, in the Netherlands, preterm infants are transferred to a secondary hospital relatively early, accounting for very little data in our cohort of infants born at 29 weeks gestation and limited data of infants after 30 weeks gestation. Although white matter injury is already visible on scans after a few days, brain atrophy is often only noticeable after weeks to months.[263] Our short follow–up time could explain why we did not find an association between expected clinical variables, such as sepsis and days on mechanical ventilation, and CCF or CC growth rate. Second, including all scans between 24 and 32 weeks postmenstrual age may have influenced the reliability of the growth charts; that preterm infants lose weight after birth and start to grow days later is a common finding. Brain growth may be limited before regain of birth weight [usually after 10 days]. This limitation may have increased variation in CC and CCF lengths. Extremely preterm and clinically unstable infants have a longer NICU stay and are likely to undergo more CUS. This might have biased our growth charts. On the other hand, our data reflect clinical practice in a neonatal intensive care setting.

In future studies, it would be interesting to compare fetal and preterm CCF growth. Currently, we are scanning fetuses in the second and third trimester of pregnancy to develop reference curves for fetal brain growth, which could also serve as an ideal growth curve for preterm infants. We were not able yet to assess the association between feeding regimens and growth during NICU stay and CCF growth trajectories. This is of interest because it may have clinical implications for nutritional practices. Moreover, CCF length can possibly be used as an outcome measure in nutritional and other intervention studies. It would be of main interest to assess whether CCF length, possibly combined with other available markers of brain growth such as CC length, could serve as predictor of neurodevelopmental outcome. The clinical applicability may extend beyond the NICU stay into the outpatient follow–up period because the anterior fontanelle can be used as an acoustic window until approximately 6 months in most infants.

CONCLUSION

There is a lack of bedside markers for brain growth in preterm infants during NICU stay. We propose a feasible, new sonography measurement called 'corpus callosum – fastigium length' with high reproducibility for monitoring of brain growth in preterm infants during hospital stay. This marker may help clinicians determine whether preterm infants show adequate postnatal brain growth and may eventually be used as an outcome measure in nutritional and other intervention studies. Further research is warranted to assess whether this marker could also serve as an early predictor for short–term and long–term neurodevelopmental outcome.

NEW ULTRASOUND MEASUREMENTS TO BRIDGE THE GAP BETWEEN PRENATAL AND NEONATAL BRAIN GROWTH ASSESSMENT

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ABSTRACT

BACKGROUND AND PURPOSE Most ultrasound markers for monitoring brain growth can only be used in either the prenatal or the postnatal period. We investigated whether corpus callosum length and corpus callosum – fastigium length could be used as markers for both prenatal and postnatal brain growth.

MATERIALS AND METHODS A three–dimensional [3D] ultrasound study embedded in the prospective Rotterdam Periconception Cohort was performed at 22, 26, and 32 weeks gestational age in fetuses with fetal growth restriction [FGR}, congenital heart defects [CHD}, and controls. Postnatally, cranial ultrasound was performed at 42 weeks postmenstrual age. First, reliability was evaluated. Second, associations between prenatal and postnatal corpus callosum length and corpus callosum – fastigium length were investigated. Third, we created reference curves and compared corpus callosum length and corpus callosum – fastigium length growth trajectories of controls with growth trajectories of fetuses with FGR and CHD.

RESULTS We included 199 fetuses; 22 with FGR, 20 with CHD, and 157 controls. Reliability of both measurements was excellent [intraclass correlation coefficient ≥0.97]. Corpus callosum growth trajectories were significantly decreased in FGR and CHD fetuses [β=−2.295; 95%CI −3.320;−1.270, p<0.01; β=−1.267; 95%CI −0.972;−0.562; p<0.01, respectively] compared with growth trajectories of controls. Corpus callosum – fastigium growth trajectories were decreased in fetuses with FGR [β=−1.295; 95%CI −2.595;0.003, p=0.05].

CONCLUSIONS Corpus callosum length and corpus callosum – fastigium length may serve as reliable markers for monitoring brain growth from the prenatal into the postnatal period. The clinical applicability of these markers was established by the significantly different corpus callosum and corpus callosum – fastigium growth trajectories in fetuses at risk for abnormal brain growth compared with those of controls.

INTRODUCTION

In preterm and those small–for–gestational age, brain growth is an important predictor of neurodevelopmental outcome.[98,233–235] Although prenatal growth often predicts postnatal growth, there is a traditional division between fetal and neonatal growth charts.[108] This is mainly due to the lack of consistent measures of brain growth that can be used in both the prenatal and postnatal period.

Markers of brain growth that can theoretically be used in both the prenatal and postnatal periods include head circumference and a few ultrasound [US] and MR imaging measures. Head circumference measured postnatally, however, lacks precision and does not correspond well with neurodevelopmental outcome.[99,236] Prenatal and postnatal US markers are largely based on individual brain structures, only reflecting growth of a specific part of the brain.[237–241] Moreover, these brain structures are not measured consistently during the prenatal and postnatal periods due to the absence of corresponding standard US planes. Although MR imaging provides more precise measures of brain growth, volume, and development, this technique is expensive and therefore not suitable for serial measurements.

Recently, we demonstrated that corpus callosum – fastigium [CCF] length is a reliable bedside–available US marker that can be used to monitor brain growth in preterm infants during neonatal intensive care unit stays.[264] CCF length is considered a composite marker of diencephalon and mesencephalon size and thereby adds information to the more widely used corpus callosum [CC] length.[264] We hypothesized that these two cranial ultrasound measures are feasible for use during prenatal US examinations. Thereby, these markers would provide a continuum for monitoring brain growth, bridging the period before and after birth.

Our main aim was to investigate whether CC and CCF length can be used as reliable US markers for monitoring fetal and neonatal brain growth. First, we assessed the reliability of the measurements. Second, we created reference curves from 22 weeks to 42 weeks gestational age [GA] by combining fetal and neonatal measurements. Finally, as a first step to evaluate the clinical applicability of these US markers, we investigated CC and CCF growth trajectories in fetuses at risk of abnormal brain growth and compared them with those of control fetuses.

METHODS

STUDY DESIGN

This three–dimensional [3D] US study was embedded in the Rotterdam Periconceptional Cohort [Predict study], an ongoing prospective cohort study at the Department of Obstetrics and Gynecology, Erasmus MC University Medical Center, Rotterdam, the Netherlands.[104] At enrollment, all participating women and their partners gave written informed consent on behalf of themselves and their unborn child. This study was approved by the regional medical ethical and institutional review board of the Erasmus MC, University Medical Center in Rotterdam [MEC 2004–227; date of approval, January 25, 2013].

Pregnant women were enrolled between November 2013 and July 2015. They were either enrolled before 12 weeks GA or between 22 and 32 weeks GA. Controls were enrolled before 12 weeks GA and were defined as fetuses without fetal growth restriction [FGR] before 32 weeks GA, born after 37 weeks GA, and without congenital anomalies. Cases included those pregnancies referred to our outpatient clinic with FGR or an isolated fetal congenital heart defect [CHD] between 22 and 32 weeks GA. The diagnosis was confirmed by an extended structural US examination at our hospital. FGR was defined as abdominal circumference or estimated fetal weight percentile of <5 according to Hadlock.[265]

For this analysis we excluded pregnancies ending in intra–uterine fetal death, termination of pregnancy, or only preterm birth [without FGR or CHD]. We also excluded fetuses with congenital anomalies other than CHD, with trisomy 21, and without US images.

STUDY PARAMETERS

According to Dutch clinical practice, GA in spontaneously conceived pregnancies was calculated based on first trimester crown–rump length measurements before 13 weeks GA.[172] In pregnancies conceived through in vitro fertilization, with or without intracytoplasmic sperm injection procedures, GA was calculated from the date of oocyte retrieval plus 14 days or from the day of embryo transfer plus 17 or 18 days after cryopreserved embryo transfer, depending on the number of days between oocyte retrieval and cryopreservation.

Data were collected on maternal characteristics, medical and obstetrical history, pregnancy course, and neonatal outcome from self–administered questionnaires in the first trimester, second trimester, and around delivery. Follow–up data on pregnancy outcomes were validated on the basis of an US report of the routine second trimester anomaly scan and on obstetric medical records.

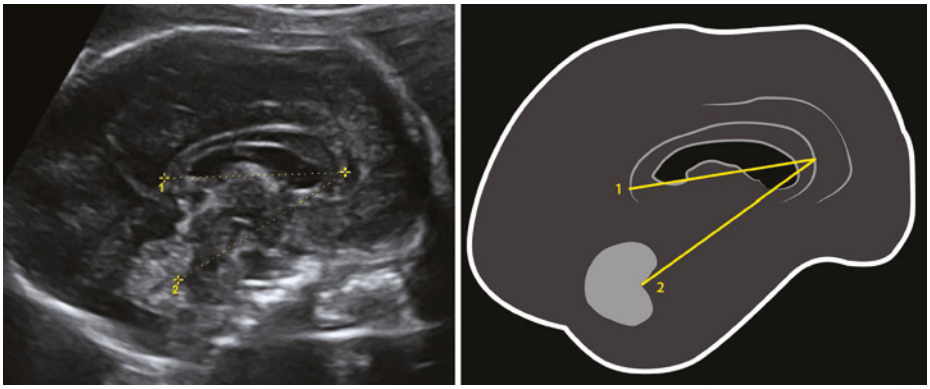
PRENATAL SONOGRAPHY

Prenatal 3D US examinations were performed on the Voluson E8 system [GE Health Care, Milwaukee, Wisconsin] by using a 1 to 7 MHz transabdominal transducer or a 6 to 12 MHz transvaginal transducer. Primarily, we used an abdominal approach, but a transvaginal approach was considered when the fetus was in head–down presentation. Serial prenatal 3D US examinations and measurements were performed at 22, 26, and 32 weeks of gestation by 1 certified sonographer [IK]. Standard biometry were measured, including bi–parietal diameter, head circumference, abdominal circumference, and femur length. An estimation of fetal weight was calculated with the Hadlock equation.[265] Biometry was followed by detailed 3D neurosonography. Standard planes were obtained according to the International Society of Ultrasound in Obstetrics and Gynecology guidelines.[266] CC and CCF length measurements were performed off–line in an exact mid–sagittal plane [**Figure 1**]. CC length is measured from genu to splenium, outer–outer border. CCF length represents the length between the genu of the CC and the fastigium [roof of the fourth ventricle].[264] CCF length was only measured in images in which CC measurement was performed successfully. Manipulation of the 3D US volume to ensure an exact mid–sagittal plane for the measurements was performed in 4D View, Version 5.0 [GE Healthcare].

POSTNATAL ASSESSMENTS

After birth, cranial ultrasound was planned between 42^{+0} and 42^{+6} weeks postmenstrual age, independent of GA at birth. Cranial ultrasounds were performed by an experienced team of researchers with MyLab 70 [Esaote, Genoa, Italy] with a convex neonatal probe [7.5 MHz]. CC and CCF length measurements were performed offline by 1 researcher [JR] according to the method described above, with MyLab software. To enhance precision, we repeated all prenatal and postnatal measurements three times. The mean values were used in the statistical analyses.

FIGURE 1 | PRENATAL MEASUREMENT OF CC AND CCF LENGTH



Legend: [1] Corpus callosum [CC] length, outer–outer border; [2] Corpus callosum – fastigium length [CCF], from genu to the fastigium [roof of the 4th ventricle].

STATISTICAL ANALYSIS

For data analyses we used SPSS [Release 21 for Windows; IBM, Armonk, New York] and R statistical and computing software, version 3.1.3 [http://www.r–project.org]. Results with p values <0.05 were considered statistically significant. Previously, we demonstrated that postnatal measurements of CC and CCF length had good intra– and interobserver agreement.[264] To evaluate the reliability and reproducibility of prenatal measurements, we randomly selected 30 US examinations of 30 different fetuses, equally divided across the three prenatal time points from the whole study population. CC and CCF length measurements were then performed in threefold by two independent observers [IK [1] and JR [2]]. We performed analyses for intra– and interobserver reliability, calculating the mean differences with 95% confidence intervals [CI] and intraclass correlation coefficients. Moreover, the extent of agreement was examined with the Bland–Altman method.

Generalized Additive Models for Location and Scale were used to create reference ranges of CC and CCF length measurements between 22 and 42 weeks GA in controls.[267] To investigate whether cases showed deviations in CC and CCF growth, we created growth trajectories for each subject of the serial measurements of CC and CCF length between 22 and 42 weeks GA. A maximum–likelihood approach was used to test

whether polynomials of GA contributed to the best model fit. In the same manner, we tested the contribution of random and fixed effects of the intercept and slopes for all included polynomials. A quadratic model of GA with random intercept and slopes was designated as the best model. We placed the origin of the GA scale at 140 days GA. In this model, the variable indicating whether a fetus was FGR, CHD, or control was used as the covariate of interest [model 1]. Last, the final model [model 2] was adjusted for serial measurements of fetal weight and sex.

RESULTS

STUDY POPULATION

In total, 227 pregnant women were enrolled prenatally. After excluding pregnancies ending in intra–uterine fetal death [n=1], termination of pregnancy [n=1], preterm birth [n=14], congenital anomalies other than CHD [n=4], trisomy 21 [n=2], and withdrawals [n=6], the study population consisted of 199 pregnancies. Of these 199 fetuses, 22 fetuses had FGR, 20 had CHD, and 157 were controls. The general characteristics of the study populations are listed in **Table 1**.

TABLE 1 | BASELINE CHARACTERISTICS

	Controls [n=157]	FGR [n=22]	CHD [n=20]	Missing
Maternal characteristics				
Age at enrollment [years]	32.3 [21;44]	29.7 [21;41]	33.0 [22;48]	7
Nulliparous	69 [44]	13 [68]	11 [58]	6
Mode of conception [IVF/ICSI]	48 [31]	2 [10]	2 [11]	3
Geographical background				6
Western	126 [81]	15 [79]	18 [90]	
Non–Western	29 [19]	4 [21]	2 [10]	
Educational level				8
Low	20 [13]	4 [20]	0	
Intermediate	56 [36]	12 [60]	7 [39]	
High	79 [51]	4 [20]	11 [61]	
Prepregnancy BMI [kg/m^2]	22.9 [15.2;39.7]	22.9 [17.6;43.4]	23.4 [18.0;35.8]	19
Periconception folic acid initiation [yes]	149 [96]	15 [79]	18 [95]	6
Periconcepion smoking [yes]	25 [16]	3 [16]	3 [16]	8
Periconception alcohol consumption [yes]	44 [29]	4 [21]	10 [53]	9
Neonatal characteristics				
Birth weight [grams]	3345 [2035;4380]	1400 [400;2900]	3420 [1650 – 4140]	2
Gestational age at birth [weeks^{+days}]	39^{+1} [37^{+0};41^{+5}]	34^{+2} [26^{+3};39^{+3}]	38^{+6} [28^{+4};41^{+5}]	2
Males	82 [52]	11 [50]	13 [65]	0

Legend: Data are presented in median [interquartile range] or n [%]. Missing data were due to incomplete questionnaires.
Abbreviations: BMI, body mass index; IVF/ICSI, in vitro fertilization with or without intracytoplasmic sperm injection.

SUCCESS RATES AND RELIABILITY ANALYSES

Of 542 prenatal 3D US scans, 377 contained a high quality mid–sagittal plane eligible for CC and CCF length measurements. Means and success rates of CC and CCF length measurements per gestational age are listed in **Table 2**. Success rates ranged between 61 and 75% for prenatal CC length measurements and between 59 and 72% for prenatal CCF length measurements. Postnatally, CC and CCF length measurements were successful in 97%. In 83% of the subjects, CC length was measured at least at two time points during the whole study period, and CCF length in 65%.

