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Maternal and Infant Adiposity

Metabolic Health Related Consequences

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Maternal and Infant Adiposity: Metabolic Health Related Consequences

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Maternal and Infant Adiposity: Metabolic Health Related Consequences

Adipositas tijdens de zwangerschap en de vroege kindertijd: gevolgen voor de metabole gezondheid

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Maternal and Infant Adiposity: Metabolic Health Related Consequences

Adiposidade materna e infantil:
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Manuscripts that form the basis of this thesis

Chapter 2.1
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Chapter 2.2
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Chapter 2.3
Santos S, Severo M, Gaillard R, Santos AC, Barros H, Oliveira A. The role of prenatal exposures on body fat patterns at 7 years: intrauterine programming or birthweight effects? *Nutr Metab Cardiovasc Dis 2016;26(11):1004:1010.*

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Chapter 3.1
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Chapter 3.2
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Chapter 3.3
Santos S, Gaillard R, Oliveira A, Barros H, Hofman A, Franco OH, Jaddoe VW. Subcutaneous fat mass in infancy and cardiovascular risk factors at school-age: The Generation R Study. *Obesity (Silver Spring) 2016;24(2):424-429.*

Contents

CHAPTER 1

General introduction, aims and design

Obesity is a major public health problem in the general adult population.[1] The prevalence of obesity worldwide has more than doubled in the last decades. The World Health Organization (WHO) estimated that 13% of the world´s adult population were obese in 2014.[1] Overweight and obesity have been considered the fifth leading global risk for mortality in the world.[2] Common health consequences of overweight and obesity include cardiometabolic diseases (e.g., mainly diabetes, stroke and heart diseases), musculoskeletal disorders and certain types of cancers such as breast and colon cancer.[3] Globally, the burden of diabetes, ischemic heart disease and certain cancers is about 44%, 23% and 7-41% attributable to overweight and obesity, respectively.[2]

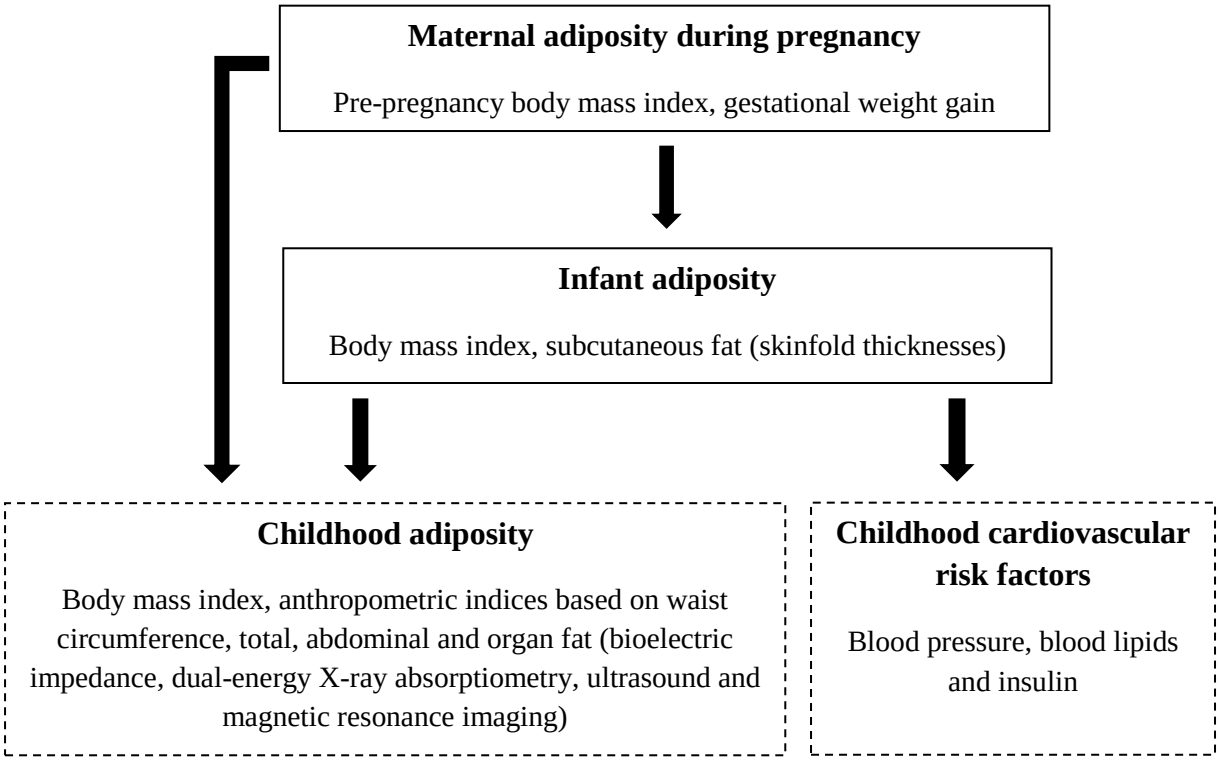
Concurrent with this, the prevalence of overweight and obesity in childhood is also reaching alarming levels worldwide. The WHO estimated that 41 million children worldwide under the age of 5 years were overweight or obese in 2014.[4] Childhood obesity is associated with a higher risk of obesity, cardiovascular disease, premature death and disability in adulthood.[5] In addition to increased future risks, obese children experience breathing difficulties, increased risk of fractures, early markers of cardiovascular disease (e.g., hypertension, non-alcoholic fatty liver disease, adverse lipid profile, insulin resistance), depression and other psychological effects.[5]

In the past few decades, there has been mounting evidence suggesting that obesity and cardiometabolic diseases in childhood and adulthood at least partly originate in fetal or early postnatal life.[6-11] The Developmental Origins of Health and Disease (DOHaD) hypothesis proposes that alterations in the intrauterine environment during critical periods of cellular proliferation and differentiation can affect the developing fetus leading to structural and functional alterations in cells, tissues and organ systems. These alterations may have lifelong consequences for body composition and cardiometabolic health in later life.[6] Maternal adiposity during pregnancy, among other lifestyle prenatal factors, has been identified as an important risk factor that might lead, through intrauterine programming, to obesity and cardiometabolic dysfunction in the offspring.[6] Along with intrauterine life, infancy seems also to be a critical period for the development of obesity and cardiometabolic diseases.[7] A large body of evidence has suggested that nutrition and growth in infancy are related to obesity and cardiovascular disease risk in later life.[9-11] In particular, rapid weight gain in infancy is associated with an adverse cardiovascular profile in adulthood.[8]

Assessing the contribution of maternal adiposity during pregnancy and infant adiposity to later adverse body composition and cardiometabolic risk status broadens the understanding on their short- and long-term health consequences. Furthermore, this may help

to develop future preventive strategies aimed at improving body composition and cardiovascular health throughout the life course and in future generations. Therefore, studies included in this thesis were designed to clarify the adiposity and cardiovascular health related consequences of maternal and infant adiposity (**Figure 1.1**).

Figure 1.1 Overview of the assessed associations in this thesis



Maternal adiposity during pregnancy

Obesity in women of reproductive age is increasing in prevalence worldwide.[12] Maternal pre-pregnancy obesity is an important risk factor for pregnancy and birth complications.[13-16] In addition to short-term adverse health effects, maternal obesity seems also to affect long-term health of the offspring. A meta-analysis of published studies showed an increased risk of overweight in offspring of mothers with overweight and obesity, as compared to offspring of mothers with normal weight.[17] Next to maternal obesity, excessive gestational weight gain also seems to be associated with an increased risk of childhood obesity.[18-20] Gestational weight gain is a complex trait, which reflects multiple components including maternal

nutritional status, the fetus, amniotic fluid, placenta, uterine and mammary tissue expansion, increased blood volume, and extracellular fluid.[21] Intrauterine programming might be involved in the associations of maternal obesity and excessive gestational weight gain with a greater risk of obesity in later life. It has been suggested that a maternal obesogenic environment during pregnancy leads to higher maternal plasma concentrations of glucose, amino acids and free fatty acids that are transferred to the developing fetus and might cause permanent changes in the development of adipocytes, and in the appetite control system.[6] Maternal pre-pregnancy body mass index and gestational weight gain are important modifiable factors that might greatly impact maternal health and health of the offspring. Thus, obtaining a better understanding of the influence of maternal pre-pregnancy body mass index and gestational weight gain on more detailed offspring fat measures, such as abdominal and organ fat, already from early infancy onwards and their underlying mechanisms is of utmost importance for development of preventive strategies.

Infant adiposity

Infant growth patterns, such as rapid postnatal weight gain, are recognized as critical for the development of overweight and obesity and for an adverse body fat distribution in later life.[7,9,11,22,23] Also, infant overweight and obesity are important risk factors for overweight and obesity in childhood.[24,25] Besides the effect on adiposity, early infant growth seems also to affect cardiovascular disease risk throughout the life course. Rapid weight gain in the first 3 months of life has been associated with an unfavorable cardiovascular and metabolic health profile in adulthood.[8]

Infancy seems to be characterized by a specific body fat development. During the first 4 months of life approximately 90% of body fat is located subcutaneously,[26] and preperitoneal fat mass seems to increase only from the second year of life onwards.[27] Skinfold thickness, which is a valid measurement of total and regional subcutaneous fat,[28] might be particularly relevant in infancy due to the pattern of fat deposition observed in this period.

Altogether, these studies suggest that infancy may be a critical period that influences the development of an adverse body fat profile and cardiovascular disease in later life. Thus, obtaining a better understanding of the influence of more detailed fat mass measures during infancy on fat mass measures and cardiovascular risk factors later in life helps to further understand the stability of body fat measures across the life course and to identify early

critical periods of fat development important for the risk of cardiovascular disease in adulthood.