TABLE 2 | SUCCESS RATES AND MEANS OF CC AND CCF LENGTH PER GESTATIONAL AGE

	GA	US scans [N]	Measurements [N]	Success Rate [%]	Mean [SD] [mm]
CC	22	166	124	75	26.35 [1.22]
	26	188	138	73	34.33 [1.86]
	32	188	115	61	41.56 [2.19]
	42	143	138	97	48.09 [3.15]
CCF	22	124	89	72	33.09 [1.61]
	26	138	82	59	39.39 [1.92]
	32	115	81	70	45.86 [2.07]
	42	143	138	97	52.26 [3.12]

Legend: Presented are the success rates, means and corresponding SD values of the CC and CCF measurements per full week of gestation. The success rates for CC length and postnatal CCF length were calculated by the number of successful measurements divided by the total number of US images. Success rates of prenatal CCF measurements were calculated by dividing the number of successful CCF measurements by the number of midsagittal images eligible for CC length.
Abbreviations: CC, Corpus Callosum length in millimetres; CCF, Corpus Callosum – Fastigium length in millimetres; GA, gestational age in weeks; US, ultrasound; N, number.

The intra– and interobserver reliability and agreement are shown in **Table 3**. CC lengths measured by observer 1 were slightly smaller [mean difference, –1.109 mm; mean percentage difference, –3.4%] than those measured by observer 2. Ninety–five percent limits of agreement for all measurements represent excellent agreement when the CC and CCF length measurements were repeated by the same observer and good agreement when repeated by a second observer. Intraclass correlation coefficient values of both intra– and interobserver were ≥0.97, which represents excellent reliability.

TABLE 3 | INTRA– AND INTEROBSERVER RELIABILITY FOR PRENATAL MEASUREMENTS OF CC AND CCF LENGTH

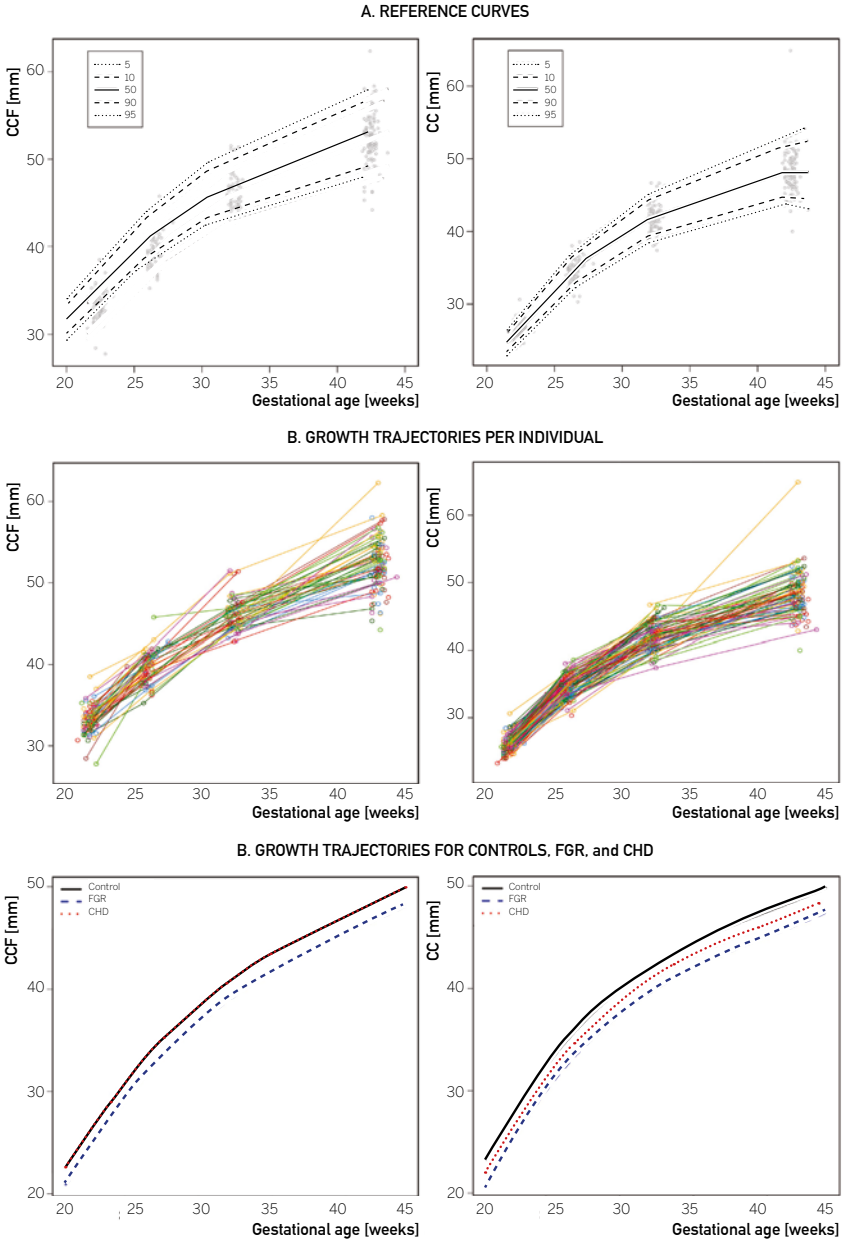
		Absolute differences				Relative difference		
		Mean difference [mm]	95% CI Mean difference [mm]	p value	95% Limits of agreement [mm]	Mean difference [%]	95% Limits of agreement [%]	ICC
Intraobserver	**CC**	0.011	−0.228;0.250	0.923	−1.373;1.396	0.1	−4.1;4.3	>0.99
	CCF	0.180	−0.157;0.517	0.284	−1.711;2.071	0.4	−4.7;5.4	>0.99
Interobserver	**CC**	−1.109	−1.702;−0.515	0.001	−4.546;2.329	−3.4	−14.9;8.1	0.97
	CCF	−0.125	−0.741;0.492	0.684	−3.589;3.340	−0.4	−9.5;8.6	0.97

Legend: Intra– and interobserver reliability analyses for prenatal CC and CCF measurements in a random selection of 30 ultrasound scans.
Abbreviations: CC, Corpus Callosum length; CCF, Corpus Callosum – Fastigium length; mm, millimeters; 95%CI, ninety–five percent confidence interval; %, percentage; ICC, intraclass correlation coefficient.

LINEAR MIXED MODEL ANALYSES

In **Figure 2A and 2B**, the reference curves and individual growth trajectories of CC and CCF lengths are shown. The results of the linear mixed models estimating differences in the mean growth trajectories of CC and CCF length among controls and fetuses with FGR and CHD are shown in **Table 4**. Growth trajectories of CC length were significantly decreased in FGR and CHD fetuses compared with growth trajectories of controls. CCF growth trajectories were only significantly decreased in FGR fetuses compared with those of controls. In **Figure 2C**, these trajectories are graphically displayed.

FIGURE 2 | GROWTH CURVES



Legend:
2A Reference curves between 22 and 42 weeks of gestation for CCF [left] and CC [right] length, with 5th, 10th, 50th, 90th, and 95th percentiles.
2B. Individual growth trajectories of CCF [left] and CC [right] length between 22 and 42 weeks of gestation.
2C. Growth trajectories for controls [black], FGR [striped blue], and CHD [dotted red] fetuses.
Abbreviations: CC, corpus callosum; CCF, corpus callosum - fastigium; mm, millimeter.

TABLE 4 | LINEAR MIXED MODELS: GROWTH TRAJECTORIES OF CC AND CCF ARE INFLUENCED BY THE PRESENCE OF FETAL GROWTH RESTRICTION AND CONGENITAL HEART DEFECTS

		Model 1			Model 2		
		β	95% CI	p value	β	95% CI	p value
CC	FGR	−2.384	−3.262;−1.505	**<0.01**	−2.295	−3.320;−1.270	**<0.01**
	CHD	−1.252	−1.954;−0.549	**<0.01**	−1.267	−1.972;−0.562	**<0.01**
CCF	FGR	−1.413	−2.500;−0.326	**0.01**	−1.295	−2.595;0.003	0.05
	CHD	0.012	−0.829;0.963	0.98	0.000	−0.835;0.835	0.99

Legend: Data are presented in beta values [ß] with corresponding 95%CI and p values, compared to controls. Significant results [p<0.05] are in **bold**. Model 1 represents the crude model using GA and its polynomials as predictor and type of case as covariate of interest. Model 2 is the fully adjusted model adjusted for the covariates in model 1 and for serial measurements of fetal weight and sex.
Abbreviations: CI, confidence interval; CC, corpus callosum length; CCF, corpus callosum fastigium length; FGR, fetal growth restriction; CHD, congenital heart defect.

DISCUSSION

Here, we demonstrate that CCF and CC length may serve as reliable markers for monitoring prenatal and postnatal brain growth. Fetuses with FGR showed decreased growth of both CC and CCF length, while in fetuses with CHD, only CC growth was decreased between 22 and 42 weeks GA.

Our findings suggest that we are able to bridge the traditional division between fetal and neonatal US growth charts. To date, studies that combine fetal and neonatal US markers of brain growth in a single cohort are scarce. One explanation is that standard prenatal US planes containing easily recognizable landmarks of the brain do not correspond well with the standardized planes accessible by cranial ultrasound. This lack of correspondence results in differences in prenatal and postnatal measures and measuring methods. For example, head circumference assessed prenatally, calculated from the biparietal diameter and occipital frontal diameter, correlates poorly to direct postnatal measurement with a tape measure.[99,268] Furthermore, changes in head shape can be induced by delivery [e.g., skull molding, edematous swelling, and hematomas].

In contrast to other prenatal US measurements, excellent reliability was shown for CC and CCF length, comparable with the reliability of the postnatal measurements.[264] On the basis of our data, we suggest that CCF length is the most reliable and relevant marker for monitoring brain growth. CCF length can be assumed to be a composite marker of multiple brain structures with different embryological origins. Therefore, CCF length may be a better representative of global brain growth than previous sonographic markers based on individual brain structures.[237–241]

Growth trajectories of CC and CCF length were decreased in fetuses at risk for abnormal brain growth and impaired long–term neurodevelopmental outcome. While studies using CCF length have not been published in literature before, CC length findings are in line with those in previous literature. The decreased CC growth trajectories in FGR fetuses are in accordance with findings of a recent MR imaging study that showed significantly reduced CC length in fetuses with FGR compared to CC length of appropriate–for–gestational–age controls.[269] Results from our previous study in preterm infants demonstrated a similar association between CCF length and birth weight SD score.[264] There are, to the best of our knowledge, no publications on CC length in CHD fetuses with which to compare our results, though previous studies did report anomalies of the CC and reduction of

CC volume in children with CHD.[270,271] The decreased growth trajectory of CC length in fetuses with CHD is, however, supported by accumulating evidence reporting that fetuses with CHD are at risk for abnormal brain growth and development.[272–275]
Brain growth is an important predictor of neurodevelopmental outcome.[98,234,276] We hypothesize that the decreased CC and CCF growth trajectories observed in cases may have consequences for long–term outcome. In preterm infants, a shorter CC length is related to a higher risk of an adverse neurodevelopmental outcome at 2 years corrected age.[235] Moreover, a significantly smaller corpus callosum was found in individuals with schizophrenia and autism.[277,278] CCF length represents the diencephalon and thus includes thalamus development, which is crucial for cognitive functioning. Derangements in thalamus development are associated with adverse neurodevelopmental outcome.[251] Yet, the clinical relevance for neurodevelopmental outcome of differences in CC and CCF growth trajectories needs further investigation.

CLINICAL APPLICABILITY

The landmarks used for CC and CCF length measurements are relatively easy to distinguish on US images. Prenatally, the main challenge is obtaining an exact midsagittal plane. The prenatal success rates are predominantly influenced by acoustic shadowing and the position of the fetus. 3D US can enhance precision by manipulating volumes to reconstruct the exact midsagittal plane.[279–281] When a midsagittal plane is obtained, both measurements take <1 minute in experienced hands. Postnatally, a standard midsagittal plane is easy to obtain through the anterior fontanelle; also, the offline measurements of CC and CCF length take <1 minute. Newly developed software that enables the identification of the midline automatically could still improve the measurements for clinical practice.[282]

STRENGTHS AND LIMITATIONS

Some considerations should be taken into account. First, our study was conducted in a tertiary hospital population, with a relatively high maternal age, mainly of western origin, and a high educational level. Therefore, replication of the data is warranted to validate our findings for the general population. Second, the small number of cases limits the conclusions of our study. We cannot exclude that absence of statistically significant findings may be due to lack of power. Third, the growth charts are based on measurements at four time points and may improve by including intermediate

time points to further smooth the curves. Finally, the US scans and measurements were performed by experienced observers, which potentially enhanced quality of the midsagittal images and thereby success rates and reliability. Clinical applicability may be overestimated as a consequence. Success rates of the measurements were mostly influenced by fetal position, which we assume to be independent of the variables in this study. We consider the prospective and longitudinal study design as strength of our study. Combining prenatal and postnatal measurements in one reference curve is an innovative method to facilitate monitoring of fetuses at risk of impaired brain growth.

FUTURE IMPLICATIONS FOR CLINICAL CARE AND RESEARCH

Tight collaboration between obstetric and neonatal researchers and caregivers is needed for bridging the gap when monitoring fetal and neonatal brain growth. This is of great importance for optimizing neurodevelopmental care in fetuses and infants at risk for abnormal brain growth and neurodevelopmental impairment. Easily applicable US tools that can be used independent of the prenatal or postnatal period will have clinical implications. We consider our reference curves useful for age–equivalent preterm infants as they are largely comparable to the postnatal reference curves between 24 and 32 weeks in preterm infants from Roelants et al.[264] In addition, CC and CCF length measurements may be applicable from midgestation onward and may theoretically be prolonged until closure of the anterior fontanelle in the first year of life. Future research should correlate these measurement to commonly used MR imaging markers and explore the link between the growth measures and functional neurodevelopmental outcome.

CONCLUSIONS

In this prospective cohort we demonstrated that CC and CCF length measurements are reliable markers for brain growth from the fetal into the early neonatal period. By combining prenatal and postnatal CC and CCF length measurements in 1 reference curve, we created a continuum for monitoring brain growth, irrespective of the intra– or extra–uterine environment. We demonstrated that fetuses at risk for abnormal brain growth [i.e., those with CHD and FGR] showed significantly decreased CC and CCF growth between 22 and 42 weeks GA. Whether these markers could serve as early predictors for neurodevelopmental outcome in later life warrants further research.

TWO–YEAR FOLLOW–UP OF A RANDOMIZED CONTROLLED NUTRITION INTERVENTION TRIAL IN VERY–LOW–BIRTH–WEIGHT INFANTS

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ABSTRACT

BACKGROUND Very–low–birth–weight [VLBW] infants are at risk for neurodevelopment impairment. This study assessed the effect of early aggressive parenteral nutrition [PN] on long–term outcome in VLBW infants.

MATERIALS AND METHODS Directly after birth, VLBW infants [birth weight <1500 g, n=142] were randomized to five different PN regimes. Controls [n=46] received glucose and standard–dose amino acids [AAs; 2.4 g/kg/d] from birth onward and pure soybean oil fat emulsion [SOY] on the second day of life. Two intervention groups received glucose, standard–dose AAs, and lipids from birth onward; SOY [n=24] or mixed fat emulsion [MIX, n=25]. The two other intervention groups received glucose, high–dose AAs [3.6 g/kg/d], and lipids from birth onward; SOY [n=24] or MIX [n=23]. The primary outcome of this follow–up study was the composite outcome of "death or major disability" at 2 years corrected age. Secondary outcomes were death, major disabilities, neurodevelopmental scores, and anthropometry.

RESULTS Follow–up rate was 92% [n=134]. Thirty–five [26%] infants had died or had a major disability, with no differences between intervention groups and controls. Increased odds for death were observed in the standard–dose AA–MIX group [OR 5.4, 95% confidence interval 1.1;27.0]. Neurodevelopmental scores and incidence of major disabilities did not differ between groups. Growth in the high–dose AA–MIX group was enhanced compared with controls at 2 years corrected age [+0.51 [0.01;1.02] weight SD score].

CONCLUSIONS This randomized controlled hypothesis–generating study demonstrated no beneficial effect of early high–dose AA administration and mixed fat emulsions on survival and neurodevelopmental outcome in VLBW infants, although growth was enhanced.

INTRODUCTION

Preterm birth is the leading cause of perinatal morbidity and mortality and contributes significantly to long–term neurodevelopmental impairment and growth failure.[42,283] Nutrition in the early postnatal phase is pivotal for growth and development of all organs, including the brain. This is especially true for very low–birth–weight [VLBW] infants. Over the past decades, major changes in nutritional regimes have been implemented in clinical practice aiming to prevent iatrogenic malnutrition. Current regimes include early parenteral feeding with carbohydrates and amino acids [AAs] from birth onwards, and the start of fat emulsions soon after birth.[48]

Short–term effects of these "aggressive" parental feeding strategies have been well studied.[284–286] Stable isotope and nitrogen balance studies showed higher anabolic rates after early, aggressive parenteral nutrition [PN].[52,53,285] Long–term outcomes of nutrition intervention trials have been little studied.[287–289] Several large cohort studies showed, however, that nutrient delivery in the first week of life has long–term implications for growth, neurodevelopment, and the development of neonatal diseases.[290–292] Randomized controlled trials [RCTs] assessing the long–term effect of high–dose AAs in combination with lipids from different sources from birth onward in VLBW infants have not been performed yet.

In this report we describe the 2–year survival and neurodevelopmental outcome in VLBW infants who received different PN regimes in an earlier performed RCT. We hypothesized that giving high–dose AAs combined with lipid administration directly after birth would result in improved survival, neurodevelopmental outcome, and growth up to 2 years corrected age. Secondly, we hypothesized that mixed fat emulsions would improve neurodevelopmental outcome compared with pure soybean oil fat emulsions.

METHODS

STUDY DESIGN

This study describes the planned follow–up of an RCT on early PN in VLBW infants.[53] This RCT was conducted between December 2008 and January 2012 at the level IV neonatal intensive care unit [NICU] of the Erasmus MC – Sophia Children's Hospital, Rotterdam, the Netherlands [registered at TrialRegister.nl: NTR1445]. Inborn infants with a birth weight <1500 grams were eligible for inclusion. The exclusion criteria were congenital anomalies [including chromosomal defects]; metabolic diseases; renal, hepatic or endocrine disorders; and other disorders interfering with growth or neurodevelopment. The local medical ethical review board approved the study protocol. Written informed consent was obtained prior to inclusion. The methods, including randomization procedure, and short–term outcome results [safety and efficacy assessed by nitrogen balances and stable isotope quantification] of this RCT were published previously.[52,53,207] The nutritional interventions are summarized below.

NUTRITION PROTOCOL

Immediately after birth, all infants received 6 mg/kg/min glucose and 2.4 g/kg/d AAs as standard care. Within 6 hours after birth, they were randomized, in a factorial trial design, to one of five study groups [**Supplemental table 1**]:

CONTROL GROUP Glucose and standard AA administration was continued during the first 2 days of life. Lipid administration [pure soybean oil fat emulsion, SOY] was started at 1.4 g/kg/d on the second day of life and increased to 2.8 g/kg/d the next day.

STANDARD [ST] AA + SOY/MIX Glucose and AA dosage was similar to that of the control group. Lipids were started at 2 g/kg/d immediately after birth and increased to 3 g/kg/d on the second day of life. Infants were 1:1 randomized to either SOY or mixed fat emulsion [MIX].

HIGH AA + SOY/ MIX Standard glucose was given, similar to the other study groups. In addition, a higher dose of AAs [3.6 g/kg/d] and 2 g/kg/d lipids were given from birth onward. Lipids were increased to 3 g/kg/d on the second day of life. Infants were 1:1 randomized to either SOY or MIX.

After day 3, the intervention ended and thereafter the attending physician followed the local standardized protocol.[195] The enteral nutrition [EN] protocol included minimal enteral feeding at day 1 and a daily gradual increment of approximately 20 mL/kg/d of enteral bolus feeding from day 2 or 3 onwards until 150–180 mL/kg/d was reached. PN was ceased when 130 mL/kg/d of enteral feeding was tolerated. Breastfeeding was strongly encouraged, but if expressed breast milk was insufficiently available, preterm formula was supplemented to realize the intended enteral intake [Nenatal Start; Nutricia, Zoetermeer, the Netherlands]. Breast milk was fortified when infants tolerated at least 100 mL/kg/d enterally until discharge. Formula fed infants received Nenatal 1 at discharge home [Nutricia, Zoetermeer, the Netherlands].[195]

All infants received the same AA solution: Primene 10% [Baxter, Utrecht, the Netherlands]. Infants in the control group received lipids based on pure soybean oil, Intralipid 20% [SOY], while infants in the intervention groups were randomized to either Intralipid 20% or SMOFlipid 20%, which is a MIX containing 30% soybean oil, 30% medium–chain triacylglycerol, 25% olive oil, and 15% fish oil [both Fresenius Kabi, Bad Homburg, Germany]. According to the local protocol, parenteral AA administration was temporarily lowered if plasma urea concentrations were >10 mmol/l and temporarily stopped if plasma urea concentrations were >14 mmol/l. Similarly, parenteral lipid administration was temporarily lowered when triacylglycerol concentrations were >3 mmol/l, and temporarily stopped if triacylglycerol concentrations were >5 mmol/l.[8] Nutritional intakes were in accordance with the study protocol.[53] Data of all infants were analyzed based on the "intention–to–treat" principle.

OUTCOME MEASURES

The primary outcome [adverse outcome] was defined as the composite outcome of death or major disability at 2 years corrected age. Secondary outcomes were death; major disabilities; neurodevelopmental scores [mental and psychomotor score] based on the Bayley Scales of Infant and Toddler Development, Third Edition [BSID III] at 2 years corrected age; and anthropometry at 6 weeks and 2 years corrected age.

Major disabilities included cerebral palsy [CP, Gross Motor Function Classification System level [GMFCS] ≥2][293], severe hearing loss [deaf or neurosensory hearing loss partly corrected by hearing aids], severe visual impairment [blind or severe visual

impairment], and mental or psychomotor score <70 points [<−2 standard deviation [SD]].

All surviving infants were examined at the outpatient clinic at 6 weeks and 2 years corrected age as part of our standard assessment program on neurodevelopment and growth after NICU admission. Study group allocation was blinded to all examiners. Certified psychologists and developmental therapists assessed the neurodevelopmental outcome using the composite BSID III scores of cognition [mental score] and motor development [psychomotor score]. The diagnosis CP was confirmed by an experienced pediatric−neurologist.

Anthropometric data at birth, discharge or 40 weeks corrected age, whichever came first [both Fenton growth chart], and at 6 weeks and 2 years corrected age [both World Health Organization [WHO] growth chart] included sex and gestational age−corrected SD scores for weight, length, and head circumference [HC] of surviving infants.[171 222] Weight and HC data at birth were available. Length was not measured at birth. Mean delta [Δ] SD scores for weight and HC were calculated between birth and 6 weeks and between birth and 2 years corrected age, taking into account the time between the measurements. The following formulas were used:

Mean ΔSD/week: $$\frac{\text{[SD score 6−weeks − SD score birth]}}{\text{[[date of measurement − date of birth]/7]}}$$

Mean ΔSD/month: $$\frac{\text{[SD score 2−years − SD score birth]/}}{\text{[[date of measurement − date of birth]/31]}}$$

In case of missing data, all available clinical information was reviewed on the presence of major disabilities. The occurrence of an adverse outcome, death or major disability [yes/no] in infants who were not tested at two years corrected age was established from medical records and information from parents if available. They were left out of the scores for the separate tests of the BSID III.

CLINICAL DATA

Obstetrical data, neonatal data on gestational age at birth, birth weight, sex, socio–economic status [SES], and data on neonatal morbidity and neonatal outcomes were collected during the study. Definitions of neonatal outcomes have been published before.[53] SES SD scores were validated ZIP–code–derived SD scores based on local average income, low–income rate, low educational level rate, and unemployment rate.[294]

STATISTICAL ANALYSIS

The data are presented as mean [SD], median [interquartile range [IQR]] or number [percentage]. A 2–tailed p value of <0.05 was considered statistically significant. Sample size calculation was based on the original trial's primary, short–term outcome: nitrogen balance.[53]

Differences in baseline characteristics between children included in the analyses and those lost to follow–up were analyzed using Mann–Whitney test or the Fisher exact test, as appropriate.

Associations between the interventions and the primary and secondary outcomes were analyzed using multivariable logistic or linear regression analysis as appropriate and expressed in odds ratios [OR] or effect sizes [β] with 95% confidence intervals [CIs]. If the count of outcomes was zero in a group and regression analysis was therefore not possible, univariable analysis was performed using the Fisher's exact test. Based on the literature and availability of prospectively collected data we identified the following potentially relevant confounders for the multivariable analyses: gestational age at birth, birth weight SD score [or HC SD score for analyses using HC as an endpoint], sex, and SES SD scores. All intervention groups were added to the model as a dummy variable. Collinearity diagnostics were performed on these covariates before definite decision on their use in the multivariable regression model. Correlations between the covariates were <0.60. All residuals of the linear regression analyses were distributed approximately normally. Potential effect modification between the AA and lipid interventions [AA dosage * lipid source] on the primary and secondary outcomes was assessed by adjustment for interaction terms.

Differences between groups in mental and psychomotor scores were assessed with univariable analysis using Kruskal Wallis and multivariable analysis using linear regression. No correction for multiple testing was applied.[295]

Data were collected prospectively and analyzed using SPSS package 21.0 [IBM SPSS Statistics, Armonk, NY].

RESULTS

Of the 144 infants included in the original trial, two infants were excluded because of congenital disorders interfering with neurodevelopmental outcome. Eight infants [6%] were lost to follow–up, mainly due to emigration [**Supplemental figure 1**]. The BSID III was administered in 103 infants [93% of the surviving infants]. These children all completed the mental assessment. Ninety of these infants [87%] also completed the psychomotor assessment. In **Table 1**, baseline and neonatal characteristics are given for the whole group [n=134] and for the different study groups. Baseline characteristics did not differ between included and those lost to follow–up [data not shown].

PRIMARY OUTCOME

In total, 35 infants [26%] had an adverse outcome at 2 years corrected age; 23 infants had died and 12 infants had one or more major disabilities. No association was found between study group allocation and the primary outcome [**Table 2**]. The addition of an interaction–term to evaluate potential effect modification between the AA and lipid interventions did not alter the results of the analyses.

SECONDARY OUTCOMES

The presence of major disabilities at 2 years corrected age did not differ between study groups [**Table 2 and 3**]. However, after adjustment for potential confounders, infants in the standard AA and mixed fat emulsion group had a 5.4 [95%CI 1.1;27.0, p=0.040] times higher odds for death before 2 years corrected age than infants in the control group. Effect modification between AA and lipid interventions was not found.

The mental and psychomotor scores in all infants were 100 [IQR, 90;105] and 100 [IQR, 91;107], respectively. No differences were found between study groups [**Supplemental table 2**].

Weight SD score dropped between birth and 6 weeks corrected age in all groups. Hereafter, catch–up growth was seen for all groups up to 2 years corrected age [**Table 4 and Figure 1**]. HC SD score was stable or had even slightly increased between birth and 6 weeks corrected age, and also showed catch–up growth between 6 weeks and 2 years corrected age. At 6 weeks corrected age, SD and Δ SD scores for weight and HC

were significantly higher in the infants given high–dose AA and mixed fat emulsion [high AA + MIX] compared to those in the control group. At 2 years corrected age, only the weight scores were higher in the high AA + MIX group [**Table 4**]. Length SD scores did not differ between groups at both time points [data not shown].

TABLE 1 | BASELINE CHARACTERISTICS FOR TOTAL COHORT [n=134] AND PER STUDY GROUP

	All infants [n=134]	Control group [n=44]	St AA + SOY [n=21]	St AA + MIX [n=24]	High AA + SOY [n=24]	High AA+MIX [n=21]
Sex [male]	64 [48]	23 [52]	10 [48]	11 [46]	13 [54]	7 [33]
GA [weeks+days]	26^{+6} [25^{+5};29^{+0}]	27^{+3} [26^{+2};29^{+3}]	26^{+2} [25^{+2};28+1]	27^{+1} [25^{+6};28^{+6}]	26^{+5} [25^{+2};28^{+2}]	27^{+1} [26^{+2};28^{+2}]
BW [grams]	848 [680;1000]	863 [651;1013]	808 [665;920]	846 [726;1000]	775 [680;988]	850 [685;1078]
BW SD score	−0.4 [−1.3;0.3]	−0.7 [−1.6;0.1]	−0.2 [−1.4;0.5]	−0.4 [−0.8;0.3]	−0.6 [−1.2;0.2]	−0.4 [−1.2;0.5]
SGA at birth [<–2 SD for weight]	8 [6]	6 [14]	0 [0]	0 [0]	1 [4%]	1 [5%]
Prenatal steroids [0/1/2 doses]	3/26/105 [2/19/79]	1/5/38 [2/11/87][1]	1/2/18 [5/9/86]	0/9/15 [0/38/62]	1/5/18 [4/21/75]	0/5/16 [0/24/76]
Caesarean section	87 [65]	29 [66]	14 [67]	16 [67]	14 [58]	14 [67]
Apgar score [5 min]	8 [6;9]	8 [7;9]	8 [7; 9][3]	8 [6;9]	7 [6;9]	8 [7;9]
CRIB score	4 [1;8]	4 [1;9]	4 [1;8]	4 [1;7]	4 [1;9]	5 [1;8]
SES SD score	−0.5 [−1.8;0.4]	−0.3 [−1.8;0.5]	−0.5 [−2.1;0.4]	−0.5 [−1.7;0.4]	−0.7 [−1.7;0.3]	−0.4 [−1.2;0.3]
NEC stage≥ IIA	5 [4]	2 [5]	2 [10]	1 [4]	0 [0]	0 [0]
Late–onset sepsis	40 [30]	7 [17]	11 [48]	6 [26]	9 [41]	7 [32]
ROP gr III or higher	4 [3]	2 [5]	0 [0]	0 [0]	2 [8]	0 [0]
Mechanical ventilation days	5 [1;18]	5 [0;12]	11 [1;24]	4 [1;7]	7 [1;22]	5 [0;16]
Severe brain injury[2]	7 [5]	2 [5][3]	0 [0]	2 [8]	1 [4]	2 [10][3]

Legend: All data is presented in n,% or median [interquartile range]. [1] One mother received four doses of prenatal steroids. [2] Severe brain injury is defined by intraventricular hemorrhage grade III or IV [based on Papille], periventricular leukomalacia grade II or higher, venous infarction. [3] 1 missing value.
ROP and days on mechanical ventilation were not scored in infants who had died. NEC was not scored in the infants who died of another cause than NEC.
Abbreviations: AA, amino acid; BW, birth weight; CRIB, clinic risk index for babies; GA, gestational age at birth; MIX, mixed lipid emulsion; NEC, necrotizing enterocolitis; ROP, retinopathy of prematurity; SD score, standard deviation score based on Fenton; SES, socio–economic status; SOY, soybean oil lipid emulsion; St, standard–dose.