Childhood adiposity and cardiovascular risk factors

The majority of studies addressing the adiposity related consequences of maternal adiposity during pregnancy and infant adiposity have used body mass index, which is a safe, inexpensive and an easy to obtain population-level diagnostic tool for defining general adiposity. However, body mass index is a suboptimal measure of body fat mass, especially in children, and provides no information about body fat distribution.[29]

Previous studies have suggested that body fat distribution, rather than body mass index, is related to the risk of cardiovascular and metabolic diseases.[30] Waist circumference and other indices based on waist circumference, such as waist-to-height ratio and waist-to-hip ratio, have been used in clinical practice to estimate abdominal fat. However, waist circumference has been more strongly correlated with body mass index and total body fat than with abdominal visceral fat,[31,32] raising doubts about its use and the use of waist-to-height ratio for assessing abdominal fat. Waist-to-hip ratio has been suggested to be a good predictor of cardiovascular disease risk because it includes a measurement of hip circumference, which has been negatively associated with diabetes, cardiovascular disease and mortality.[33,34] In line with this, in a meta-regression analysis of 15 prospective studies, waist-to-hip ratio tended to be more strongly associated with cardiovascular events than waist circumference.[35] Although easier to obtain and cheaper, anthropometric measurements are more likely to be affected by measurement error. As an alternative, dual-energy X-ray absorptiometry is an imaging technique that quantifies total and regional body fat content with high precision.[28]

These measures do not give any insight into the amount of subcutaneous, visceral and organ fat compartments, which have been described as better indicators of cardiometabolic risk.[30,36,37] Large cohort studies such as the Framingham Heart Study and the Jackson Heart Study have reported that excess visceral adiposity and ectopic fat deposition (such as excess heart, liver and intrathoracic fat) is related to various cardiometabolic abnormalities, independently of total or subcutaneous fat.[38-44] Magnetic resonance imaging is a high quality imaging and gold standard technique for the measurement of intra-abdominal and organ fat deposition.[45-48] Abdominal ultrasound is also a valid method to estimate subcutaneous fat mass and preperitoneal fat mass, as a proxy for visceral fat mass.[46,49] Overall, the cardiometabolic risk associated with adipose tissue seems to be mainly related to abdominal

and organ fat and thus it is important to gain more knowledge on the associations of early life factors with these measures of adiposity later in life.

Cardiovascular disease events are more frequent from the fifth decade of life onwards but the precursors of cardiovascular disease seem to originate in childhood. Previous studies have shown that increased blood pressure levels during childhood strongly predict hypertension in young adulthood.[50] Also, a long exposure to raised concentrations of low-density lipoprotein (LDL)-cholesterol increases the risk of cardiovascular events.[51] In line with this, an adverse cardiovascular risk profile characterized by high levels of LDL-cholesterol, systolic blood pressure, body mass index and cigarette smoking in 12- to 18-year-old adolescents was associated with carotid intima media thickness in adulthood, independently of current risk factors.[52] Higher glucose, insulin and insulin resistance in childhood have also been associated with a higher risk of diabetes and an adverse cardiovascular profile in adulthood.[53,54] Exposure to cardiovascular risk factors early in life may contribute to the development of cardiovascular disease events in adulthood and thus it is important to obtain further insight into their determinants and underlying mechanisms.

General aim

The general aim of this thesis was to assess the adiposity and cardiovascular health related consequences of maternal and infant adiposity.

Specific aims

The specific aims of this thesis are addressed in the studies presented in **Chapter 2** and **Chapter 3**. In **Chapter 2**, studies on the influence of maternal adiposity during pregnancy on infant and childhood outcomes are described. In **Chapter 2.1**, we reviewed the childhood cardiometabolic, respiratory and cognitive-related health consequences of maternal obesity during pregnancy. In **Chapter 2.2**, we studied the influence of maternal body mass index, and gestational weight gain on body mass index and subcutaneous fat at the ages of 1.5, 6 and 24 months. In **Chapter 2.3**, we addressed the associations of maternal gestational weight gain, diabetes and smoking on total and regional adiposity at the age of 7 years and explored whether these associations were through direct intrauterine mechanisms or through birth weight. The associations of maternal body mass index, and gestational weight gain on body mass index, and total, abdominal, pericardial and liver fat at the age of 10 years are described in **Chapter 2.4**. In **Chapter 3**, studies on the influence of infant adiposity on childhood outcomes are described. In **Chapter 3.1**, we examined the accuracy of single and combined

anthropometric indices for assessing body fat and clarified the use of indices based on waist circumference as measures of adiposity in children. The influence of infant body mass index and subcutaneous fat on childhood adiposity and cardiovascular risk factors is studied in **Chapter 3.2** and **Chapter 3.3**, respectively.

General design

The studies presented in this thesis were embedded in the Generation XXI, Porto, Portugal and in the Generation R Study, Rotterdam, the Netherlands.

Generation XXI

The Generation XXI is a population-based birth cohort assembled in Porto, Portugal.[55] The Generation XXI was designed to chart the growth and development of children born at the dawn of the new millennium seeking to better understand health during childhood and later in adolescence and adulthood, thereby contributing to health gains among the population. Mothers resident in the catchment area who delivered a live-born child, with a gestational age≥24 weeks, in one of the public maternity units covering the metropolitan area of Porto, between April 2005 and August 2006, were eligible for enrolment in this study. Mothers were invited to participate, 24 to 72 hours after delivery, and of the invited mothers, 91% accepted to participate. A total of 8647 children and 8495 mothers were enrolled at baseline. Follow-up evaluations at ages 6, 15 and 24 months were restricted to sub-samples of children. Re-evaluations of the entire cohort were conducted at 4 years old (between 2009 and 2011; 86% of all children re-evaluated) and at 7 years old (between 2012 and 2014; 80% of all children re-evaluated). Currently, they are being evaluated at 10 years old. The Generation XXI has been collecting parental and child data through interviewer- and self-administered questionnaires and diaries, performing detailed physical examinations and collecting biological samples. The studies presented in this thesis used data from enrolment and from the 7-year-old re-evaluation.

Generation R Study

The Generation R Study is a population-based prospective cohort study from fetal life until young adulthood in Rotterdam, the Netherlands.[56] The Generation R Study was designed to identify early environmental and genetic determinants of growth, development and health in fetal life and childhood. All pregnant women living in the study area with a delivery date between April 2002 and January 2006 were eligible for enrolment in this study. Enrolment

was aimed at early pregnancy, but was possible until the birth of the child. In total, 9778 mothers were enrolled in the study, of whom 8880 (91%) were included during pregnancy. Assessments were planned in early-, mid-, and late-pregnancy (<18, 18-25 and ≥25 weeks of gestation, respectively) and included parental physical examinations, blood and urine collection, fetal ultrasound examinations, and self-administered questionnaires. Assessments of the newborn at birth included a physical examination and cord blood tests. Additional detailed assessments of fetal and postnatal growth and development were conducted in a subgroup of Dutch mothers and their children from late pregnancy onwards. From birth to 4 years of age, data on growth, development and health of the participating children were obtained by questionnaires and visits to the routine child health care centers. At the age of 6 and 10 years, all children were invited to participate in detailed body composition and cardiovascular follow-up measurements. Currently, they are being evaluated at 13 years old. The studies presented in this thesis used data from pregnancy, birth, infancy and 6 and 10-year-old re-evaluations.

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CHAPTER 2

Maternal adiposity during pregnancy

CHAPTER 2.1

Maternal obesity, excessive gestational weight gain and childhood cardiometabolic, respiratory and cognitive-related health outcomes

Romy Gaillard, Susana Santos, Liesbeth Duijts, Janine F. Felix

Adapted from *Ann Nutr Metab 2016;69(3-4):171-180.*

Abstract

Background: Obesity is a major public health problem among women of reproductive age. In a narrative review, we examined the influence of maternal obesity during pregnancy on fetal outcomes and childhood adiposity, cardio-metabolic, respiratory and cognitive-related health outcomes. We discuss results from recent studies, the causality and potential underlying mechanisms of observed associations and challenges for future epidemiological studies.

Summary: Evidence from observational studies strongly suggests that maternal pre-pregnancy obesity and excessive gestational weight gain are associated with increased risks of fetal pregnancy complications and adverse childhood cardio-metabolic, respiratory and cognitive-related health outcomes. It remains unclear whether these associations are due to intrauterine mechanisms or explained by confounding family-based sociodemographic, lifestyle and genetic factors. The underlying mechanisms have mainly been assessed in animal studies and small human studies, and are yet to be further explored in large human studies.

Key Message: Maternal obesity is an important modifiable factor during pregnancy that is associated with a variety of adverse offspring health outcomes. Further studies are needed to explore the causality and underlying mechanisms of the observed associations. Ultimately, preventive strategies focused on reducing maternal obesity and excessive weight gain during pregnancy may reduce common diseases in future generations.

Introduction

Obesity is a major public health problem worldwide.[1] The World Health Organization estimated that 11% men and 15% women of the world's adult population were obese in 2014.[1] The strong increase in obesity prevalence also affected women of reproductive age. Over time, studies from the US and UK showed an increase in maternal obesity at the start of pregnancy from approximately 10% around 1990 to approximately 16-22% in the early 2000s.[2, 3] To date, the obesity prevalence rate in pregnant women is estimated to be as high as 30% in Western countries.[4-7] In these countries, an even higher percentage of women gain an excessive amount of weight during pregnancy based on the US Institute of Medicine (IOM) guidelines, which define optimal ranges of maternal weight gain during pregnancy according to a mother's pre-pregnancy body mass index (BMI) based on evidence from observational studies (**Table 1**).[5, 8-11] An accumulating body of evidence suggests that maternal obesity and excessive weight gain during pregnancy are not only associated with adverse maternal and fetal pregnancy outcomes, but also have a long-term adverse influence on common health outcomes in the offspring.[11, 12]

In this narrative review, we provide a review update of the findings from recent observational studies and meta-analyses focused on the associations of maternal obesity and excessive weight gain during pregnancy with fetal outcomes and offspring adiposity, cardio-metabolic, respiratory and cognitive-related health outcomes throughout childhood.[11, 12] We also discuss the causality and potential mechanisms underlying the observed associations as well as challenges for future research.