TABLE 2 | LOGISTIC REGRESSION ANALYSES BETWEEN CONTROL GROUP AND THE FOUR STUDY GROUPS SEPARATELY

	Major disabilities				Death				Adverse outcome			
	Crude OR [95%CI]	p value	Adjusted OR [95%CI]	p value	Crude OR [95%CI]	p value	Adjusted OR [95%CI]	p value	Crude OR [95%CI]	p value	Adjusted OR [95%CI]	p value
St AA + SOY	NA[1]	0.30[2]	NA[1]	NA[1]	1.8 [0.4;7.7]	0.41	2.1 [0.4;11.5]	0.41	0.9 [0.2;3.4]	0.90	0.7 [0.2;3.0]	0.62
St AA + MIX	0.4 [0.05;4.1]	0.47	0.3 [0.03;3.3]	0.35	2.6 [0.7;9.6]	0.15	5.4 [1.1;27.0]	0.04	1.6 [0.5;5.0]	0.42	1.9 [0.5;6.7]	0.33
High AA + SOY	1.4 [0.3;7.0]	0.66	1.2 [0.2;6.2]	0.83	2.1 [0.5;8.0]	0.30	2.1 [0.4;10.4]	0.36	1.9 [0.6;6.0]	0.25	1.7 [0.5;6.0]	0.38
High AA + MIX	2.3 [0.5;10.5]	0.26	1.8 [0.4;9.1]	0.44	1.3 [0.3;6.0]	0.74	2.1 [0.3;12.4]	0.42	1.9 [0.6;6.2]	0.26	2.5 [0.7;9.3]	0.16

Legend: Shown are odds ratios [OR], 95% confidence intervals [CI] and p values for the outcomes in the separate intervention groups compared to the control group. An OR < 1 favors the intervention and an OR > 1 favors the control. Adjusted for: birth weight standard deviation score, gestational age at birth, sex and socio–economic status. Definitions of outcomes are defined in the method section. Significant results [p<0.05] are printed in **bold**. [1] No major disabilities in intervention group 'st AA + SOY'. [2] Analyzed using the Fisher's exact test.
Abbreviations: AA, amino acid; CI, confidence interval; MIX, mixed lipid emulsion; NA, not applicable; OR, odds ratio; SOY, pure soybean oil emulsion; St, standard–dose.

TABLE 3 | ADVERSE OUTCOMES FOR TOTAL GROUP AND PER STUDY GROUP

	All infants [n=134]	Control group [n=44]	St AA + SOY [n=21]	St AA + MIX [n=24]	High AA + SOY [n=24]	High AA + MIX [n=21]	Number missing/ available infants
Death	23 [17]	5 [11]	4 [19]	6 [25]	5 [21]	3 [14]	0/134
Mental score <70	5 [4]	2 [5]	0 [0]	1 [4]	1 [4]	1 [5]	8/111
Psychomotor score <70	4 [3]	2 [5]	0 [0]	1 [4]	0 [0]	1 [5]	21/111
Severe visual problems	1 [3]	1 [2]	0 [0]	0 [0]	0 [0]	0 [0]	3/111
Severe hearing problems	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]	3/111
Cerebral palsy	7 [5]	1 [2]	0 [0]	1 [4]	2 [8]	3 [14]	0/111
Major disability	12 [9]	4 [9]	0 [0]	1 [4]	3 [13]	4 [19]	0/134
Adverse outcome	35 [26]	9 [20]	4 [19]	7 [29]	8 [33]	7 [33]	0/134

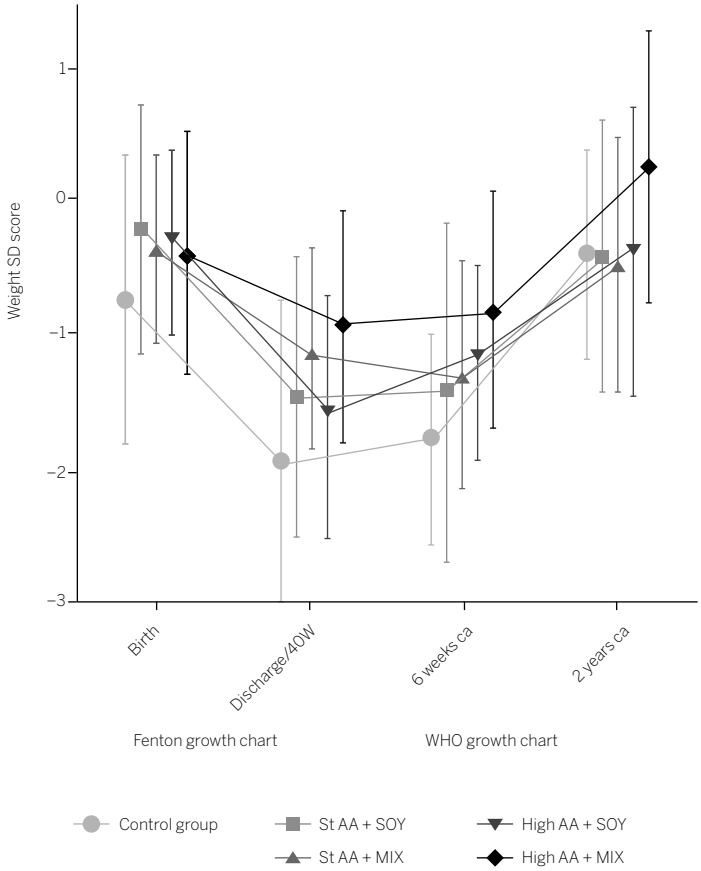
Legend: All data are presented in number [%].
Multiple major disabilities in one patient is possible. An adverse outcome is defined as one or more major disabilities or death before 2 years corrected age. Of the patients alive with missing data, information was obtained from medical records and parents to categorize them in major disability yes/no.
Abbreviations: AA, amino acid; MIX, mixed lipid emulsion; n, number; SOY, soybean oil lipid emulsion; St, standard–dose.

TABLE 4 | ANTHROPOMETRIC ANALYSES AT 6 WEEKS AND 2 YEARS CORRECTED AGE USING MULTIVARIABLE LINEAR REGRESSION

			St AA + SOY	St AA + MIX	High AA + SOY	High AA + MIX
			Adjusted β [95%CI] p value	**Adjusted β [95%CI] p value**	**Adjusted β [95%CI] p value**	**Adjusted β [95%CI] p value**
6 weeks	**Weight**	SD score	0.11 [−0.42;0.64] p=0.68	0.27 [−0.24;0.79] p=0.29	0.42 [−0.10;0.94] p=0.11	0.81 [0.29;1.33] **p=0.003**
		ΔSD/week	0.006 [−0.02;0.03] p=0.68	0.014 [−0.01;0.04] p=0.30	0.023 [−0.004;0.05] p=0.09	0.043 [0.02;0.07] **p=0.002**
	HC	SD score	0.03 [−0.62;0.69] p=0.92	0.08 [−0.56;0.71] p=0.82	0.32 [−0.35;0.99] p=0.35	0.83 [0.20;1.47] **p=0.011**
		ΔSD/week	-0.01 [-0.04;0.03] p=0.96	0.00 [−0.03;0.03] p=0.99	0.02 [−0.02;0.05] p=0.31	0.05 [0.00;0.08] **p=0.008**
2 years	**Weight**	SD score	−0.18 [−0.69;0.33] p=0.48	−0.22 [−0.73;0.29] p=0.40	−0.03 [−0.54;0.48] p=0.90	0.51 [0.01;1.02] **p=0.048**
		ΔSD/month	−0.006 [−0.03;0.01] p=0.51	−0.008 [−0.03;0.01] p=0.44	−0.001 [−0.02;0.02] p=0.90	0.02 [0.00;0.04] **p=0.047**
	HC	SD score	−0.69 [−1.4;0.01] p=0.053	−0.48 [−1.23;0.26] p=0.20	−0.30 [−1.00;0.41] p=0.40	0.61 [−0.07;1.28] p=0.08
		ΔSD/month	−0.025 [−0.05; 0.00] p=0.06	−0.018 [−0.046;0.01] p=0.20	−0.010 [−0.04;0.02] p=0.44	0.02 [−0.002;0.05] p=0.07

Legend: Shown are the results of multivariable linear regression [effect size, 95% confidence interval and p value] of standard deviation [SD] score and SD score change from birth to 6 weeks corrected age per week [n=109] and from birth to 2 years corrected age per month [n=103] per study group compared to the control group. Significant results [p<0.05] are printed in **bold**. Data are adjusted for birth weight SD score or head circumference SD score [if head circumference was used as outcome], gestational age at birth, sex, and socio−economic status.
Abbreviations: AA, amino acid; MIX, mixed lipid emulsion; SOY, pure soybean oil emulsion; St, standard−dose.

FIGURE 1| GROWTH PER STUDY GROUP FROM BIRTH TO 2 YEARS CORRECTED AGE



Legend: On the x–axis are depicted the 4 different time points of growth assessment: birth, discharge or 40 weeks corrected age [term age], 6 weeks corrected age, and 2 years corrected age. On the y–axis is depicted the SD score of weight using the Fenton growth chart [birth and discharge] and the WHO growth chart [6 weeks and 2 years corrected age].
Abbreviations: AA, amino acid; ca; corrected age; MIX, mixed lipid emulsion; SD, standard deviation; SOY, soybean oil lipid emulsion; st, standard–dose.

DISCUSSION

To our knowledge, this is the first RCT investigating the long–term effect of high–dose AA and mixed lipid administration in the first days of life in VLBW infants. This hypothesis–generating study did not find a beneficial effect of the interventions on survival with normal neurodevelopmental outcome at 2 years corrected age. Neither did it find a beneficial effect of any of the interventions on neurodevelopmental scores and incidence of major disabilities. However, infants who had received high–dose AA and mixed fat emulsion from birth onward showed enhanced growth up to 2 years corrected age. Surprisingly, the odds for death were higher in the standard–dose AA and mixed fat emulsion group, which will be discussed below.

Small nutritional interventions in the first days of life can have long–lasting effects.[296] When we were designing this study, we hypothesized that the interventions in this trial would influence long–term outcome based on the following mechanisms. First, increased AA provision leads to increased protein synthesis and thus growth and development, provided sufficient energy is supplied.[297] Early start of parenteral lipids can, at least partly, improve the caloric intake. Second, mixed fat emulsions might be more beneficial for long–term development than pure soybean oil emulsion, based on three biological mechanisms: [1] a higher supply of very long–chain polyunsaturated fatty acids [e.g., docosahexaenoic acid [DHA]], essential for central nervous system development; [2] administration of fewer phytosterols, which are thought to be the hepatotoxic component of soybean oil fat emulsion; and [3] possibly decreased rates of septicemia, which could prevent brain injury.[298,299–301] The short–term results of this study indeed showed improved nitrogen balances, enhanced growth, higher albumin synthesis rates, and lower phytosterol levels in blood. Effects on other possible mechanistic factors for later outcome, such as septicemia, bilirubin levels, or duration of intravenous fluids, were not found.[52,53,300]

In this follow–up study, we found insufficient evidence for any positive effect of early high–dose AA administration, early lipid administration, or the use of a mixed fat emulsion on neurodevelopmental outcome. These results need to be interpreted with caution because of the low power: absence of evidence is not evidence of absence. The failure to detect a neurodevelopmental effect might be due to several reasons. First,

the actual differences in intake might have been too small and the intervention period might have been too short to generate a long–lasting effect on neurodevelopmental outcome in a relatively small sample size.[53] Second, the effect might also be diluted by the heterogeneous study group of infants. Extremely low–birth–weight infants can be expected to benefit most from the interventions. Unfortunately, the small sample size did not allow for subgroup analysis in this specific patient group. Third, the instruments used to detect neurodevelopmental impairment might not be sensitive enough to detect small differences in cognitive outcome. Specifically the BSID III may lack sensitivity to detect subtle differences in clinical outcome that may still be clinically relevant.[302]

It is certainly also possible that there was truly no effect of the interventions on neurodevelopment. Other trials with comparable AA intakes, but without lipid interventions, also found no effect on neurodevelopmental outcome.[288,289] The ideal composition of AA solutions and the balance between energy and AA intake, necessary for optimal protein synthesis and long–term effects, are still unknown. In addition, recent insights on the developmental origins of health and disease [DOHaD] hypothesis suggest that the effect of fetal programming may be more relevant on the long–term outcome than interventions postnatally.[4]

Unexpectedly, we found increased odds for death in the study group that had received standard–dose AA together with mixed fat emulsion. We wonder whether this finding reflects a true effect, as no such effect was seen in the other groups with comparable AA dosage [st AA + SOY] or in the other group that also received a mixed fat emulsion [high AA + MIX]. Moreover, proper judgement on mortality is difficult due to the small sample size. Correction for multiple testing would lead to the conclusion that the finding was not statistically significant. The finding cannot easily be explained by differences in baseline characteristics between the randomized study groups.[53] Also in current literature comparable findings cannot be found: mixed lipid emulsions from birth onward were not associated with an increased risk of adverse outcome in prior research.[303,304] Moreover, previous literature questioned the safety of high–dose AA administration, but not of standard–dose AA administration.[212,213] A biochemical explanation for a harmful interaction thus seems not likely.
However, to further explore the trend for mortality and to increase power, we performed three multivariable post–hoc analyses on the three outcomes. In these analyses, we

combined intervention groups according to [1] lipid source: MIX [n=45] vs SOY [n=45], [2] AA dosage: high–dose [n=45] vs standard dose [n=45], and [3] timing of lipid introduction as follows: early lipid [all intervention groups combined, n=90] vs late introduction [controls, n=44]. In the first two post–hoc analyses, which only included intervention groups, no significant associations were found for all outcomes [data not shown]. The third analysis showed a nonsignificant adjusted OR for death of 2.7 [95%CI 0.76;9.4, p=0.128]. For the other outcomes, we did not find a significant association either [data not shown].
In conclusion, we believe that the increased risk for death in the standard AA + MIX group most likely reflects no true effect but rather a false–positive finding [type I error]. Large multicenter trials are needed to confirm our findings, but those are difficult to perform in this specific vulnerable study population. Therefore, meta–analysis of multiple RCTs is likely necessary to draw conclusions on long–term effects of early nutrition interventions.

The neurodevelopmental scores in our study may seem rather high for preterm infants, even taking into account the general overestimation of the BSID III compared to the BSID II.[305–307] An overall improvement of neurodevelopmental outcome over time may explain this finding. Note, however, that fewer motor scores than mental scores were available. This reflects refusal of assessment or incomplete assessment due the fatigue in most cases. Fatigue may also have influenced the results of tests that were fully performed, possibly resulting in lower scores. However, the contrary is also plausible; infants who are less capable of performing the test might have had a higher refusal rate, which might have led to selection and overestimation of the capacities of the group. The BSID III is widely applied in clinical practice and in research but does not seem a very sensitive test for assessment of neurodevelopment in infants. Follow–up into school age may reveal more subtle differences in brain function.

Between birth and discharge, weight SD score decreased in all study groups, while HC SD scores remained relatively stable. Between discharge and 6 weeks and 2 years corrected age, weight and HC showed catch–up growth. In the first period, in which brain sparing is observed, this might be the result of non–individualized nutrition practices and restrictions in nutrient intake by fluid restriction and [biochemical] intolerance of PN and EN. The catch–up growth after 6 weeks corrected age might be the result of

a high caloric and protein intake via discharge formula and of nutrition practices more geared to the infants' needs and personal appetite.

Of interest is the higher growth from birth onward observed in the high AA + MIX group. Analyses showed an increased SD change at both time points for the high AA + MIX group: +0.04 SD score/week at 6 weeks and +0.02 SD score/month at 2 years corrected age. This means that an infant in the high AA + MIX group, born, for example, at 28 weeks, has on average a 0.04 x [40 – 28 + 6] = 0.72 higher SD score for weight at 6 weeks corrected age compared to an infant in the control group born at the same gestational age. We realize that we did not correct for multiple testing, and therefore the effect might be a false positive finding. However, the observed difference is rather large and may well be of clinical relevance. As the increased growth was not accompanied with measurable improved neurodevelopmental outcome, one can debate whether this increase in growth benefits metabolic health. Unfortunately, we do not have data on body composition or other markers of cardiometabolic health in this study.

LIMITATIONS

Some aspects of the design are worth mentioning. Despite the high follow–up rate [92%], the sample size remained relatively small, which is reflected by the wide confidence intervals. As a result of the small sample size, we were not able to perform per protocol analysis in addition to the intention–to– treat analysis. As discussed above, due to the limited power in our study, we consider our data as hypothesis generating.

We decided to adjust for different maternal and perinatal characteristics and not for postnatal variables. Hereby, we prevented adjustment for factors that may actually be the result of the nutrition interventions, but we may have overlooked relevant confounders.

We did not correct for multiple testing, as it is still unclear when and how correction for multiple testing should be performed. We advise to judge the clinical relevance of the different findings on the effect sizes and confidence interval rather than on p values and their cut–offs.[295,308]

The question of which growth chart to use in the neonatal period and during follow–up of preterm infants is still under debate. As recommended, we used two consecutive references, the Fenton and the WHO growth charts, which complicates the interpretation

of growth between discharge and 6 weeks corrected age.[197] Focus should therefore primarily be on the post–discharge long–term growth. We did not have information on EN after the intervention period, such information may have provided more insight in the long–term growth findings.

CONCLUSION

We reported on the 2–year follow–up of an earlier performed nutrition intervention RCT on the effect of parenteral high–dose AA combined with mixed fat emulsion administration from birth onward in VLBW infants. No beneficial effects of the interventions on survival and neurodevelopmental outcome at 2–years corrected age could be demonstrated, although enhanced growth was observed. We recommend future researchers studying nutrition interventions in VLBW infants to focus on follow–up with sensitive tests for the detection of neurodevelopmental impairment and to strive for large study populations.

SUPPLEMENTAL TABLE 1 | NUTRITION PROTOCOL DURING INTERVENTION PERIOD

			Day 1	Day 2	Day 3
Controls	Glucose	mg/kg/min	6	6	6
	AA	g/kg/d	2.4	2.4	2.4
	Lipids	g/kg/d	0	1.4	2.8
	Protein energy	kcal/kg/d	9.6	9.6	9.6
	Total energy	kcal/kg/d	44.6	50.2	63.4
	Protein:energy ratio	%	21.5	19.1	15.1
Standard–dose AA	Glucose	mg/kg/min	6	6	6
	AA	g/kg/d	2.4	2.4	2.4
	Lipids[1]	g/kg/d	2	3	3
	Protein energy	kcal/kg/d	9.6	9.6	9.6
	Total energy	kcal/kg/d	62.6	66.3	71.6
	Protein:energy ratio	%	15.3	14.5	13.4
High–dose AA	Glucose	mg/kg/min	6	6	6
	AA	g/kg/d	3.6	3.6	3.6
	Lipids[1]	g/kg/d	2	3	3
	Protein energy	kcal/kg/d	14.4	14.4	14.4
	Total energy	kcal/kg/d	67.4	71.1	76.4
	Protein:energy ratio	%	21.3	20.2	18.8

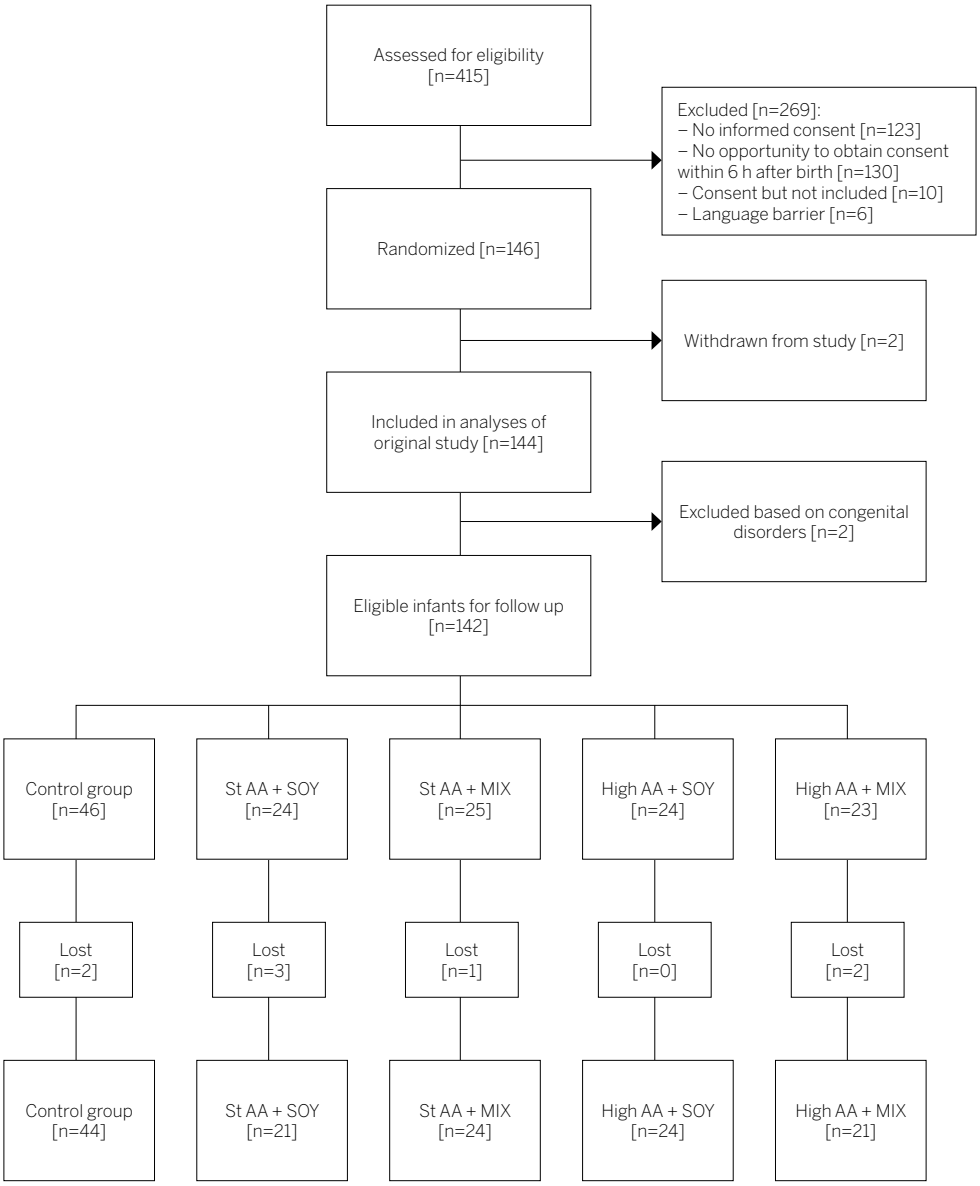
Legend: [1]Lipids are either mixed lipid emulsion or pure soybean oil lipid emulsion.
Abbreviations: AA, amino acid; d, day; mg, milligram; min, minute; kcal, kilocalories; kg, kilogram.

SUPPLEMENTAL TABLE 2. MEDIAN [IQR] MENTAL AND PSYCHOMOTOR SCORES IN CHILDREN AT 2 YEARS CORRECTED AGE, SHOWN FOR ALL INFANTS AND PER STUDY GROUP

	All infants [n=103&90]	Control group [n=36&33]	St AA + SOY [n=16&13]	St AA + MIX [n=17&17]	High AA+SOY [n=17&15]	High AA+MIX [n=17&12]
Mental score	100 [90;105]	98 [86;105]	100 [95;105]	100 [93;105]	95 [88;100]	100 [90;110]
Psychomotor score	100 [91;107]	100 [91;107]	100 [97;105]	97 [91;107]	97 [91;107]	104 [92;109]

Legend: No significant differences between groups.
Abbreviations: AA, amino acid; MIX, mixed lipid emulsion; n, number of developmental scores; IQR, interquartile range; SOY, soybean oil emulsion; St, standard–dose.

SUPPLEMENTAL FIGURE 1 | CONSORT FLOW DIAGRAM



PART III

1

GENERAL DISCUSSION

PARTLY BASED ON:

OUTCOME AFTER PRETERM BIRTH: DESIGN OF A LONGITUDINAL FOLLOW–UP STUDY ON BODY COMPOSITION, NEURODEVELOPMENT AND THE CIRCADIAN SYSTEM

Submitted

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This thesis describes research that crosses the borders between prenatal and postnatal life – an important step in joining both fields at a scientific level. Although from the maternal perspective, embryonic, fetal, and neonatal life may be a continuum; clinical care is traditionally divided into different departments for pregnancy and neonatal care. The staff's different background, training, and goals and points of view can complicate close cooperation. For the individual patient but also on a population scale, loss of information and lack of knowledge about previous [for neonatologists] or future [for obstetricians] care can have implications for health on the long term. In this chapter, we attempt to link the prenatal to the postnatal life.

IDENTIFYING PREGNANCIES AT RISK OF AN ADVERSE OUTCOME

PREGNANCY OUTCOME

In clinical practice, embryonic growth and development are assessed using two–dimensional [2D] ultrasonography [US] between 9 and 14 weeks of gestation. The development of three–dimensional [3D] US techniques has tremendously improved imaging of the embryo, making it now possible to visualize the embryo from 6 weeks of gestation onward.[111] 3D measures are more sensitive for detection of altered growth trajectories than 2D measures: crown–rump length [CRL], a 2D measure, increases from approximately 1 centimeter at 7 weeks to 5 cm at 11 weeks of gestation – a 5–fold increase. Embryonic volume, a 3D measure, increases 30–fold within the same time frame [**Chapter 2**]. We hypothesized that by using 3D measures and virtual reality it becomes possible to identify pregnancies at risk of an adverse birth outcome already early in the first trimester. In **Chapter 2**, we investigated the association between embryonic size and growth parameters and the risk of preterm birth, small for gestational age, congenital anomalies, and perinatal mortality [adverse birth outcomes]. We showed that already early in the first trimester, a small sized embryo has increased odds of an adverse birth outcome: with each cm^3 increase in embryonic volume the odds of an adverse birth outcome decreases with 12%. These data add to findings of previous studies conducted at the end of the first trimester.[108,115,116] We additionally evaluated the association between embryonic growth [growth rate between time points] and birth outcome. For CRL growth between 6 and 13 weeks of gestation, we did not observe a statistically significant association. Embryonic volume growth in this period was negatively associated with the odds of being small for gestational age at birth.

These data confirm that embryonic growth is not uniform and that pregnancies at increased risk of an adverse outcome can be identified already early in the first trimester. These findings add to the growing body of evidence on the relevance of the periconceptional period for embryonic, fetal, and neonatal health.

An important aspect of the methodology of this study is the procedure of pregnancy dating. Information on the menstrual cycle is often lacking, requiring pregnancy dating based on CRL measurement. This methodology assumes that embryonic development is uniform until the moment of dating – which we, and previous studies, showed not to be true.[309] To minimize the bias of ignoring variation in growth, pregnancy dating on the basis of CRL should be performed as early in pregnancy as possible. Our study suggests, however, that no large effect of bias has been introduced by dating based on CRL [1/4th of our cohort]. This might be the result of dating around 9 weeks of gestation, rather than at 11 – 14 weeks which is usual in clinical care.[27]

Using current 3D techniques we are now able to reliably measure embryonic growth from around 7 – 8 weeks of gestation onward.[29,111] We therefore advocate to date early in the first trimester. In clinical care this is challenging as the Dutch obstetric care system follows pregnancies from around 10 – 14 weeks of gestation onward. Moreover, high–quality US devices and expertise are necessary for reliable measurements this early in pregnancy and those are not yet widespread available.

Prediction of pregnancies at risk of an adverse outcome would preferably be done as early in pregnancy as possible, to extend the time window for interventions. Despite the use of 3D US, using current markers we are not able to detect differences in growth before 9 weeks of gestation [**Chapter 2**]. Improvements of 3D techniques and measures may further improve detection of growth differences already in this very early period.

Prediction of pregnancies at risk of an adverse birth outcome should not only include measures of growth and development, but also risk factors such as [epi]genetic profiles, maternal age, the occurrence of prior adverse pregnancy outcomes, and geographical background.[309–312] Other markers that should be considered are measures of environmental conditions such as maternal inflammation levels, and later in pregnancy measures of placental health, volume, and functioning.[313,314]

NEONATAL ADIPOSITY

Obesity is a multifactorial disorder, with genetics, epigenetics, and healthy and lifestyle conditions playing a role in its development.[118] In early life, adiposity is considered an important marker of obesity and cardiometabolic health. As obesity may be difficult, if not impossible, to reverse, we would also like to identify embryos and fetuses at risk for adiposity. Previous studies identified several fetal markers of neonatal adiposity. Those markers include maternal characteristics [e.g., maternal BMI], laboratory measures [e.g., glucose and HbA1c] and 2D and 3D US measures. Based on our systematic review, we could not recommend the use of specific measures to identify fetuses at risk of neonatal adiposity. The use of 3D US measures seemed nevertheless promising [**Chapter 3**]. We therefore looked into the use of fetal fractional thigh volume, a 3D US measure, as marker of neonatal adiposity and showed that this measure was negatively associated with neonatal adiposity at 22 weeks of gestation. Additionally, we showed that also fractional thigh volume growth between 22 and 32 weeks of gestation was negatively associated with neonatal adiposity [**Chapter 4**].

Most studies assessed the use of US measures as markers of neonatal adiposity only in the third trimester of pregnancy [**Chapter 3**]. This is probably the result of the physiological fetal growth pattern, with in the first and second trimester only lean mass accretion and fetal fat accretion starting in the third trimester.[5] This does, however, not imply that [tissue–specific] measurements should not be performed before the third trimester. Important differences in body composition might already be present and detectable early in pregnancy, especially because high adiposity levels may either be the result of increased fat mass or of decreased lean mass [**Chapter 4**].

Despite lack of useful prenatal markers of neonatal adiposity in current literature, it is important to identify fetuses at risk of neonatal adiposity. Risk profiles for neonatal adiposity should include risk factors for increased adiposity levels, such as maternal BMI, ethnicity, and age, but also genetic profiles [**Figure 1**].[118,153] 3D US measures to assess differences in body composition should also be included. Fractional thigh volume, assessed in **Chapter 3**, is a relatively feasible marker for clinical practice, as it is measured on a standard US plane and quickly performed.[167] Our results on the use of fractional thigh volume are, however, not completely in line with other studies on fractional thigh volume.[147,169] The use of this marker for clinical practice has thus not been

established yet. Another interesting marker, because of the underlying hypothesis and more consistent findings across studies, is fetal liver blood flow. An increased fetal liver blood flow is associated with increased hepatic nutrient synthesis and adiposity.[145,315] We did not study this marker because a major disadvantage of this marker is that under circumstances of nutrient excess, fat accretion is suggested to be less dependent on hepatic nutrient synthesis, making the marker less reliable in this patient group.[315] Thus, as currently available 3D measures of body composition showed either limited association with body composition, inconsistent findings, or are only useful in a selected patient population, we propose that new measures should be developed.