Fetal outcomes

Maternal pre-pregnancy obesity and excessive gestational weight gain are important risk factors for multiple adverse fetal outcomes (**Figure 1**).[11-13] Several large meta-analyses have shown that a higher maternal pre-pregnancy or early pregnancy BMI is associated with increased risks of fetal death, stillbirth, neonatal death and the development of various congenital anomalies.[14, 16] Thus far, increased gestational weight gain seems not to be associated with fetal death or still birth. Both higher maternal pregnancy BMI and increased gestational weight gain are well-known risk factors for delivering large size for gestational age infants. A meta-analysis among 13 studies showed that, as compared to normal maternal pre-pregnancy weight, maternal pre-pregnancy obesity was associated with a 2-fold higher risk of delivering a large size for gestational age infant.[17] Similarly, a meta-analysis among 15 cohort and case-control studies showed that excessive gestational weight gain based on the

IOM criteria was associated with a 2-fold higher risk of macrosomia.[18] Based on studies that assessed the associations of gestational weight gain during specific periods of pregnancy, it appears that especially higher second and third trimester maternal weight gain are associated with an increased risk of delivering a large size for gestational age infant.[19, 20] Also, both maternal pre-pregnancy BMI and gestational weight gain seem to be associated with common adverse neonatal outcomes, such as preterm birth, a low Apgar score, neonatal hypoglycemia and referral to the neonatal intensive care unit, with stronger and more consistent associations for maternal pre-pregnancy BMI than for gestational weight gain.[20-25]

Thus, both maternal pre-pregnancy obesity and excessive gestational weight gain lead to increased risks of fetal complications throughout pregnancy. Overall, the associations for maternal pre-pregnancy obesity with adverse fetal outcomes seem to be more consistent and stronger than for excessive gestational weight gain.[13, 20]

Childhood outcomes

An accumulating body of evidence suggests that maternal obesity during pregnancy adversely affects a variety of childhood health outcomes.[11] Most studies that assessed the influence of maternal gestational obesity on common childhood health outcomes focused on childhood adiposity and cardio-metabolic development (Figure 1).[12] Across studies from different countries, maternal pre-pregnancy obesity and excessive gestational weight gain are associated with an increased risk of obesity throughout childhood.[26] Two meta-analyses based on results from observational studies showed that both maternal pre-pregnancy obesity and excessive gestational weight gain according to the IOM criteria were associated with a 3-fold higher risk of childhood obesity, as compared to normal weight women and to a recommended amount of gestational weight gain, respectively.[27, 28] However, during childhood, BMI might not be an appropriate measure of fat mass because an increase in BMI may reflect an increase in lean mass instead of fat mass.[29, 30] It has been shown that detailed fat mass measures, such as total body fat mass and abdominal fat mass, are more strongly associated with cardio-metabolic risk factors in childhood and adulthood and the risk of mortality in later life.[31-33] Several studies also showed that a higher maternal pre-pregnancy BMI and total gestational weight gain are independently associated with a higher childhood waist circumference, total body fat mass and abdominal fat mass levels, although the associations for total gestational weight gain are less consistent.[10, 34-39] Maternal weight gain in early pregnancy appears to be a specific critical period for the development of adiposity in childhood.[12] Three population-based prospective cohort studies showed that especially

maternal gestational weight gain in early pregnancy was associated with a higher childhood BMI, total body fat mass and abdominal fat mass from the age of 4−9 years.[10, 19, 35] These associations appeared to be independent from maternal pre-pregnancy BMI and weight gain later in pregnancy.

Both maternal pre-pregnancy obesity and excessive gestational weight gain are associated with a suboptimal childhood cardio-metabolic profile.[12] In the Generation R Study, a population based prospective cohort study in Rotterdam, the Netherlands, we observed that a higher maternal pre-pregnancy BMI was associated with a higher childhood systolic blood pressure, left ventricular mass, aortic root diameter and insulin levels, and lower high-density lipoprotein (HDL)-cholesterol levels at the age of 6 years.[34, 40] As compared to children from normal weight mothers, children from obese mothers had a 3-fold higher risk of an adverse childhood cardio-metabolic risk profile, which included high abdominal fat mass, high blood pressure, high insulin and triglycerides levels and low HDL-cholesterol level.[34] Similarly, higher maternal early pregnancy weight gain, but not weight gain later in pregnancy, was associated with an increased risk of an adverse childhood cardio-metabolic risk profile.[35] Another Dutch study among 1,459 mothers and their 5-6 year old children showed that a higher maternal pre-pregnancy BMI was associated with higher childhood systolic blood pressure and overall metabolic score, as a measure of a metabolic syndrome like phenotype, but not with childhood sympathetic drive, parasympathetic drive or heart rate.[39, 41] A study among 5,154 UK mother-offspring pairs showed that higher maternal pre-pregnancy weight and gestational weight gain in mid-pregnancy were associated with higher childhood levels of triglycerides, HDL-cholesterol, apolipoprotein A1 and interleukin (IL)-6 at the age of 9 years.[10] A study among 1,090 mother-child pairs participating in a pre-birth cohort in the USA showed that a higher maternal pre-pregnancy BMI was associated with higher mid-childhood leptin, high sensitivity C-reactive protein and IL-6 levels, and lower adiponectin levels, whereas a higher total gestational weight gain was only associated with higher mid-childhood leptin levels.[36] Across different studies, these associations with cardio-metabolic risk factors are not explained by birth weight, but seem to be largely mediated by childhood BMI.[12]

A higher maternal pre-pregnancy BMI and increased gestational weight gain may affect respiratory outcomes throughout childhood (Figure 1). A meta-analysis among 14 European birth cohort studies showed that maternal overweight and obesity during pregnancy were associated with the risk of ever wheezing and recurrent wheezing until the age of 2 years.[42] Accordingly, a more recent meta-analysis among 14 studies with over 108,000

mother–child pairs showed that maternal obesity during pregnancy was associated with a 31% increased risk of asthma or ever wheezing in children aged 14 months to 16 years.[43] Each 1-kg/m^2 increase in maternal BMI led to a 3% increased risk of childhood asthma.[43] High gestational weight gain was associated with a 16% higher risk of ever asthma or wheeze, but not with current asthma or wheeze.[43] In both meta-analyses, the observed associations could not be explained by multiple socioeconomic, lifestyle and birth characteristics or by the child's BMI at the time of assessment of the outcome.[42, 43] Asthma is partly considered an atopic disorder. However, thus far, the associations of maternal pre-pregnancy obesity with other atopic disorders including allergic rhinitis, hay fever, atopic dermatitis or inhalant and food allergen sensitization are inconsistent.[44-48] In addition, only few studies assessed the associations with more detailed childhood lung function measurements directly and have shown inconsistent results.[45, 46]

Less is known about the associations of maternal obesity during pregnancy with cognitive outcomes in the offspring (Figure 1).[49-59] Several studies showed that maternal pre-pregnancy obesity is associated with a lower cognitive function in children, but results are not consistent.[49-57] Total weight gain during pregnancy seems not be associated with childhood cognitive function.[54, 56, 60] However, a study among 5,191 mother-offspring pairs restricted to term deliveries from the UK showed small positive associations of maternal weight gain in each trimester of pregnancy with IQ scores at 8 years of age, without remarkable differences in strength of the effect estimates for different periods of maternal weight gain.[61] Similarly, a study among normal weight Scandinavian mothers and their children, showed that maternal third trimester weight gain was only associated with child IQ scores at 5 years of age when the sample was limited to term deliveries.[62] In a meta-analysis among 5 observational studies, maternal obesity during pregnancy was associated with a 1.5-fold higher risk of childhood autism spectrum disorder.[63] A Swedish study among 333,057 participants also showed that both low and high maternal gestational weight gain were associated with an increased risk of autism spectrum disorder in children.[64] A combined study among 12,556 school-aged children and their mothers from 3 prospective Scandinavian cohorts showed that maternal pre-pregnancy obesity was associated with an increased risk of childhood attention deficit hyperactivity disorder (ADHD) symptoms rated by teachers.[65] No associations of maternal gestational weight gain with ADHD symptoms seem to be present.[66]

Thus, in line with the risks of adverse fetal outcomes, maternal pre-pregnancy obesity and excessive gestational weight gain also lead to increased risks of adverse adiposity, cardio-metabolic, respiratory and cognitive-related outcomes in childhood. Most consistent

associations have been reported for childhood adiposity outcomes, whereas especially the associations with childhood cognitive outcomes seem to be weaker and less consistent across studies. The observed associations seem not to be restricted to obesity or excessive gestational weight gain only, but present across the full range of BMI and gestational weight gain.

Causality or confounding

It remains unclear whether the associations of maternal obesity during pregnancy with common childhood outcomes are explained by direct intrauterine mechanisms or confounded by environmental, lifestyle or genetic characteristics.[11, 12] Most previous observational studies adjusted their analyses for a variety of pregnancy-related characteristics and maternal and offspring socio-demographic, nutrition and lifestyle-related characteristics.[11, 12] Despite adjustment for potential confounding factors in these observational studies, residual confounding may still be a major issue to consider.[11, 12] As described previously, several methods in epidemiological research can be used to better control for confounding characteristics in observational studies.[11, 12]

Multiple studies have compared the strength of associations of maternal and paternal BMI with childhood outcomes as an aid to further disentangle underlying mechanisms.[12] Stronger associations for maternal BMI suggest direct intrauterine mechanisms, whereas similar or stronger associations for paternal BMI suggest a role for shared genetic or family-based, lifestyle-related characteristics.[12] Stronger associations of maternal pre-pregnancy BMI with birth weight have been reported than for paternal BMI.[67] Although studies comparing associations of maternal and paternal BMI with childhood BMI have shown conflicting results,[68] studies examining these associations with more detailed childhood fat mass measures and other cardio-metabolic risk factors have shown that maternal pre-pregnancy BMI tends to be more strongly associated with childhood total fat mass, android/gynoid fat mass ratio and clustering of cardio-metabolic risk factors than paternal BMI.[34, 69] A study among 940 Swedish children and 873 adolescents showed that higher maternal BMI was more strongly associated with offspring cardiorespiratory fitness, after taking offspring fatness into account, than paternal BMI.[70] No such studies have yet been performed with other childhood respiratory outcomes. With regard to childhood cognitive outcomes, few studies that do not show strong evidence for a potential intrauterine effect have been performed. A study among 1,783 Danish parents and their 5-year old children observed similar associations for maternal and paternal BMI with childhood IQ.[50] A study

among 2,379 infants and their parents from 2 Southern-European birth cohorts showed that although the association for maternal BMI with offspring cognition was stronger than for paternal BMI, the confidence intervals of the maternal and paternal effect estimates were not statistically different. These findings suggest that maternal pre-pregnancy BMI may, at least partly, influence offspring birth weight and childhood cardio-metabolic health through direct intrauterine mechanisms, but this remains to be further explored for other childhood health outcomes. A limitation of this statistical method is the assumption that a mother and father contribute equally to the shared lifestyle-related characteristics between parents and their offspring.[68] However, the influence of the parents on the offspring's diet and exercise may differ between mother and father.[68]

Another approach used to obtain further insight into the role of confounding in these observed associations is by a sibling comparison study.[11, 12] A sibling comparison study allows control for environmental characteristics as well as maternal genotype that are shared among siblings.[12] Important sibling comparison studies have been performed among children from mothers who had high levels of pre-pregnancy weight loss due to biliopancreatic diversion bariatric surgery. These studies showed that among children born to mothers before surgery, the risk of macrosomia, obesity, reduced insulin sensitivity and suboptimal lipid levels was higher than among those children born to mothers after surgery.[71] These findings provide evidence to suggest that some of the effect of extreme maternal obesity on offspring outcomes may be through direct intrauterine mechanisms. However, it remains unclear whether this effect is also present for less extreme maternal pre-pregnancy BMI levels and a variety of childhood health outcomes. Two large sibling comparison studies from the USA showed that children born to mothers who gained a large amount of weight during pregnancy had a higher birth weight and higher childhood BMI than children born to mothers who gained less weight during pregnancy.[72, 73] The association with childhood BMI was only partly mediated by offspring birth weight. A sibling comparison study among Swedish men aged 18 years showed that among siblings from overweight and obese mothers, higher total gestational weight gain was associated with higher offspring BMI.[74] However, in the same study population, no evidence was found for an association of maternal BMI with offspring BMI among siblings.[75] Thus far, no sibling comparison studies have focused on childhood respiratory outcomes and only few sibling comparison studies focused on childhood cardio-metabolic outcomes or cognitive outcomes. A study among 4,908 brother-pairs from Sweden showed no associations of maternal gestational weight gain and offspring blood pressure or risk of hypertension at 18 years among siblings.[76] A study among 333,057 participants from

Sweden showed that maternal BMI at the first antenatal visit was not associated with autism spectrum disorder among siblings, whereas excessive gestational weight gain was associated with the risk of autism spectrum disorder within a matched sibling analysis.[64] Another study among 673,632 individuals from Sweden showed that at the population level, maternal pre-pregnancy obesity was associated with an increased risk of offspring ADHD, but not among siblings.[77] Thus, findings from sibling comparison studies among less extreme obese populations suggest that especially gestational weight gain may affect offspring health outcomes through direct intrauterine mechanisms, whereas the associations for maternal pre-pregnancy BMI with childhood outcomes may be explained by unmeasured confounding factors. An important limitation of sibling comparison studies is that besides the major exposures of interest, maternal pre-pregnancy BMI and gestational weight gain, other lifestyle-related characteristics may differ between siblings.[12]

A Mendelian randomization approach uses genetic variants, which are robustly associated with the exposure of interest and not affected by confounding, as an instrumental variable for a specific exposure.[12] A study among 30,487 mothers-offspring pairs from 18 cohort studies showed that a genetically higher maternal BMI was associated with a higher birth weight, which suggests that genetically higher maternal BMI may be causally related to birth weight.[78] On the contrary, a study among 4,091 mother-offspring pairs showed no association of maternal FTO with childhood fat mass at the age of 9 years, which suggests that maternal obesity may not be causally related to childhood adiposity outcomes.[69] However, this study needs to be interpreted cautionary, as this study may be limited by a relatively small sample size. The need for a large sample size to have adequate power for these types of analyses is an important limitation of the Mendelian Randomization approach. However, the findings from these studies mark the importance for further Mendelian randomization studies with a larger sample size and using multiple maternal genetic variants as instruments focused on a variety of childhood health outcomes.

Randomized controlled trials are considered as the golden standard to assess causality.[11, 12] However, with randomized controlled trials, we are only able to test a specific exposure which is amenable to intervention. Therefore, previous randomized controlled trials have focused on influencing determinants of maternal obesity and excessive weight gain during pregnancy, such as dietary factors and physical activity levels, since directly randomized studies are difficult to perform with maternal pre-pregnancy obesity and excessive gestational weight gain as major exposures of interest.[12] A randomized controlled trial among 574 obese infertile women, which provided a 6-month lifestyle intervention

program prior to fertility treatment showed a small reduction in maternal pre-pregnancy weight but no effect on rates of healthy singleton life born children.[79] A meta-analysis among 44 randomized controlled trials focused on dietary and physical activity interventions during pregnancy suggested that especially dietary interventions during pregnancy, and not physical activity interventions, may lead to a small reduction in gestational weight gain and to a slightly lower risk of adverse pregnancy outcomes.[80] A Cochrane review which included 65 randomized controlled trials suggested that interventions during pregnancy focused on diet or exercise, or combined, can reduce the risk of excessive gestational weight gain.[81] However, the effect of these interventions on childhood outcomes remains to be explored. A small randomized controlled trial among 254 mothers and their children, which provided both dietary advice and exercise to obese mothers during pregnancy, showed no difference in their infant BMI or metabolic risk factors.[82] Long-term follow-up of participants in these trials is important as it will provide insight into the causality of observed associations as well as the effectiveness of maternal lifestyle interventions during pregnancy for improving common health outcomes in offspring.[12]

Altogether, these epidemiological studies specifically designed to explore the causality for the associations of maternal obesity with common childhood health outcomes show inconsistent results. Each method has important methodological limitations and combined results from these different approaches will be needed to obtain further insight into the causality of these observed associations.

Underlying mechanisms

The mechanisms underlying the associations of maternal pre-pregnancy obesity or excessive gestational weight gain with offspring health outcomes remain unclear. Maternal pre-pregnancy BMI and gestational weight gain are complex traits, which are inversely correlated.[20] In general, women with a higher pre-pregnancy BMI gain less weight during pregnancy as compared to women with a lower pre-pregnancy BMI.[8] Most observational studies mutually adjusted their analyses for maternal pre-pregnancy BMI and gestational weight gain to explore the independent effects of these maternal exposures on offspring outcomes.[11, 12] However, both maternal pre-pregnancy BMI and gestational weight gain reflect multiple components.[12] Maternal pre-pregnancy obesity not only reflects maternal fat accumulation, but also other maternal characteristics, including maternal nutritional status, insulin and glucose metabolism and low-grade systemic inflammation.[12] Similarly, maternal weight gain during pregnancy reflects maternal fat accumulation, but also maternal and

amniotic fluid expansion and growth of the fetus, placenta and uterus.[12] Both maternal pre-pregnancy obesity and excessive gestational weight gain as well as the correlated maternal exposures may lead to programming effects in the offspring through several pathways.[12]

Not much is known about the potential programming effects by maternal obesity or excessive gestational weight gain. The fetal overnutrition hypothesis suggests that increased placental transfer of nutrients to the developing fetus in obese mothers and mothers with high levels of gestational weight gain, may subsequently affect fetal development, fetal fat deposition and the development of the hypothalamic-endocrine system that controls appetite and energy metabolism.[12] Inflammation and immunological mechanisms due to maternal obesity during pregnancy may also affect offspring cardio-metabolic, pulmonary and brain development.[65, 83, 84] This may predispose individuals to a greater risk of adverse health outcomes in later life. Accumulating evidence suggests that epigenetic mechanisms may play a key role in these programming mechanisms.[85] Thus far, animal studies provide support for programming effects of maternal obesity and excessive gestational weight gain through epigenetic mechanisms.[86] Also, several small human studies using a epigenome-wide approach showed associations of maternal pre-pregnancy BMI or gestational weight gain with DNA methylation in offspring cord blood.[87-91] However, the mechanisms proposed have not been tested yet in large epidemiological studies. Further mechanistic studies are important to obtain a better understanding of the underlying mechanisms.

Challenges for future epidemiological research

Although current evidence suggests that maternal obesity during pregnancy adversely affect common childhood health outcomes, there remain major issues to be addressed in future studies, as previously described.[11, 12]

First, despite extensive adjustment for potential confounding factors in most studies and the use of more sophisticated study designs in some observational studies, the causality of the observed associations remains unclear. Large observational studies with sophisticated designs, such as parent-offspring comparison studies, sibling comparison studies and Mendelian randomization studies are needed for insight into the causality of the observed associations.[11, 12] These studies need to move beyond childhood adiposity as an outcome of interest and also focus on a variety of offspring health outcomes, including cardio-metabolic, respiratory and cognitive outcomes. Long-term follow-up of participants in trials focused on reducing maternal weight throughout pregnancy will also provide further insight into the causality of the observed associations.[11, 12]

Second, the underlying mechanisms of the observed associations of maternal obesity during pregnancy with childhood health outcomes need to be further explored.[11, 12] Although animal studies and small human studies have suggested several pathways that may be involved in the observed associations, these pathways remain to be studied in large human studies.[11, 12] Identification of potential underlying pathways is complicated, as maternal pre-pregnancy obesity and excessive gestational weight gain both reflect several lifestyle-related and biological components.[11, 12] Future studies need to obtain detailed assessments of the maternal exposures of interest from the start of pregnancy onwards, such as repeated measurements of maternal weight, body composition and metabolic status.[11, 12] For the offspring outcomes, more detailed measurements of growth, body composition, cardio-metabolic, respiratory and cognitive development, such as cardiac structures, endothelial function, lipid spectrums, insulin/glucose metabolism, spirometry parameters, fractional exhaled nitric oxide levels, bronchial hyper-responsiveness and cranial structures assessed by MRI might also lead to further insight into the underlying mechanisms present in the observed associations. Since epigenetic mechanisms are one of the major underlying mechanisms of interest, repeated offspring blood samples throughout the life course are needed to assess the influence of the specific maternal exposures on offspring epigenetic adaptations. Long-term follow-up of participants in observational studies is needed to assess the influence on common adverse health outcomes throughout the life-course.[11, 12]

Third, further research focused on optimizing maternal pre-pregnancy BMI and weight gain during pregnancy for the prevention of adverse health outcomes in offspring is needed.[11, 12] Further insight needs to be obtained into the optimal amounts of maternal weight gain for short- and long-term maternal and offspring health outcomes to further improve the IOM recommendations for gestational weight gain.[11, 12] By conducting long-term follow-up studies of mothers and their children participating in randomized trials focused on reducing maternal weight throughout pregnancy by dietary and physical activity interventions, we will gain insight into the effectiveness of these maternal lifestyle interventions during pregnancy for improving a variety of long-term health outcomes in offspring.[11, 12]

Conclusions

Maternal pre-pregnancy obesity and excessive weight gain during pregnancy are common and important modifiable risk factors for adverse fetal outcomes and childhood adiposity, cardio-metabolic, respiratory and cognitive related health outcomes. To explore the causality of these associations, parent offspring comparison studies, sibling comparison studies,

Mendelian randomization studies and randomized controlled trials are needed. Further mechanistic studies, especially in large human populations are needed to obtain insight in the underlying mechanisms. Finally, the potential for prevention of common diseases in future generations by reducing maternal obesity and excessive weight gain during pregnancy needs to be explored. Preventive strategies focused on improving maternal health in the preconception period and in pregnancy by optimizing preconception care may improve long-term health outcomes in the offspring.