EARLY HUMAN BRAIN GROWTH

Markers for fetal and neonatal brain growth are very scarce. Prenatally, brain growth is regularly monitored using bi–parietal diameter and occipital frontal diameter, which are used to estimate head circumference. These are however very rough measures. Moreover, they are not used postnatally, while ideally the same markers could be used throughout pregnancy and in early infancy. Therefore, we created an US measure based on the corpus callosum and the fastigium [roof of the 4th ventricle] [**Chapter 7**]. Corpus callosum – fastigium length is an US measure that reflects a large part of the brain, covers brain structures of different embryonic origins, and is measured on a standard midsagittal US plane, making it feasible for both prenatal and postnatal clinical care. We showed that this marker is feasible and can reliably be measured in both the prenatal and postnatal environment [**Chapter 7 & 8**]. The corpus callosum is the largest white matter structure in the human brain, connecting the left and right cerebral hemisphere, thereby enabling interhemispheric communication.[245,316] Being formed in the first trimester, it keeps on growing up to adulthood – making it an ideal marker for serial monitoring.[317] A disadvantage of corpus callosum – fastigium length is that it can only be visualized from 20 weeks of gestation onward, while alterations in brain development may already be present earlier.[258]

We showed in **Chapter 8** that fetuses with fetal growth restriction had decreased corpus callosum – fastigium length growth trajectories, supporting the theory that this measure might be useful to detect differences in brain growth in the prenatal period. Before implementation in clinical care, the predictive value of the measure for long–term outcome should be established.

INTERVENTIONS

As shown above, it is generally believed that pathways leading to increased risk of adverse birth outcomes are often related to prenatal maternal health and lifestyle factors – often referred to as environmental factors, as the mother is the environment of the fetus – in the periconceptional period.[41] Ideally, prevention should thus take place in the preconceptional period. Improving environmental factors will likely improve embryonic and fetal health, but the effect of interventions need further study. Also means of effective implementation should be focus of research, as many parents–to–be are unaware of the relevance of a healthy lifestyle for health of their offspring and future generations. To increase awareness, local, national, and European governments should in our opinion focus more on the relevance of a healthy lifestyle for becoming healthy pregnant and delivering a healthy neonate. There is also an important task for general practitioners, midwifes, and other healthcare professionals dealing with parents and their offspring, in informing people about the detrimental effects of poor lifestyle behavior on short–term and long–term health. The use of mobile health [mHealth] might be helpful in providing tailored and repeated information to parents–to–be and monitoring of the effects of lifestyle improvements.[318]

Realistically, improving lifestyle behavior will not be that easy and the prevalence of adverse birth outcomes will thus only slowly decline. As plasticity is high in the first 1000 days, it might be possible to reverse programming by [lifestyle] interventions in pregnancy or even after birth. Early identification of pregnancies at risk of an adverse outcome may therefore still be beneficial for health on the long term. By using risk profiles throughout gestation, and specifically monitoring of growth and biomarkers, risks can be re–estimated and the effect of interventions can be evaluated. This longitudinal evaluation might help obstetricians and neonatologists when making decisions on timing of termination of pregnancies.

An important aspect of this monitoring is placental functioning. Postnatally assessed placenta size [length, thickness, and weight] is associated with birth weight, but does not add to the prenatal prediction of pregnancy outcome. US measures of placental functioning mainly consist of Doppler measures of the uterine artery.[319] Prior research looked into the use of placental growth factor and soluble fms–like tyrosine kinase–1 as biomarkers of placental functioning, but mainly focused on its use for predicting severity of pre–eclampsia, and not for their usefulness in predicting pregnancy outcome.[320,321]

Thus, more research is thus needed to adequately monitor placental functioning during pregnancy, especially because the placenta is also an important target for interventions by improving nutrient and oxygen transfer to the fetus.

PRETERM INFANTS

SETTING THE SCENE

Improvements of neonatal care in the last decades have resulted in change of focus in research concerning preterm infants; from survival toward improving long–term health. Health outcome of preterm infants is influenced by many factors in early life, including gestational age at birth, neonatal morbidity, and parental health and lifestyle. The relevance of nutrition and growth in early life on neurodevelopmental outcome and cardiometabolic health has been well recognized, but many questions remain on optimal nutrition and growth for long–term outcome.

The general recommendation that fetal growth rates should be mimicked postnatally in preterm infants for optimal health outcomes is lacking solid evidence.[55,56] Clear evidence is available of the harmful effects of growth retardation and the associated nutritional deficits on the presence of major neonatal morbidities and organ development and functioning of, amongst others, the brain, bones, and kidney.[50,322,323] Moreover, also consequences of postnatal growth retardation on cardiometabolic health have been described – supporting the DOHaD paradigm in neonatal life.[324] To date, we still don't know whether optimal outcome is achieved when fetal growth rates are mimicked postnatally. First, because in most published research, fetal growth rates are not mimicked during neonatal intensive care unit [NICU] stay, and second, because mostly only short–term follow–up is performed.

Let's assume that fetal growth rates should be mimicked postnatally to achieve optimal health outcomes. Besides differences between the intra–uterine and extra–uterine environment which hamper growth in early life as mentioned in **Chapter 1**, there are also some practical issues that greatly influence the chance of meeting the targets. After healthy term birth, weight loss of ~10% is considered a physiological phenomenon, and within 2 weeks, healthy infants catch up their loss and continue growing on their initial growth trajectory.[325] In our study, we confirmed that preterm infants experience a similar drop in weight in the first week after birth, but their pattern of recovery is different [**Chapter 5**]. This postnatal weight loss was an important

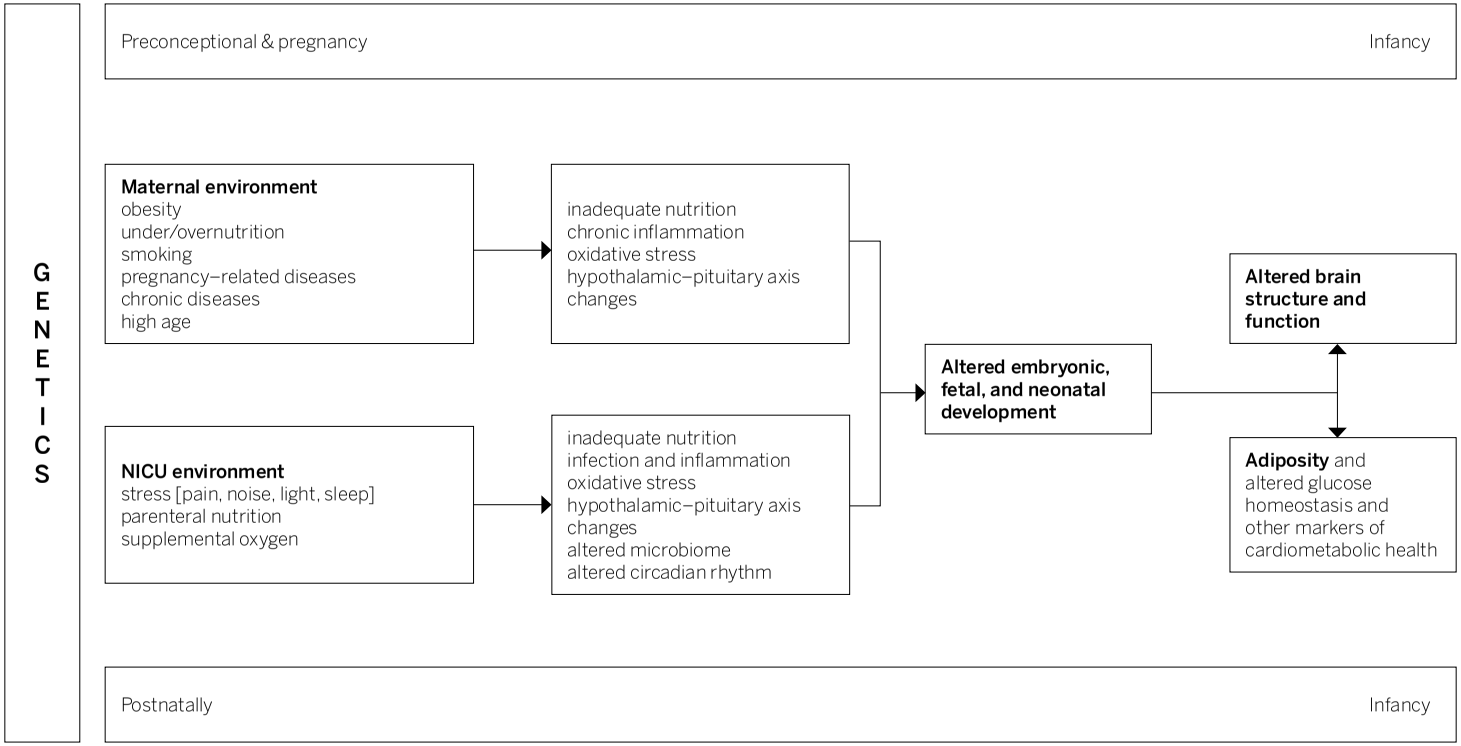
determinant of growth retardation in the first month of life. It explains why growth targets are not met when calculated from birth onward, but met when this period of weight loss was not taken into account in growth evaluation [**Chapter 5**]. Despite also other studies observing this phenomenon in preterm infants, knowledge is still lacking on the influence of this early weight loss for long–term outcome: should we aim for less weight loss postnatally, or should it be considered a physiological phenomenon necessary for adapting to the extra–uterine life after preterm birth?[326] These findings emphasize the importance of establishing which growth periods are most important for cardiometabolic and neurodevelopmental outcome.

After the initial period of growth retardation, we and others observed that many infants show rapid or so–called catch–up growth, sometimes already during hospital stay, but mostly in the first months thereafter [**Chapter 6 and 9**].[67] Nowadays, increasing concerns are expressed on the influence of rapid growth on long–term cardiometabolic health. Rat studies showed that enhanced intake in growth restricted rats resulted in catch–up growth, with improved neurodevelopmental outcome but also increased body adiposity.[327,328] This supports the hypothesis that catch–up growth in preterm infants might be beneficial for neurodevelopmental outcome, but harmful for cardiometabolic health on the long–term.[68] Timing of catch–up growth seems to be relevant for its influence on outcome. In term born infants, the first three moments of life have been identified as critical window for cardiometabolic health.[219,329] In preterm infants, however, the critical window has not been determined yet. Some studies show beneficial, while others found harmful effects of catch–up growth in the first 2 years.[69,324,330] In **Chapter 9**, we showed that increased parenteral amino acid and lipid intakes in the first days after birth resulted in higher growth rates in infancy and early childhood, but without beneficial effects on neurodevelopmental outcome. Although we do not have information on body composition in those infants, if the rapid growth consisted merely of fat and not of lean mass, it may reflect increased cardiometabolic risk as a result of rapid growth in the first 2 years of life.
In **Chapter 6** we showed that growth in early postnatal life is associated with body composition at 6 weeks after term age, while body composition around 6 months after term age is more determined by growth after term age. Because we only have short–term outcome measures, the effect on long–term health – which should be considered the most important outcome measure – remains unclear. Previous studies demonstrated

that relative fat mass is higher around term age in preterm infants, but normalizes or decreases even below levels of term born infant 3 – 4 months hereafter.[77,231] In our cohort, relative fat mass was comparable or already lower than relative fat mass of term born infants. At 6 months after term age, relative fat mass was almost 1 SD lower.[219] It is still unclear why relative fat mass – often referred to as adiposity – of preterm infants is high in early life, while later it normalizes or even goes below adiposity of term born infants in early infancy, and then increases again in childhood and adulthood.[77,82,330] Although the effect in childhood is thought to be related to programming in early postnatal life [DOHaD paradigm], the underlying mechanism of the increased adiposity around term age is still unclear. Some suggestions are a direct effect of programming due to an imbalance between nutrient supply in–utero and ex–utero; inadequate protein:energy ratio or amino acid composition during neonatal intensive care unit [NICU] stay, resulting in fat rather than lean mass growth; and increased fat accumulation for energy storage and thermal regulation.[58] The decrease in adiposity in infancy may reflect differences in energy expenditure, due to impaired neurodevelopment and related physical activity patterns, or early metabolic or endocrine changes.

The importance of parenteral nutrition on short–term outcomes such as survival and growth has been well established, but long–term outcome data remain scarce. In a randomized controlled trial [RCT] we found no beneficial effects of early high–dose [3.6 g/kg/d] amino acid and mixed lipid administration after birth on neurodevelopmental outcome. In the standard–dose [2.4 g/kg/d] amino acid and mixed lipid emulsion group we observed increased odds for death. This unexpected finding can be considered an accidental finding in our study with low power [**Chapter 9**]. However, also other studies showed worrying results on the use of parenteral nutrition and specifically of early high–dose amino acid administration in preterm infants.[212–214,331] Also in critically ill term born infants increasing concerns are expressed on the use of parenteral nutrition and amino acids.[216,332] Undoubtedly, supply of amino acids is essential in early life of preterm infants, as the infant otherwise depends on its own limited protein stores for obligatory protein catabolism. The optimal amino acid dosage, which may partly depend on the postnatal age and the level of illness, has however not been established yet. Probably, there are circumstances during which [high] amino acid provision is harmful, for example in periods of septicaemia.[332,333] This supports more individualized nutritional practices in the NICU setting to improve outcomes and survival rates.

FIGURE 1 | DEVELOPMENTAL ORIGINS OF ADIPOSITY AND BRAIN ALTERATIONS IN EARLY LIFE



ENTERAL NUTRITION

The relevance of parenteral nutrient supply in early life for growth is very well established.[182] After the acute phase, enteral nutrition is an important determinant of growth. Own mothers milk is believed to be most beneficial for neurodevelopment and cardiometabolic health.[334] Still, many infants depend on formula milk during NICU stay, of which the optimal composition has not been established yet. Also in own mothers and donor milk, composition of the milk is not always optimal for the developing preterm infant.[335] Personalized supplementation of milk, based on breast milk analyses, may improve growth and health outcomes.[336] In **Chapter 6** we showed that high weight gain in the stable growing phase is associated with high relative fat mass – emphasizing the relevance of adequate enteral nutrition for an optimal growth and body composition in this period.

UNDERLYING MECHANISMS OF ADVERSE HEALTH OUTCOMES AFTER PRETERM BIRTH

Underlying mechanisms of the risk for an adverse long–term outcome after preterm birth still need to be unraveled, especially as it may provide targets for interventions. Different pathways are suggested to be involved in the increased risk of abnormal organ development and functioning. Proposed pathways are presented in **Figure 1** and include genetic predisposition; epigenetic changes induced by prematurity; rapid telomere shortening due to prematurity and environmental conditions related to prematurity; epigenetic changes by early life conditions such as nutrition and stress; and changes in the hypothalamic–pituitary adrenal [HPA]–axis.[337–340] Disturbances of physiological processes have also been suggested to increase the risk for an adverse outcome. A physiological process of interest is the circadian rhythm.[341] The circadian rhythm is the biological clock, regulating day–night rhythm of cells and organs.[342] This rhythm can be disturbed by environmental conditions such as stress, light, and nutrition. In preterm born infants, the [development of] a circadian rhythm is likely disturbed as a consequence of the shorter maternal clock guidance and the unnatural NICU environment, with unnatural light–dark cycles, noise, artificial feeding schemes, and disturbed sleep.[343,344] As the circadian rhythm is involved in glucose homeostasis, insulin secretion, and energy metabolism, chronodisturbance can have detrimental effects on cardiometabolic health.[345,346] Animal and human adult studies also showed adverse effects of chronodisturbance on brain development.[347–349]

Another system involved in both neurodevelopmental and cardiometabolic outcome is the gut–brain axis. The gut microbiome – a collective word for millions of bacteria that colonize the gut – interacts with the brain via immunological, endocrine, and neural pathways. The microbiome is suggested to influence the risk of an adverse neurodevelopmental and cardiometabolic health via the HPA axis, via upregulation of energy from the diet, and by the induction of chronic inflammation.[350,351]

Important to mention is that brain structure, volume, and functioning are related to obesity levels in animals, adults and children.[352,353] The exact underlying mechanisms are unclear, but may relate to brain alterations interfering with glucose homeostasis and control of food intake [**Figure 1**], thereby increasing metabolic derangement, increasing obesity levels which will increase inflammation levels, which in turn will again influence brain functioning.[354] Whether this association is also present in preterm infants needs to be established, as no solid conclusions can be drawn based on current literature.[355–357]

INTERVENTIONS

Preterm infants all have different phenotypes, based on their prenatal condition, gestational age and weight at birth, neonatal morbidities, feeding type, and geographical and socio–economic background. Based on these factors, the risk of an adverse outcome varies widely between preterm infants. Ideally, risk profiles will be developed in the future to identify those at risk of an adverse health outcome, including parental, prenatal, and postnatal conditions. Using this profiling, more adequate estimations can be given in early life to parents with regard to long–term outcome, which may support ethical decision–making. Later in life, more targeted therapy and advices can be given to parents, including advices on lifestyle, nutrition, and exercise, and guidance by physio– and speech therapists.