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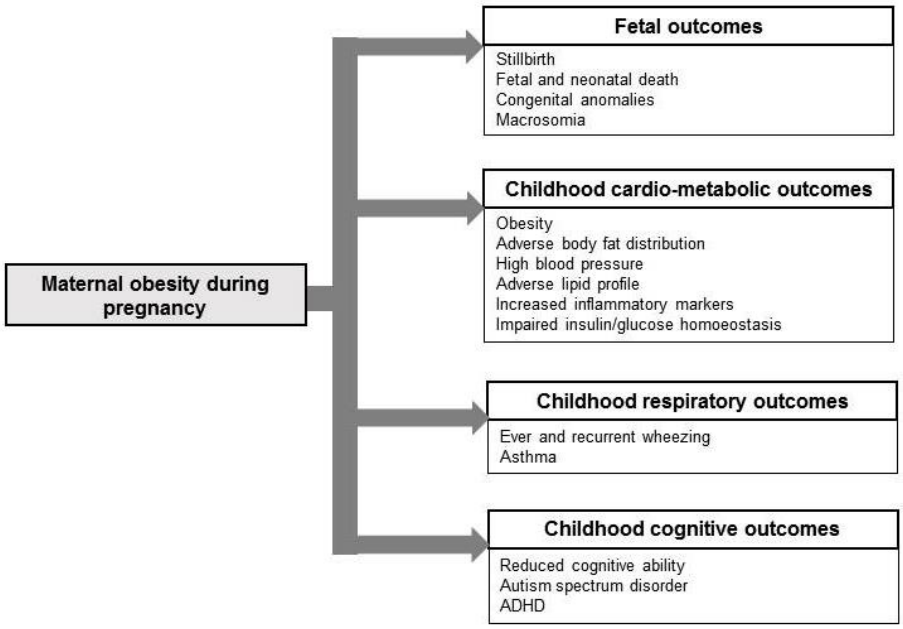
Table 1. IOM criteria for gestational weight gain[1]

Pre-pregnancy body mass index	**Recommended amount of total gestational weight gain, kg**
Underweight (Body mass index< 18.5 kg/m^2)	12.5–18
Normal weight (Body mass index ≥ 18.5–24.9 kg/m^2)	11.5–16
Overweight (Body mass index ≥ 25.0–29.9 kg/m^2)	7–11.5
Obesity (Body mass index ≥ 30.0 kg/m^2)	5–9

[1] Recommended gestational weight gain guidelines according to women's pre-pregnancy body mass index. Adapted from the IOM criteria.[8]

Figure 1. Maternal obesity during pregnancy and adverse childhood outcomes



CHAPTER 2.2

Maternal obesity, excessive gestational weight gain and subcutaneous fat mass in infancy

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Abstract

Background: Not much is known about the associations of maternal obesity and excessive gestational weight gain with body fat in infancy.

Objective: To examine the associations of maternal pre-pregnancy body mass index and gestational weight gain with infant subcutaneous fat.

Methods: In a population-based prospective cohort study among 845 mothers and their infants, we obtained maternal pre-pregnancy body mass index and measured maternal weight during pregnancy. At 1.5, 6 and 24 months, we estimated infant total subcutaneous fat (sum of biceps, triceps, suprailiacal and subscapular skinfold thicknesses) and central-to-total subcutaneous fat ratio (sum of suprailiacal and subscapular skinfold thicknesses/total subcutaneous fat).

Results: Maternal body mass index was positively associated with higher infant body mass index from 6 months onwards. Maternal body mass index was not associated with infant subcutaneous fat measures at 1.5 or 6 months. A 1-standard deviation scores (SDS) higher maternal body mass index was associated with a 0.09 (95% Confidence Interval 0.01, 0.17) SDS higher infant total subcutaneous fat at 24 months, but not with central-to-total subcutaneous fat ratio. No associations were present for maternal total or period-specific gestational weight gain with infant fat.

Conclusion: Maternal body mass index was positively associated with infant body mass index and total subcutaneous fat in late infancy. Maternal total and period-specific gestational weight gain were not associated with infant body fat mass measures.

Introduction

Maternal pre-pregnancy obesity and excessive weight gain during pregnancy are associated with an increased risk of obesity in childhood.[1,2] Body mass index is a suboptimal measure of body fat mass and provides no information about body fat distribution.[3] Several studies have shown that compared to body mass index, central fat distribution is more strongly associated with an adverse cardiovascular risk profile.[4] Previously, we reported that maternal obesity and excessive weight gain especially in early-pregnancy seem to be associated with an adverse body fat distribution, such as higher android-to-gynoid fat mass ratio, at 6 years.[5,6] We have also shown that maternal pre-pregnancy body mass index tended to be more strongly associated with childhood total and abdominal fat than paternal body mass index, suggesting that intrauterine mechanisms might be involved.[6] Thus far, previous studies did not assess the associations and explore the underlying mechanisms of maternal obesity and excessive weight gain during pregnancy with detailed offspring fat mass measures already from early infancy onwards, which is a well-known critical period for adiposity development in later life.[7] Skinfold thickness is a valid measurement of total and regional subcutaneous fat mass in infancy.[8] We have previously shown that subcutaneous fat mass measured by skinfolds tends to track throughout infancy and is positively associated with cholesterol levels at school-age children.[9,10]

Therefore, we examined in a population-based prospective cohort study among 845 parents and their infants, the associations of maternal pre-pregnancy body mass index and weight gain in different periods of pregnancy with subcutaneous fat mass measures throughout infancy. We also compared the strength of the associations of maternal and paternal body mass index with infant fat mass measures to obtain further insight in the underlying mechanisms.

Methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards among 9,778 mothers and their children living in Rotterdam, the Netherlands.[11] The local Medical Ethical Committee approved the study. Written informed consent was obtained from parents. Additional detailed assessments of growth and development were conducted in a subgroup of Dutch mothers and their children from late pregnancy onwards. Of all approached women, 80% agreed to participate. From the

total of 1,205 mothers and their singleton children participating in the subgroup study, 1,033 mothers had information about pre-pregnancy body mass index. Missing information about pre-pregnancy body mass index was mainly because of later enrolment in the study and nonparticipation in the first questionnaire. Body mass index or skinfold thicknesses measured at the age of 1.5, 6 or 24 months were available in 845 children (Flow chart is given in **Supplemental Figure S1**). Missing body fat mass measurements during infancy were due to loss to follow-up or crying behavior.

Parental anthropometrics

As previously described, maternal pre-pregnancy weight was obtained by questionnaire at enrolment.[11] Maternal height (cm) and paternal height (cm) and weight (kg) were measured without shoes and heavy clothing at enrolment. Body mass index (kg/m^2) was calculated. Maternal and paternal body mass index were categorized into 4 categories (underweight (<20 kg/m^2), normal weight (20–24.9 kg/m^2), overweight (25–29.9 kg/m^2), and obese (≥30 kg/m^2)). We measured maternal weight without shoes and heavy clothing at median 12.8 (95% range 9.9,17.0), 20.4 (95% range 18.6,22.7) and 30.4 (95% range 28.5,32.5) weeks of gestation. In a subgroup of 509 mothers, information about maximum weight during pregnancy was assessed by questionnaire 2 months after delivery. Based on the timing of maternal weight measurements within our study cohort, we defined early-, mid- and late-pregnancy weight, using self-reported and measured maternal weight data, as: at 13 weeks of gestation (median 12.8, 95% range 9.9,18.9); at 26 weeks of gestation (median 29.9, 95% range 20.4,31.6); and at 40 weeks of gestation (median 39.0, 95% range 32.6,42.0), respectively. Using this method, information about early-, mid- and late-pregnancy weight was available for 762, 824 and 493 mothers, respectively. Among the subgroup of mothers with maximum weight during pregnancy available, we defined excessive gestational weight gain in relation to maternal pre-pregnancy body mass index according to the Institute of Medicine (IOM) guidelines.[12]

Body fat measurements during infancy

We measured weight to the nearest gram in naked infants at the age of 1.5 and 6 months by using an electronic infant scale and at 24 months by using a mechanical personal scale (SECA, Almere, the Netherlands). Body length at the age of 1.5 and 6 months was measured in supine position to the nearest millimeter by using a neonatometer and body height at 24 months was measured in standing position by using a Harpenden stadiometer (Holtain

Limited, Dyfed, UK). Body mass index (kg/m^2) was calculated. We measured skinfold thicknesses at the ages of 1.5, 6 and 24 months on the left side of the body at the biceps, triceps, suprailiacal and subscapular area by using a skinfold caliper (Slim Guide, Creative Health Products).[9] We calculated total subcutaneous fat mass from the sum of all four skinfold thicknesses, and central subcutaneous fat mass from the sum of suprailiacal and subscapular skinfold thicknesses.[13] Measurements of body fat quantity and distribution require appropriate adjustment for body size or total fat mass, respectively, in order to undertake informative comparisons between children and within children over time. To create total subcutaneous fat mass independent of length or height and central subcutaneous fat mass independent of total subcutaneous fat mass, we estimated the optimal adjustment by log-log regression analyses.[14] Based on these analyses, total subcutaneous fat mass was only weakly correlated with length at 1.5 and 6 months or height at 24 months, and was not adjusted for it. A central-to-total subcutaneous fat mass ratio was calculated as central divided by total subcutaneous fat mass.

Covariates

Information on maternal and paternal age, educational level and parity was obtained at enrolment.[11] Information on maternal smoking was assessed by questionnaires during pregnancy. First trimester maternal nutritional information was obtained by food frequency questionnaire. Information about pregnancy complications, mode of delivery, child's sex, gestational age and weight at birth was obtained from medical records.[15] Information about breastfeeding duration and timing of introduction of solid foods was obtained by questionnaires in infancy.