Effective interventions to improve health outcomes of preterm infants may be related to nutrition.[72] In **Chapter 5** we showed that energy intakes in the first day of life are lower than recommended by the ESPGHAN guideline, and also lower than necessary for targeted growth. Improving energy supply, for example by increasing early parenteral lipid intake, may be beneficial for early growth and thereby for long–term health outcomes. Other important aspects of parenteral nutrition which can be improved are the protein:energy ratio and the amino acid composition of the parenteral and enteral solutions, as plasma amino acid levels determine protein synthesis rates.[358] Tailored

made parenteral solutions, providing an optimal amount of amino acids and energy for protein synthesis, might in the future improve both short–and long–term outcomes.

Another nutritional target of interest are probiotics, which showed to reduce the occurrence of necrotizing enterocolitis, and may be beneficial for brain and cardiometabolic health by decreasing morbidities rates and by reducing inflammation.[359,360]

The development of a circadian rhythm may also be an interesting target for interventions. Prevention of chronodisturbance may have beneficial effects on both neurodevelopmental and cardiometabolic health.[361,362] It can be influenced via several pathways: light–dark cycling combined with reduced noise may promote the development of a circadian rhythm, and thereby improve growth.[362] But also nutrient supply may greatly influence this; parenteral nutrition can be given in intervals rather than continuously, or with varying dosages over the day, and also provision of enteral nutrition can be improved. Human milk composition changes over time, and also during the day, to meet the infants' changing nutritional demands.[363] Although in milk of mothers who have delivered prematurely this is less well investigated, it might be that the non–physiological feeding schemes, including breast milk given disrespected of moment of expression, greatly influence the development of the infant's circadian system, making it an easy target for improvement.

METHODOLOGICAL CONSIDERATIONS

The studies described in **Chapter 2, 4, and 8** have been conducted in the Predict study and the embedded DREAM study. These prospective observational cohort studies are conducted in a tertiary hospital population that is not representative of the general population.[104] Studying a high–risk population is advantageous for unravelling underlying mechanisms, but limits the external validity of the findings. Many data used in these studies were obtained via self–administered questionnaires. Although the most common health and lifestyle factors, such as alcohol and smoking, were additionally checked by the research team at the first visit, misclassification may have occurred. In our study, for example, smoking status may have been underreported as the prevalence was only 16% in periconceptional period [**Chapter 2**].[364] Moreover, a well–trained research team performed the 3D ultrasounds and the subsequent 3D measurements, likely increasing the reliability and precision of the measurements.

The BOND study, described in **Chapter 5 and 6**, is conducted on a large tertiary NICU. Generalizing our results to intensive care settings with lower level of care and less extremely preterm infants is difficult. In addition, discharge policies in the Netherlands are different than in other countries, resulting in a relatively short duration of NICU stay. This may have influenced our growth data, and the external validity of our NICU findings. By including data from the high care centers and collecting post discharge growth data we were still able to study the most important period of growth.
All studies, but especially follow–up studies that require active parent participation, automatically select parents who are willing to participate in such studies. This selection may limit the generalizability to the source population and may induce selection bias, as participation correlates with social, educational, and health conditions. It was, however, previously demonstrated that this selection is less likely to introduce bias than loss to follow–up.[365] Actual loss to follow–up was limited to only two infants in our study, but not all infants did completely follow the study protocol. This was mainly caused by purely logistic problems such as availability of the devices, and not related to maternal, fetal, or neonatal characteristics. Therefore, we expect that this has barely biased our findings.

The last consideration is the use of the PEA POD® for body composition measurement. Although the PEA POD® is generally considered the gold standard, its value is limited

by only two compartments [lean and fat] that are evaluated, compared to the three compartments which can be assessed using DEXA or MRI [lean, fat, and bone].[13] Moreover, as mentioned in **Chapter 1**, the PEA POD® does not provide any information on fat distribution [subcutaneous versus visceral], which is a known and important risk factor for cardiometabolic health.[366] With regards to preterm infants, the PEA POD® has been validated from 30 weeks of gestation onward, which is relatively early, but its use in this period is hampered as infants need to be without oxygen supply for approximately 5 minutes, decreasing the feasibility during NICU stay.

CONCLUSIONS

In conclusion, we learned that embryonic size and growth parameters are associated with birth outcome in early pregnancy, with the 3D measure of embryonic volume being more sensitive for detection of deviations in growth trajectories than standard 2D measures.
Continuing into fetal life, we showed that fractional thigh volume is a promising 3D soft tissue marker for prediction of neonatal adiposity in midgestation. We additionally showed that brain growth can be monitored reliably pre– and postnatally by using the newly developed ultrasound measure corpus callosum – fastigium length.

Continuing into postnatal life of preterm infants, we showed that preterm infants are still at high risk for growth retardation in the neonatal period, despite meeting the macronutrient recommendations from birth onward. Postnatal weight loss greatly influences neonatal growth outcomes, making this an interesting target for future research. We also learned that growth rates in the first weeks to months of life are associated with adiposity levels up to 6 months after term age. Additionally, we did not demonstrate a beneficial effect of parenteral high–dose amino acid and mixed lipid emulsion administration from birth onward in very–low–birth–weight infants on neurodevelopmental outcome, despite enhanced growth.

FUTURE RESEARCH ON IDENTIFYING PREGNANCIES AT RISK

Research on identification of pregnancies at risk of an adverse outcome is still in its infancy, and many opportunities remain for future research. A few important considerations should be taken into account when designing future research projects.

THE THIRD DIMENSION

The use of 3D techniques in prenatal care is increasing, but the additional value is limited by the evaluation on a 2D screen. In **Chapter 2**, we used a virtual reality system allowing for 3D evaluation by creating a hologram; a 3D image created via photographic projection. The recent development of a desktop version of this system is promising for future research and clinical care, as it allows for real–time bedside evaluation of 3D scans.[121]

SIZE OR GROWTH?

Many people use growth interchangeably for size and growth. But what exactly is size, and what is growth? And should we indeed consider growth, and not size as an important marker of health status? Size represents weight or length at a specific moment, not taking into account the size before that specific moment. In clinical care, size is most often assessed, although sometimes the terminology suggests otherwise [e.g., '[intra– or extra–uterine] growth retardation']. In **Chapter 4**, we show that decreased thigh volume is associated with increased relative fat mass. In **Chapter 2**, we demonstrated that a smaller embryonic size is associated with higher odds of an adverse birth outcome. Being small thus has implications, but being small is actually the outcome of a growth trajectory. Evaluation of growth trajectories might therefore be even more relevant for short– and long–term health. In **Chapter 2, 4, and 8** we indeed showed that altered growth trajectories are associated with the occurrence of adverse birth outcomes. Future research should thus not only focus on size, but also on growth trajectories.

FROM PRENATAL TO POSTNATAL LIFE

Ideally, the same growth marker is used both prenatally and postnatally. A major limitation of continuous growth evaluation is however the lack of markers that can be used consistently in both periods, or even throughout pregnancy. New markers

should be developed that can be used both prenatally and postnatally, as in clinical care postnatal growth trajectories should be evaluated in light of the prenatal growth trajectory.

FUTURE RESEARCH ON NUTRITION AND GROWTH IN PRETERM INFANTS

Although much research has been performed on nutrition and growth, many questions remain. The focus of research on nutrition and growth in preterm infants should not be on mimicking of fetal growth rates but on establishing growth rates for optimal health outcome. To establish this, we should first determine which outcomes should be evaluated, and when they should be assessed.

WHICH OUTCOMES SHOULD BE EVALUATED?

Currently, follow–up of preterm infants mainly focusses on psychomotor and mental outcome. However, using current tests, subtle differences may not be recognized while significantly influencing everyday life. More specific measures of brain functioning, such as eye-tracking [assessment of processing of visual input], might provide additional information on functioning in daily life. Also assessment of executive functioning could be integrated in follow–up of preterm infants in childhood. Assessment of insulin sensitivity [fasting insulin and Homeostasis Model Assessment [HOMA]–IR], inflammation [CRP], stress [cortisol], and body composition might also be helpful in identification of infants and children at risk for obesity. For parents and their infants, quality of life may be most important, which should be included more often as outcome measure in research. Assessment of risk factors such as the microbiome and chronodisturbance, during the period on the NICU as well as in infancy and childhood, may provide additional information on the risk of an adverse health outcome.

TIMING OF EVALUATION

Long–term rather than short–term outcome should be considered the most important outcome as the long–term relevance of short–term outcomes is often unclear and many outcomes are not detectable yet in infancy and childhood using currently available markers. Ideally, infants should be followed up into adulthood to provide an adequate estimation on the influence of early life on health throughout the life course. A downside of long–term follow–up is the large time window between interventions and outcomes, hampering the clinical relevance and decreasing the applicability in practice at moment of publication. Moreover, follow–up over a longer time results in higher loss to follow–up rates, thereby potentially inducing bias.[365] Using assessments at regular

time intervals [for example every 3 to 4 years] may limit the numbers lost to follow–up and provides intermediate effects of the intervention.

RANDOMIZED CONTROLLED TRIALS OR COHORT STUDIES?

For well–defined clinical questions randomized controlled trials [RCTs] and meta–analyses are needed. RCTs efficiently deal with confounders that cannot sufficiently be adjusted for in cohort studies. But RCTs are no longer considered the only way to obtain unbiased evidence.[367] Clinical practice is often more complex than can be mimicked in an RCT, and the magnitude of beneficial effects found in RCTs are often not found in cohort studies after clinical implementation. Furthermore, RCTs often need external funding, which may create conflicts of interests in case of industry–sponsored research. In future research, important knowledge can be derived from cohort studies, provided that study design and analyses are chosen with care and results are interpreted carefully.[368] As multiple outcomes combined with long–term follow–up require large sample sizes, collaboration between centers is strongly recommended. This does not only provide the opportunity to include more infants in a shorter timeframe, it also increases external validity of the findings.

11

SUMMARY & SAMENVATTING

SUMMARY

The development from an embryo to a newborn and from their onward into a healthy child and adult is a complex process, with genetic and prenatal environmental conditions, including maternal health and lifestyle, increasingly being recognized as relevant influencing factors. In this thesis we focus on the influence of nutrition and growth on the development of the body [fat and lean mass] and the brain [size and functioning] of the embryo, fetus, neonate, infant, and child.

In **Chapter 1** we present background information on topics that are addressed in the following chapters: the development from an embryo into a neonate, the DOHaD paradigm, and the complex interaction between body composition, brain development, and environmental factors. We present gaps in current research on the use of three–dimensional [3D] ultrasonography in pregnancy to predict birth outcome, body composition, and for monitoring of brain growth. Additionally, we discuss clinically relevant questions regarding body composition and brain development of preterm infants, with focus on the influence of parenteral nutrition and growth.

PART I THE EARLY HUMAN BODY

In **Chapter 2** we describe the associations between embryonic size and growth parameters and the risk of the following adverse birth outcomes: preterm birth, small for gestational age, congenital anomalies, and fetal and early neonatal mortality. To describe embryonic size we used two 3D ultrasonography parameters: crown–rump length [CRL] and embryonic volume [EV]. No statistically significant associations were observed between embryonic size parameters at 7 and 9 weeks of gestation and any of the adverse birth outcomes. At 11 weeks, a larger embryo is associated with smaller odds of small for gestational age. And already at 9 weeks of gestation, embryonic size is positively associated with birth weight. These findings emphasize that embryonic growth is not uniform across pregnancies and may help to identify pregnancies at risk of an adverse birth outcome already early in pregnancy.

In a systematic review we searched for markers that can predict neonatal body composition during pregnancy [**Chapter 3**]. Of the 16 papers included in this review, only four studies were of methodological and statistical moderate to high quality. From these studies, we identified

three markers of interest: maternal prepregnancy BMI, fasting glucose level, and HbA1c level. The results of these studies are inconclusive. We therefore concluded that to date no single marker is available to adequately predict neonatal body composition during pregnancy. In our opinion, 3D ultrasonography should be considered an interesting technique for future development of prenatal markers of neonatal body composition.

In **Chapter 4** we describe a prospective cohort study in which we assessed the association between fetal fractional thigh volume, a 3D ultrasonography marker, and neonatal body composition. We measured this marker at 22, 26, and 32 weeks of gestation. We found that a smaller fetal thigh volume at 22 weeks is associated with higher relative fat mass after birth. At the other time points we did not observe an association. We concluded that fractional thigh volume is a promising marker to identify fetuses at risk of neonatal adiposity.

From prenatal life we shift into the postnatal life of preterm infants.

In a prospective cohort we evaluated the nutritional intake of preterm infants during neonatal intensive care stay, and investigated achievement of recommended growth rates in this period [**Chapter 5**]. We observed that macronutrient [carbohydrate, protein, and fat] recommendations were met from birth onward. Energy recommendations were however not met before day 5 of life in the majority of the infants. Possibly as a result of this, most infants did not reach the intended growth rates when calculated between birth and discharge from the neonatal intensive care to a secondary hospital. Most infants achieved the intended growth rates when growth rates were calculated between the moment of maximum weight loss postnatally and discharge to a secondary hospital.

In **Chapter 6** we present a prospective cohort study of 118 preterm born infants. We evaluated the influence of weight gain during hospital stay on body composition measured at 6 weeks and 6 months after term age. Weight gain during hospital stay was significantly associated with body composition in infancy. Although the association was strongest with lean mass, faster weight gain after discharge to a secondary hospital was associated with increased fat accumulation, resulting in increased relative fat mass. The long–term relevance of these findings for cardiometabolic health needs further study.

PART II THE EARLY HUMAN BRAIN

In **Chapter 7** we describe the development of a new ultrasound marker to monitor preterm brain growth. We show that the reproducibility of this marker, called corpus callosum – fastigium length, is good, and created reference curves for preterm infants. We found that boys have increased corpus callosum – fastigium length growth trajectories. Also with increasing birth weight, increasing corpus callosum – fastigium length were observed. No statistically significant associations were found between clinical outcomes such as sepsis or ventilation days and corpus callosum – fastigium length growth trajectories.

There is a need for markers that allow for monitoring of brain growth in both the prenatal and the postnatal period. Therefore we investigated the feasibility and reproducibility of corpus callosum – fastigium length in fetal life [**Chapter 8**]. These analyses showed that measurement of corpus callosum – fastigium length is also feasible and reproducible in the prenatal period. We found that growth trajectories of corpus callosum – fastigium length of fetuses with fetal growth restriction are decreased compared to those of healthy control fetuses. We additionally created reference curves between 22 and 42 weeks of gestation, which are largely comparable to the reference curves of preterm infants as presented in **Chapter 7**.

Quantity and quality of [parenteral] nutrition in early life of preterm infants are pivotal for their developmental potential. In a randomized controlled trial we assessed the long–term effects of parenteral high–dose amino acid and mixed lipid administration from birth onward in preterm infants [**Chapter 9**]. No beneficial effects of the nutritional interventions were found on neurodevelopmental outcome, but growth was enhanced 2 years after term age, suggesting a possible beneficial effect of the interventions.

In the general discussion [**Chapter 10**] we discuss the clinical implications and address limitations and strengths of the studies that are presented in this thesis. Moreover, we evaluate which questions regarding growth in prenatal and postnatal life have been answered, but also which questions remain to be answered to improve health of the developing embryo, fetus, and infant in the upcoming years.

SAMENVATTING

De ontwikkeling van een bevruchte zaadcel via een pasgeborene naar een foetus en van daar naar een gezond kind en volwassene is een complex proces. Er is steeds meer bewijs dat genetische en omgevings– en leefstijlfactoren van de moeder voor de bevalling dit proces sterk kunnen beïnvloeden. In dit proefschrift richten wij ons op de invloed van voeding en groei op de lichaamssamenstelling [verhouding vetmassa en vetvrije massa] en de hersenen [omvang en functie] van het embryo, de foetus, de pasgeborene, en het kind.

In **Hoofdstuk 1** introduceren wij de onderwerpen die in de hierop volgende hoofdstukken besproken worden. Eerst belichten wij de ontwikkeling van de foetus en het DOHaD paradigma, en de complexe verbanden tussen omgevingsfactoren, lichaamssamenstelling en hersenontwikkeling. We schetsen de bestaande hiaten in het gebruik van foetale driedimensionale [3D] echografie om zwangerschapsuitkomsten te voorspellen en om hersengroei te volgen. Daarnaast bespreken we vragen met betrekking tot de ontwikkeling van de lichaamssamenstelling en de hersenenontwikkeling van te vroeg [prematuur] geboren kinderen, met specifieke aandacht voor de invloed van infuusvoeding en groei.

DEEL I HET MENSELIJK LICHAAM IN HET VROEGE LEVEN

In **Hoofdstuk 2** beschrijven wij het verband tussen groei van het embryo in de eerste weken van de zwangerschap en de uitkomst van de zwangerschap. We vergeleken de embryonale groei van een groep met een normale zwangerschapsuitkomst, met een groep waarin het kind overleed, te vroeg werd geboren [prematuriteit], met een te laag geboortegewicht werd geboren [dysmaturiteit] en/of een ernstige aangeboren afwijking had. Als maat voor groei van het embryo maakten wij gebruik van kruin–stuit lengte en het embryonaal volume, gemeten op 3D echo's. Wij vonden geen verband tussen de metingen gedaan bij 7 en 9 weken zwangerschapsduur en de verschillende zwangerschapsuitkomsten, maar wel bij 11 weken zwangerschapsduur. Een groter embryonaal volume bij 11 weken is geassocieerd met een lagere kans op een combinatie van de verschillende gecompliceerde zwangerschapsuitkomsten. Daarnaast zagen we dat er een positief verband was tussen kruin–stuit lengte en embryonaal volume en het geboortegewicht. Deze bevindingen laten zien dat groei in de eerste weken van de

zwangerschap niet uniform is, en kunnen in de toekomst helpen met het identificeren van zwangere vrouwen met een verhoogd risico op een nadelige zwangerschapsuitkomst.

In een systematische literatuurstudie zochten wij naar factoren die al tijdens de zwangerschap de lichaamssamenstelling van pasgeborenen kunnen voorspellen [**Hoofdstuk 3**]. Van de 16 artikelen in de wereldwijde medische literatuur die dit onderwerp onderzocht hebben bleken er slechts vier van goede methodologische en statistische kwaliteit. Uit deze vier studies zijn de volgende drie relevante factoren naar voren gekomen: body mass index [BMI] gemeten voor de zwangerschap, nuchtere bloed glucosewaarde en HbA1c [maat voor gemiddelde bloedsuikerspiegel in de afgelopen weken] van de zwangere. De resultaten van deze vier studies waren niet eenduidig. Op basis van ons overzicht concludeerden wij dan ook dat er niet één ideale voorspeller is voor neonatale lichaamssamenstelling, en dat voorspellers die specifiek de fetus betroffen niet goed onderzocht zijn. Wij opperen dat 3D echografie tijdens de zwangerschap een veelbelovende techniek is voor toekomstige ontwikkeling van voorspellers van neonatale lichaamssamenstelling.

In **Hoofdstuk 4** beschrijven wij een prospectieve observationele studie, waarin wij het verband onderzochten tussen een foetale 3D echografische meting en neonatale lichaamssamenstelling. We keken specifiek naar het foetale dijvolume gemeten bij 22, 26 en 32 weken zwangerschapsduur. We zagen dat een kleiner dijvolume bij 22 weken geassocieerd is met een hogere relatieve vetmassa na de geboorte. Later in de zwangerschap vonden wij geen verband. We concludeerden dat het foetale dijvolume een veelbelovende marker is om foetussen te identificeren die een verhoogd risico hebben op neonatale adipositas.

Van het prenatale leven richten we ons vervolgens op het postnatale leven van prematuur geboren kinderen.

In een prospectieve cohort studie keken wij naar de voedingsinname van prematuur geboren kinderen tijdens de opname op de intensive care neonatologie en onderzochten of de aanbevolen groeisnelheden werden bereikt [**Hoofdstuk 5**]. Het bleek dat aan de aanbevelingen van glucose–, eiwit– en vetinname vanaf de geboorte werd voldaan. Echter, de aanbeveling voor de energie werd in het merendeel van de kinderen niet

gehaald voor de 5e levensdag. Mogelijk hierdoor haalden de meeste kinderen niet de beoogde groeisnelheid wanneer de groei werd berekend tussen geboorte en ontslag van de intensive care neonatologie. De beoogde groeisnelheid werd wel bereikt wanneer wij deze berekenden vanaf het moment van maximaal gewichtsverlies na de geboorte tot ontslag van de intensive care neonatologie.

In **Hoofdstuk 6** presenteren wij een prospectieve cohort studie waaraan 118 prematuur geboren kinderen deel hebben genomen. We evalueerden de invloed van gewichtstoename tijdens de ziekenhuisopname op lichaamssamenstelling gemeten 6 weken en 6 maanden na de uitgerekende datum. Hierbij werd gevonden dat er gewichtstoename tijdens de ziekenhuisopname significant geassocieerd was met lichaamssamenstelling in de eerste maanden na de geboorte. Hoewel het verband tussen gewichtstoename het sterkste was met vetvrije massa, vonden wij ook dat snellere gewichtstoename tot ontslag naar huis geassocieerd was met een verhoogde vetmassa in verhouding tot de vetvrije massa. De langere termijn consequenties voor cardiometabole gezondheid van deze bevindingen zullen in toekomstig onderzoek onderzocht moeten worden.

DEEL II HET MENSELIJK BREIN IN HET VROEGE LEVEN

In **Hoofdstuk 7** wordt de ontwikkeling van een nieuwe echografische meting beschreven om hersengroei te evalueren bij prematuur geboren kinderen. In dit hoofdstuk wordt getoond dat de reproduceerbaarheid van deze meting, de zogeheten corpus callosum – fastigium lengte, goed is, en presenteerden wij referentiecurves voor prematuur geboren kinderen. Daarnaast toonden wij aan dat jongens een hogere corpus callosum – fastigium lengte groeisnelheid hebben, en ook dat een hoger gewicht bij geboorte geassocieerd is met een hogere corpus callosum – fastigium lengte groeisnelheid. Er werd geen verband gevonden tussen klinische uitkomsten zoals het voorkomen van een ernstige infectie of het aantal beademingsdagen en de groeisnelheid van de corpus callosum – fastigium lengte.

Er is behoefte aan meetinstrumenten waarmee de hersengroei zowel voor als na de geboorte geëvalueerd kan worden. Daarom onderzochten wij of corpus callosum – fastigium lengte, welke na de geboorte betrouwbaar gemeten kan worden, in het foetale leven ook betrouwbaar gemeten kan worden [**Hoofdstuk 8**]. Hieruit bleek dat de meting

van de corpus callosum – fastigium lengte ook tijdens de zwangerschap goed uitvoerbaar en reproduceerbaar is. Tevens vonden wij dat de groeisnelheid van de corpus callosum – fastigium lengte tussen 22 en 42 weken zwangerschapsduur lager was in foetussen met groeivertraging dan in gezonde foetussen. Wij maakten referentiecurves welke grotendeels vergelijkbaar zijn met de referentiecurves van prematuur geboren kinderen zoals gepresenteerd in **Hoofdstuk 7**.

De hoeveelheid en kwaliteit van [infuus]voeding in de eerste dagen van het leven van prematuur geboren kinderen is cruciaal voor hun ontwikkelingspotentieel. In een geblindeerde gerandomiseerde studie keken wij naar de effecten van een voedingsinterventie direct na de geboorte op de neurologische ontwikkeling 2 jaar later. Op basis van loting kregen sommige kinderen vanaf de geboorte een hogere dosis aminozuren en/of een vetemulsie [**Hoofdstuk 9**]. De neurologische ontwikkeling werd vergeleken tussen kinderen die wel of niet deze aangepaste voeding kregen. De voedingsinterventie lijkt de neurologische ontwikkeling niet te verbeteren, maar mogelijk wel de groei, hoewel dat nog in een grotere onderzoeksgroep bevestigd zal moeten worden.

In de algemene discussie [**Hoofdstuk 10**] bediscussiëren wij het belang van onze bevindingen voor de praktijk en bespreken wij de beperkingen en kracht van het onderzoek gepresenteerd in dit proefschrift. Daarnaast laten wij zien welke vragen met betrekking tot groei in het foetale en postnatale leven zijn beantwoord, en welke nog beantwoord moeten worden om de gezondheid van de fetus en de pasgeborenen te bevorderen.

ADDENDUM

References
Abbreviations
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ABBREVIATIONS

%FM	percentage fat mass
2D US	two–dimensional ultrasonography
3D US	three–dimensional ultrasonography
AA	amino acids
AC	abdominal circumference
ADP	air displacement plethysmography
BC	body composition
BMI	body mass index
BPD	bi–parietal diameter
CA	corrected age
CC	corpus callosum
CCF	corpus callosum – fastigium
CHD	congenital heart defect
CI	confidence interval
CRL	crown–rump length
CUS	cranial ultrasound
DEXA	dual x–ray absorptiometry
EFW	estimated fetal weight
EV	embryonic volume
FGR	fetal growth restriction
FL	femur length
FFM	fat free mass
FM	fat mass
GA	gestational age
HC	head circumference
ICSI	intracytoplasmic sperm injection
IQR	interquartile range
IVF	in vitro fertilization
IVH	intraventricular hemorrhage
LMP	last menstrual period
MRI	magnetic resonance imaging
NEC	necrotizing enterocolitis
NICU	neonatal intensive care unit
PHVD	post hemorrhagic ventricular dilatation
PMA	postmenstrual age
SD[s]	standard deviation [score]
SGA	small for gestational age
TVol	fractional thigh volume

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THIS THESIS

Roelants JA, Hulst JM, Rizopoulos D, Hokken–Koelega ACS, Neelis EG, Reiss IKM, Joosten KFM, Vermeulen MJ.
Rapid growth during hospital stay: is there an association with later body composition in preterm infants?
[In preparation]

Roelants JA, Vermeulen MJ, Willemsen SP, Been JV, Eggink AE, Reiss IKM, Steegers EAP, Steegers–Theunissen RPM.
Early first trimester embryonic size and growth parameters and the association with adverse birth outcome: the Rotterdam Periconceptional Cohort.
[Submitted]

Roelants JA, Joosten KFM, Hulst JM, Chaves I, Dudink J, van der Horst GTJ, Reiss IKM, Vermeulen MJ.
Outcome after preterm birth: design of a longitudinal follow–up study on body composition, neurodevelopment and the circadian system.
[Submitted]

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First week weight dip and reaching growth targets in early life in preterm infants.
Clin Nutr. 2017 Aug 31 [Epub ahead of print].

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OTHER MANUSCRIPTS

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PHD TRAINING	YEAR	ECTS
GENERAL COURSES		
Biomedical English Writing [Erasmus MC]	2016	1.0
Scientific Integrity [Erasmus MC]	2015	0.3
Biostatistical Methods I: Basic Principles [NIHES]	2014	5.7
BROK–course [NFU BROK academy]	2014	1.0
CPO–mini–course [Erasmus MC]	2014	0.3
SPECIFIC COURSES & WORKSHOPS		
Paediatric nutrition: Beyond the Nutrients Summer School [ESPGHAN]	2017	2.0
Repeated measurements [NIHES]	2017	1.4
Cohort studies [NIHES]	2014	0.7
Workshop body composition, Amsterdam, the Netherlands	2013	0.5
LOCAL RESEARCH MEETINGS		
Annual Wladimiroff Award Meeting	2015 – 2017	0.5
Annual Sophia Research day	2014 – 2017	0.5
Weekly Neonatology research meeting	2014 – 2017	0.5
Weekly Gynecology research meeting	2014 – 2017	0.5
Monthly 'Metabolism, endocrinology and nutrition' research meeting	2014 – 2017	0.5
Annual PhD Day	2014 – 2016	0.5

PRESENTATIONS AT CONFERENCES

10th World Congress on Developmental Origins, of Health and Disease, Rotterdam, the Netherlands [poster presentation]	2017	1.0
4th International Conference on Nutrition & Growth, Amsterdam, the Netherlands [oral]	2017	1.0
6th Congress of the European Academy of Paediatric Societies, Geneva, Switzerland [poster presentation]	2016	1.0
International Society of Ultrasound in Obstetrics and Gynecology 26th World Congress, Rome, Italy [poster presentation]	2016	1.0
9e Nationale Voedingscongres, Ede, the Netherlands [oral]	2016	1.0
Wladimiroff Award Meeting, Rotterdam, the Netherlands [oral]	2016	1.0
7th Dutch Neonatal Fellow Meeting, Leiden, the Netherlands [oral]	2016	1.0
3rd International Conference on Nutrition & Growth, Vienna, Austria [poster]	2016	1.0
9th World Congress on Developmental Origins of Health and Disease, Cape Town, South–Africa [oral]	2015	1.0
Post-jENS symposium, Soesterberg, the Netherlands [oral]	2015	1.0
1st congress of joint European Neonatal Societies, Budapest, Hungary [oral & poster]	2015	1.0

TEACHING

SUPERVISING MASTER THESES

Lotte Kat, medical student Erasmus University	2017	2.0
Julia Sluiter, student dietetics Haagse Hogeschool	2016	2.0
Brigitte van der Geest, student dietetics Haagse Hogeschool	2015	2.0
Mattia Bas, medical student Erasmus University	2015	2.0

OTHER

Participation in TULIPS PhD program	2016 – 2018	0.5
Peer review of articles for international scientific journals	2014 – 2017	0.5

ABOUT THE AUTHOR

Jorine Atalante Roelants was born on the 4th of September 1988 in Nijmegen, the Netherlands.

She completed her high school at the Stedelijk Gymnasium Nijmegen in 2006. In the same year she started her medical training at the Erasmus University in Rotterdam. Her enthusiasm for research started in 2010, when she conducted her master research project on parenteral nutrition in preterm infants at the Department of Pediatrics, division of Neonatology at the Erasmus MC – Sophia Children's Hospital, Rotterdam, the Netherlands.

After spending 2 months at the Pediatric department of a regional referral hospital in Mbale, Uganda, Jorine graduated in January 2013. Hereafter, Jorine started working as a pediatric resident [ANIOS] at the Jeroen Bosch Ziekenhuis, 's–Hertogenbosch. In 2014 she started her dissertation focusing on growth and nutrition in early life at the departments of Neonatology and Obstetrics of the Erasmus MC – Sophia Children's Hospital, under guidance of prof.dr. I.K.M. Reiss, prof.dr. R.P.M. Steegers–Theunissen, dr. K.F.M. Joosten, and dr. M.J. Vermeulen. This work resulted in her PhD thesis: "Food for Thought – growth of the human body and brain in early life". In January 2018 Jorine will start her pediatric residency at the Erasmus MC – Sophia Children's Hospital.

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GEEF ME WERK DAT BIJ ME PAST
EN IK HOEF NOOIT MEER TE WERKEN
[CONFUCIUS]