Statistical analysis

First, we examined differences in subject characteristics between maternal body mass index categories with 1-way ANOVA tests and χ^2 tests. Next, we examined the associations of maternal and paternal body mass index with infant subcutaneous fat mass measures at each time period using linear regression models. We also used repeated measurement regression models to assess the associations of parental pre-pregnancy overweight with the repeatedly measured infant fat mass measures. These models take the correlation between repeated measurements of the same subject into account, and allow for incomplete outcome data. Third, we examined the associations of maternal maximum gestational weight gain and excessive gestational weight gain according to the IOM criteria with infant subcutaneous fat

mass measures using linear regression models. Since maternal weight measurements throughout pregnancy are strongly correlated, we performed conditional linear regression analyses to assess the independent associations of maternal pre-pregnancy weight and early-, mid- and late-pregnancy weight gain with infant subcutaneous fat mass measures. We obtained standardized residuals for each weight measurement from the regression of maternal weight at a specific time point on prior maternal weight measurements. These weight variables are statistically independent from each other, and can be simultaneously included in the regression models.[16]

Models were adjusted for maternal and childhood socio-demographic and lifestyle-related characteristics. Covariates were included based on associations with the exposures and outcomes of interest in previous studies, or a change in effect estimates >10%. We constructed SDS ((observed value−mean)/SD) for parental body mass index and gestational weight gain and infant fat mass measures to enable comparison of effect estimates. Since no significant interactions between parental body mass index or maternal gestational weight gain and child's sex in the associations with infant subcutaneous fat mass measures were present, no further stratified analyses were performed. Missing values in covariates were multiple-imputed, by using Markov chain Monte Carlo approach. Five imputed datasets were created and analyzed together. All statistical analyses were performed using the Statistical Package of Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Subject characteristics

Characteristics of included mothers, fathers and their children are given in **Table 1**. Non-response analyses showed that as compared to mothers who did not participate in the follow-up studies, those who did participate were slightly older, had a higher educational level and their children were breastfed for a longer period ($p<0.05$), but no differences were observed regarding maternal and paternal body mass index and maternal weight gain during pregnancy (**Supplemental Table S1**).

Parental body mass index and infant body fat

Table 2 shows the associations of maternal and paternal body mass index with infant subcutaneous fat mass measures. We observed no associations of maternal pre-pregnancy body mass index with infant body fat mass measures at 1.5 months. A higher maternal pre-

pregnancy body mass index was associated with higher infant body mass index from 6 months onwards (difference at 6 and 24 months: 0.09 (95% Confidence Interval (CI) 0.01,0.17) SDS, 0.17 (95% CI 0.09,0.26) SDS per 1-SDS higher maternal body mass index, respectively). A higher maternal pre-pregnancy body mass index was also associated with higher infant total subcutaneous fat mass at 24 months (difference: 0.09 (95% CI 0.01, 0.17) SDS per 1-SDS higher maternal body mass index), but not with infant central-to-total subcutaneous fat mass ratio. A higher paternal body mass index was only associated with higher infant body mass index at 1.5 and 24 months (p-values<0.05). Including both maternal and paternal body mass index in the same model did not change the effect estimates for infant fat mass measures at 1.5, 6 and 24 months.

Supplemental Figure S2 shows that maternal pre-pregnancy overweight was associated with higher infant body mass index growth from 6 months onwards, resulting in higher body mass index at 24 months (all p-values<0.05). Maternal pre-pregnancy overweight also tended to be associated with higher total subcutaneous fat mass and central-to-total subcutaneous fat mass ratio at 24 months (difference: 0.09 (95% CI 0.01,0.17) SDS, 0.08 (95% CI -0.01,0.17) SDS for maternal pre-pregnancy overweight as compared to maternal pre-pregnancy normal weight, respectively). No associations of paternal overweight with infant body fat mass measures were present.

Maternal gestational weight gain and infant body fat

Table 3 shows no consistent associations for maternal maximum and excessive gestational weight gain with infant body mass index and subcutaneous fat mass measures at 1.5, 6 and 24 months. Excessive maternal gestational weight gain was only associated with higher infant body mass index at 6 months (difference: 0.30 (95% CI 0.11,0.48) SDS for excessive maternal weight gain as compared to non-excessive weight gain). No associations were observed for maternal weight gain measured until 30 weeks of gestation with infant body fat mass measures (**Supplemental Table S2**). In **Supplemental Figure S3**, early-pregnancy weight gain was associated with higher total subcutaneous fat mass at 6 months, but not at older ages. No independent associations were observed for maternal mid- and late-pregnancy weight gain with infant fat mass measures.

Discussion

This study showed that maternal pre-pregnancy body mass index was positively associated with infant body mass index and total subcutaneous fat mass from 6 months onwards. Maternal total and period-specific gestational weight gain were not associated with infant body fat mass measures.

Methodological considerations

Strengths of this study were the prospective design with extensive maternal data collection from early pregnancy onwards and detailed infant body fat measurements available. Of the 1,033 mothers and their singleton children with pre-pregnancy body mass index available, 82% (845) had information on infant body fat measures. The non-response could lead to biased effect estimates if the associations of maternal obesity during pregnancy with infant body fat were different between participants included and excluded in the analyses. This seems unlikely since no differences were observed between participants and non-participants regarding parental pre-pregnancy body mass index and maternal gestational weight gain. A limitation of our study might be the generalizability of our findings to other ethnic groups, due to our homogenous ethnic study population. Maternal pre-pregnancy body mass index and maximum gestational weight gain were self-reported, which may have led to misclassification and underestimation of the reported associations. However, we observed similar results when we used maternal weight measured at enrolment (results not shown) and weight gain measured until 30 weeks of gestation. Skinfold thickness is a valid measure to estimate infants subcutaneous fat mass but provides no information about intra-abdominal fat mass.[8] However, infants body fat is mainly located subcutaneously in the first two years of life.[17] Also, inter- and intra-observer measurement error might be larger compared to other anthropometric measurements.[18,19]

Interpretation of main findings

A higher maternal pre-pregnancy body mass index is associated with higher body mass index from early childhood onwards.[1,2] Also, maternal pre-pregnancy obesity seems to be associated with an adverse offspring body fat pattern, characterized by higher total fat mass and abdominal fat mass levels from the age of 2 years onwards.[20-22] A study among 325 infants showed that maternal pre-pregnancy obesity was positively associated with total fat mass already throughout infancy.[23] In the current study, we observed that maternal pre-pregnancy body mass index was already associated with a higher infant body mass index and total subcutaneous fat mass from the age of 6 months onwards. No associations were

observed for central-to-total subcutaneous fat mass ratio at all ages. Thus, maternal obesity seems to influence total body fat mass development already from early infancy onwards.

By comparing the strength of associations of maternal and paternal body mass index with offspring fat mass outcomes, further insight into the underlying mechanisms can be obtained. Stronger maternal associations would suggest that intrauterine programming effects might be part of the underlying mechanisms, whereas similar or stronger paternal associations suggests that genetics and lifestyle-related characteristics might explain the observed associations. Previous studies comparing the strength of associations of parental body mass index with infant and childhood body mass index have reported inconsistent associations.[24-26] We have shown, among 4,871 parents and their 6-year-old children, that maternal pre-pregnancy body mass index was more strongly associated with childhood total fat mass and android-to-gynoid fat mass ratio, as compared to paternal body mass index.[6] In this current study, we observed more consistent and stronger associations for maternal pre-pregnancy body mass index with infant subcutaneous fat mass measures from the age of 6 months onwards, as compared to the associations of paternal body mass index with infant outcomes. At 1.5 months, we observed stronger associations for paternal body mass index with infant body mass index as compared to maternal body mass index. Thus, these results suggest that intrauterine programming effects may become more apparent at later ages. The intrauterine programming effects may involve increased placental transfer of nutrients during fetal development. This may subsequently cause permanent adaptations in appetite, energy metabolism and neuro-endocrine function in offspring, which predispose individuals to a greater risk of obesity in later life.[24] These findings could also be explained by a stronger influence of maternal lifestyle-related characteristics on child's lifestyles and subsequent body fat mass. However, previous studies have suggested that maternal and paternal diet and physical activity are both associated with child's diet and physical activity, without a stronger maternal influence.[27,28] Also, since we adjusted our analyses for multiple potential confounders the influence of lifestyle-related characteristics on our findings might be limited.

Next to maternal pre-pregnancy obesity, higher maternal total gestational weight gain is also associated with a higher childhood body mass index, total fat mass levels and waist circumference.[29,30] We did not observe consistent associations of maternal total gestational weight gain with early infant fat mass measures. It has been suggested that the associations of maternal weight gain with offspring fat mass outcomes may depend upon the timing of gestational weight gain. A prospective cohort study among 5,154 UK mothers and their children showed that maternal weight gain during early-pregnancy was positively associated

with childhood body mass index and total fat mass.[25] In line with these findings, we have previously shown that a higher maternal weight gain, especially in early-pregnancy, is associated with a higher childhood body mass index, total body fat mass and abdominal fat mass levels at the age of 6 years.[5] However, these associations were weaker as compared to the associations for maternal pre-pregnancy body mass index with these offspring fat mass measures. A study among 977 Greek mothers and their children aged 4 years showed that maternal weight gain during early-pregnancy was positively associated with childhood body mass index, waist circumference and sum of skinfold thickness.[31] In the current study, no associations were present for maternal early-, mid- and late-pregnancy weight gain during pregnancy with early infant fat mass measures. Thus, maternal weight gain during pregnancy seems not to influence fat mass development in early infancy, but the effects may become more apparent at older offspring ages.

Conclusions

A higher maternal pre-pregnancy body mass index is associated with higher infant body mass index and total subcutaneous fat mass from the age of 6 months onwards. Maternal gestational weight gain was not associated with infant body fat. Further studies are needed to obtain insight into the causality of the observed associations, and the underlying biological mechanisms.

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Table 1. Characteristics of mothers, fathers and their children[1]

Characteristics	Total group (N=845)	Maternal underweight (N=113)	Maternal normal weight (N=518)	Maternal overweight (N=151)	Maternal obesity (N=63)	P-value
Maternal characteristics						
Age, mean (SD), years	31.8 (4.1)	31.4 (4.8)	32.0 (4.0)	31.7 (3.8)	31.1 (4.2)	0.206
Education (higher education), n (%)	548 (65.2)	70 (61.9)	368 (71.5)	86 (57.3)	24 (38.1)	<0.001
Parity (nulliparous), n (%)	522 (61.8)	71 (62.8)	322 (62.6)	89 (58.9)	40 (63.5)	0.878
Body mass index, mean (SD), kg/m^2	23.5 (4.1)	19.0 (0.7)	22.2 (1.4)	26.9 (1.4)	34.1 (3.5)	<0.001
Maximum gestational weight gain, mean (SD), kg	15.5 (5.7)	14.7 (4.9)	15.8 (5.2)	16.2 (6.0)	12.7 (8.6)	0.005
Excessive gestational weight gain (IOM criteria), n (%)	242 (47.9)	13 (20.0)	132 (42.9)	76 (78.4)	23 (59.0)	<0.001
Weight in early-pregnancy, mean (SD), kg	70.8 (12.9)	57.6 (5.9)	67.1 (6.5)	80.8 (7.7)	100.0 (12.9)	<0.001
Weight in mid-pregnancy, mean (SD), kg	78.0 (13.3)	64.6 (6.1)	74.4 (7.7)	88.5 (8.5)	105.4 (12.9)	<0.001
Weight in late-pregnancy, mean (SD), kg	84.5 (13.5)	71.2 (6.8)	80.9 (8.3)	94.8 (9.2)	112.7 (13.7)	<0.001
Total energy intake, mean (SD), kcal	2119 (512)	2246 (504)	2131 (510)	2040 (481)	1969 (568)	0.069
Smoking during pregnancy (yes), n (%)	186 (23.3)	27 (26.0)	112 (23.0)	38 (25.9)	9 (15.0)	0.351
Gestational diabetes, n (%)	9 (1.1)	0 (0)	3 (0.6)	4 (2.7)	2 (3.2)	0.034
Gestational hypertensive disorders, n (%)	62 (7.6)	4 (3.5)	27 (5.4)	15 (10.1)	16 (29.6)	<0.001
Paternal characteristics						
Age, mean (SD), years	33.9 (5.1)	33.5 (5.6)	34.1 (5.0)	33.6 (4.2)	33.9 (6.3)	0.517
Education (higher education), n (%)	472 (64.2)	68 (68.7)	312 (68.7)	73 (55.7)	19 (37.3)	<0.001
Body mass index, mean (SD), kg/m^2	25.2 (3.2)	24.5 (2.9)	25.0 (2.9)	26.0 (3.6)	26.6 (4.4)	<0.001

Birth and infant characteristics						
Boys, n (%)	439 (52.0)	60 (53.1)	277 (53.5)	69 (45.7)	33 (52.4)	0.405
Gestational age at birth, median (95% range), weeks	40.3 (35.9-42.4)	40.0 (36.0-42.0)	40.4 (35.9-42.6)	40.4 (35.6-42.4)	39.9 (34.3-42.8)	0.013
Birth weight, mean (SD), g	3515 (537)	3342 (527)	3530 (526)	3582 (545)	3542 (576)	0.002
Caesarean delivery, n (%)	119 (14.9)	13 (11.9)	71 (14.8)	21 (14.4)	14 (22.2)	0.326
Breastfeeding duration, mean (SD), months	4.4 (3.9)	4.7 (4.2)	4.6 (3.8)	4.4 (3.9)	2.7 (3.6)	0.008
Introduction of solids foods (before 6 months), n (%)	600 (80.2)	71 (73.9)	371 (79.6)	116 (86.6)	42 (80.8)	0.004
Infant fat mass measures						
1.5 months						
Age, mean (SD), months	1.6 (0.5)	1.6 (0.4)	1.7 (0.5)	1.5 (0.4)	1.6 (0.5)	0.029
Body mass index, mean (SD), kg/m^2	15.2 (1.4)	14.9 (1.3)	15.2 (1.4)	15.3 (1.5)	15.1 (1.4)	0.273
Total subcutaneous fat mass, mean (SD), mm	24.4 (7.5)	23.6 (7.8)	24.7 (7.8)	23.9 (6.7)	24.4 (7.0)	0.538
Central-to-total subcutaneous fat mass ratio, mean (SD)	0.50 (0.05)	0.50 (0.05)	0.50 (0.05)	0.50 (0.04)	0.50 (0.04)	0.949
6 months						
Age, mean (SD), months	6.5 (0.7)	6.6 (0.7)	6.5 (0.7)	6.6 (0.8)	6.4 (0.6)	0.252
Body mass index, mean (SD), kg/m^2	16.8 (1.3)	16.6 (1.3)	16.7 (1.3)	16.9 (1.4)	17.1 (1.4)	0.068
Total subcutaneous fat mass, mean (SD), mm	27.1 (6.5)	26.3 (6.1)	27.0 (6.3)	27.7 (7.4)	27.5 (5.7)	0.420
Central-to-total subcutaneous fat mass ratio, mean (SD)	0.47 (0.06)	0.47 (0.06)	0.46 (0.06)	0.47 (0.05)	0.48 (0.06)	0.163
24 months						
Age, mean (SD), months	25.3 (1.2)	25.3 (1.1)	25.3 (1.2)	25.2 (1.2)	25.4 (1.0)	0.804
Body mass index, mean (SD), kg/m^2	15.9 (1.3)	15.6 (1.4)	15.8 (1.2)	16.1 (1.3)	16.6 (1.4)	<0.001

Total subcutaneous fat mass, mean (SD), mm	27.6 (7.3)	26.9 (6.2)	27.4 (7.3)	27.6 (7.3)	30.0 (9.2)	0.078
Central-to-total subcutaneous fat mass ratio, mean (SD)	0.43 (0.07)	0.44 (0.07)	0.42 (0.06)	0.43 (0.06)	0.45 (0.08)	0.013

[1]Values are observed data and represent means (SD), medians (95% range) or numbers of subjects (valid %). Total subcutaneous fat mass = biceps + triceps + suprailiacal + subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal + subscapular skinfold thicknesses)/total subcutaneous fat mass. SD, standard deviation. IOM criteria, Institute of Medicine criteria.

Table 2. Associations of parental body mass index with infant subcutaneous fat mass measures[1]

	Fat mass measures in standard deviation scores Difference (95% Confidence Interval)								
	1.5 months			6 months			24 months		
Model	Body mass index	Total subcutaneous fat mass	Central-to-total subcutaneous fat mass ratio	Body mass index	Total subcutaneous fat mass	Central-to-total subcutaneous fat mass ratio	Body mass index	Total subcutaneous fat mass	Central-to-total subcutaneous fat mass ratio
Maternal model									
n = 845	*n = 729*	*n = 694*	*n = 694*	*n = 739*	*n = 733*	*n = 733*	*n = 659*	*n = 631*	*n = 631*
Unadjusted model	0.05 (-0.03,0.12)	0 (-0.08,0.08)	0.04 (-0.04,0.11)	0.09 (0.02,0.17)*	0.04 (-0.04,0.11)	0.01 (-0.07,0.08)	0.19 (0.11,0.25)**	0.08 (0.01,0.16)*	0.08 (0.01,0.16)*
Adjusted model§	0.03 (-0.04,0.10)	-0.02 (-0.10,0.06)	0.04 (-0.04,0.12)	0.09 (0.01,0.17)*	0.02 (-0.06,0.10)	0.02 (-0.06,0.10)	0.17 (0.09,0.26)**	0.09 (0.01,0.17)*	0.08 (-0.01,0.17)
Paternal model									
n = 797	*n = 687*	*n = 656*	*n = 656*	*n = 700*	*n = 693*	*n = 693*	*n = 621*	*n = 597*	*n = 597*
Unadjusted model	0.12 (0.05,0.20)*	-0.02 (-0.10,0.06)	0.06 (-0.01,0.14)	0.05 (-0.02,0.13)	-0.01 (-0.09,0.06)	0.02 (-0.06,0.09)	0.10 (0.02,0.18)*	0.03 (-0.05,0.11)	0.08 (-0.01,0.16)
Adjusted model¶	0.10 (0.03,0.17)*	-0.02 (-0.10,0.05)	0.06 (-0.02,0.13)	0.04 (-0.04,0.12)	-0.03 (-0.11,0.05)	0.03 (-0.05,0.11)	0.08 (0.01,0.16)*	0.02 (-0.06,0.10)	0.07 (-0.01,0.15)
Combined maternal and paternal model									
n = 797	*n = 687*	*n = 656*	*n = 656*	*n = 700*	*n = 693*	*n = 693*	*n = 621*	*n = 597*	*n = 597*
Unadjusted model									
Maternal body mass index	0.02 (-0.06,0.09)	0 (-0.08,0.08)	0.03 (-0.05,0.11)	0.09 (0.01,0.16)*	0.04 (-0.04,0.11)	0.01 (-0.07,0.08)	0.18 (0.10,0.26)**	0.08 (0.01,0.16)*	0.07 (-0.01,0.15)
Paternal body mass index	0.12 (0.04,0.19)*	-0.02 (-0.10,0.06)	0.06 (-0.02,0.14)	0.03 (-0.05,0.11)	-0.02 (-0.10,0.06)	0.01 (-0.07,0.09)	0.06 (-0.02,0.13)	0.01 (-0.07,0.09)	0.06 (-0.02,0.14)
Adjusted model‡									

Maternal body mass index	0.01 (-0.06,0.08)	-0.02 (-0.11,0.06)	0.05 (-0.04,0.13)	0.09 (0.01,0.17)*	0.03 (-0.06,0.11)	0.02 (-0.06,0.11)	0.17 (0.09,0.26)**	0.09 (0.01,0.18)*	0.07 (-0.02,0.16)
Paternal body mass index	0.10 (0.03,0.16)*	-0.02 (-0.10,0.06)	0.05 (-0.03,0.13)	0.02 (-0.05,0.10)	-0.04 (-0.12,0.05)	0.02 (-0.06,0.10)	0.05 (-0.03,0.13)	0 (-0.08,0.09)	0.06 (-0.02,0.14)

[1]Values are regression coefficients (95% confidence interval) from linear regression models that reflect differences in subcutaneous fat mass measures in standard-deviation scores at 1.5, 6 and 24 months per standard-deviation scores change in maternal and paternal pre-pregnancy body mass index. Total subcutaneous fat mass = biceps + triceps + suprailiacal + subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal + subscapular skinfold thicknesses)/total subcutaneous fat mass.

§Maternal model includes maternal age and educational level, parity, maternal total energy intake, smoking habits and weight gain until 30 weeks of gestation, gestational diabetes, gestational hypertensive disorders, child's sex and gestational age-adjusted birth weight standard-deviation scores, cesarean delivery, breastfeeding duration and timing of introduction of solid foods (for 6 and 24 months outcomes).

¶Paternal model includes the same potential confounders as maternal model but paternal age and educational level instead of maternal age and educational level.

‡Combined maternal and paternal model includes all potential confounders.

*P-value<0.05; **P-value<0.01.

Table 3. Associations of maternal maximum gestational weight gain with infant subcutaneous fat mass measures[1]

	Fat mass measures in standard deviation scores Difference (95% Confidence Interval)								
	1.5 months			6 months			24 months		
	Body mass index	Total subcutaneous fat mass	Central-to-total subcutaneous fat mass ratio	Body mass index	Total subcutaneous fat mass	Central-to-total subcutaneous fat mass ratio	Body mass index	Total subcutaneous fat mass	Central-to-total subcutaneous fat mass ratio
Maximum gestational weight gain model									
n = 501	*n = 447*	*n = 430*	*n = 430*	*n = 466*	*n = 460*	*n = 460*	*n = 433*	*n = 414*	*n = 414*
Unadjusted	0.10 (0.01,0.19)*	-0.03 (-0.12,0.07)	-0.02 (-0.11,0.08)	0.11 (0.02,0.20)*	0.06 (-0.04,0.15)	0.04 (-0.05,0.14)	0 (-0.10,0.09)	-0.05 (-0.14,0.05)	-0.10 (-0.20,-0.01)*
Adjusted	0.05 (-0.04,0.14)	-0.04 (-0.14,0.06)	0.01 (-0.09,0.12)	0.09 (-0.01,0.19)	0.09 (-0.01,0.19)	0.07 (-0.03,0.17)	0.01 (-0.09,0.10)	-0.04 (-0.14,0.06)	-0.11 (-0.21,-0.01)*
Excessive gestational weight gain model¶									
n = 242	*n = 219*	*n = 212*	*n = 212*	*n = 225*	*n = 222*	*n = 222*	*n = 213*	*n = 202*	*n = 202*
Unadjusted	0.19 (0.01,0.37)*	0 (-0.19,0.19)	0.04 (-0.15,0.23)	0.32 (0.14,0.50)**	0.11 (-0.07,0.30)	0.11 (-0.08,0.29)	0.12 (-0.07,0.30)	-0.01 (-0.20,0.19)	-0.11 (-0.31,0.08)
Adjusted	0.12 (-0.06,0.29)	-0.03 (-0.23,0.18)	0.09 (-0.11,0.30)	0.30 (0.11,0.48)**	0.13 (-0.06,0.32)	0.16 (-0.03,0.35)	0.16 (-0.04,0.35)	0.03 (-0.17,0.23)	-0.14 (-0.34,0.07)

[1]Values are regression coefficients (95% confidence interval) from linear regression models that reflect differences in subcutaneous fat mass measures in standard-deviation scores at 1.5, 6 and 24 months per standard-deviation scores change in maternal maximum gestational weight gain or for excessive weight gain as compared to the reference group (insufficient and sufficient weight gain). Total subcutaneous fat mass = biceps + triceps + suprailiacal + subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal + subscapular skinfold thicknesses)/total subcutaneous fat mass.

¶Adjusted for maternal age and educational level, parity, pre-pregnancy body mass index (for models with maximum gestational weight gain as a continuous variable), maternal total energy intake, smoking habits during pregnancy, gestational diabetes, gestational hypertensive disorders,

child's sex and gestational age-adjusted birth weight standard-deviation scores, cesarean delivery, breastfeeding duration and timing of introduction of solid foods (for 6 and 24 months outcomes).
*P-value<0.05; **P-value<0.01

Supplementary materials

Figure S1. Flow chart of participants in study

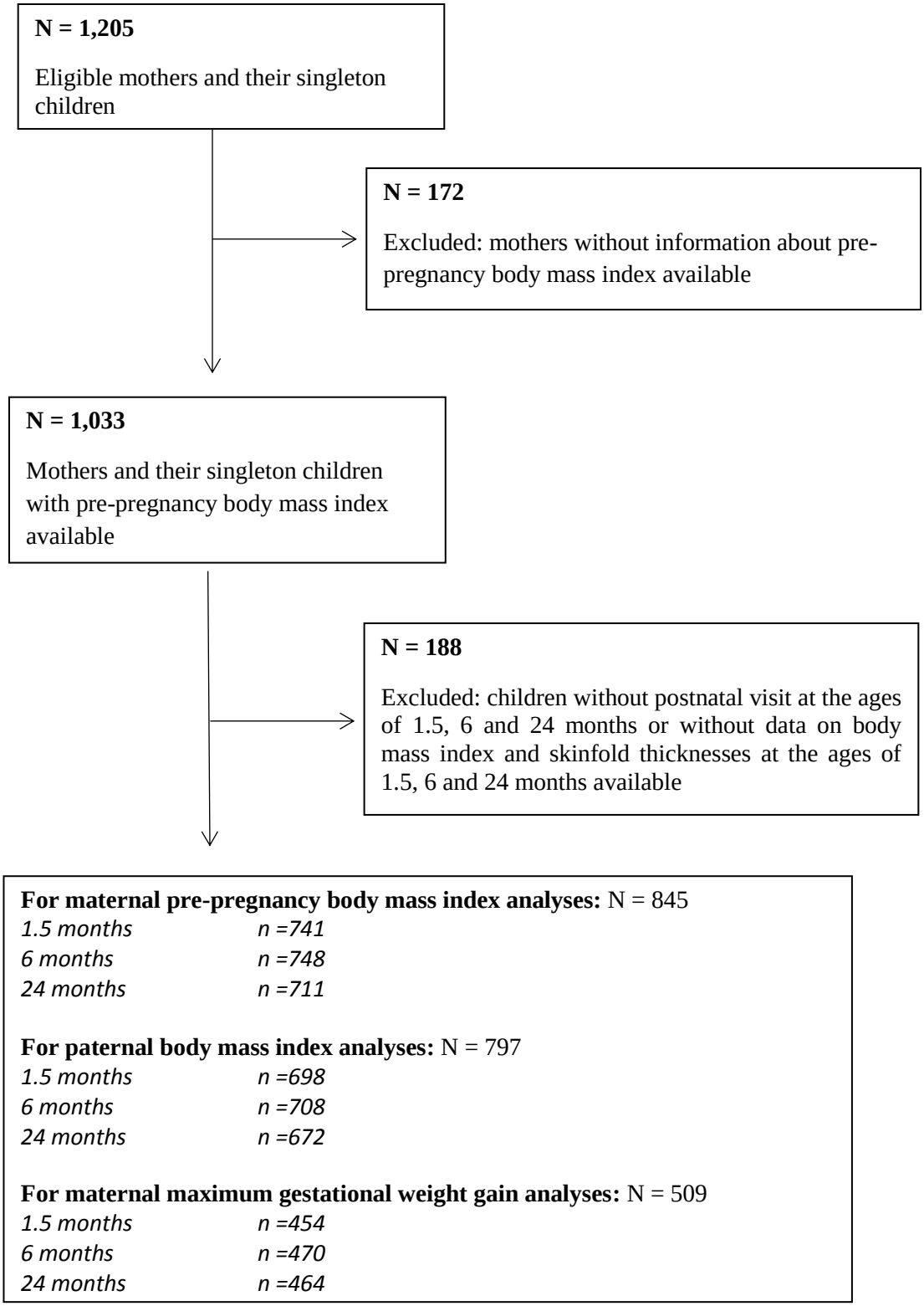


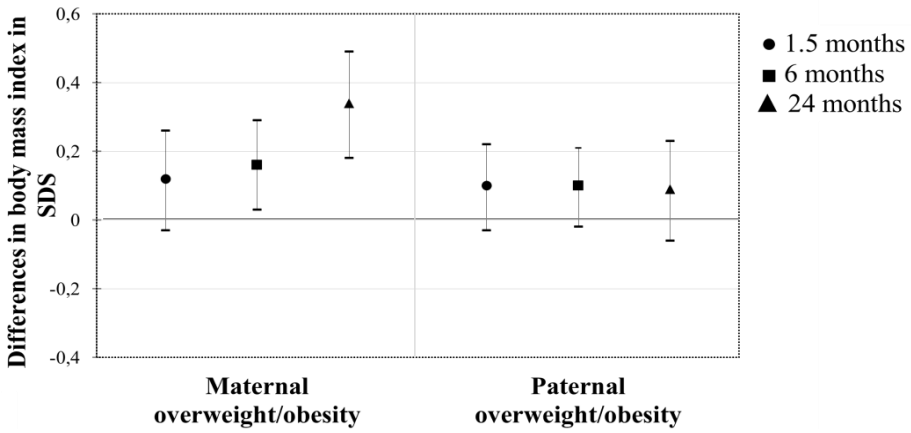
Table S1. Comparison of subject characteristics between children included and not included in the analyses[1]

Characteristics	Participants (N=845)	Non-participants (N=188)	P-value
Maternal characteristics			
Age, mean (SD), years	31.8 (4.1)	30.5 (4.5)	<0.001
Education (higher education), n (%)	548 (65.2)	103 (55.7)	0.042
Parity (nulliparous), n (%)	522 (61.8)	113 (60.1)	0.671
Body mass index, mean (SD), kg/m^2	23.5 (4.1)	23.5 (4.3)	0.855
Maximum gestational weight gain, mean (SD), kg	15.5 (5.7)	14.9 (5.6)	0.424
Excessive gestational weight gain (IOM criteria), n (%)	242 (47.9)	23 (41.1)	0.449
Weight in early-pregnancy, mean (SD), kg	70.8 (12.9)	71.2 (13.2)	0.752
Weight in mid-pregnancy, mean (SD), kg	78.0 (13.3)	78.3 (13.2)	0.832
Weight in late-pregnancy, mean (SD), kg	84.5 (13.5)	83.1 (13.4)	0.462
Total energy intake, mean (SD), kcal	2119 (512)	2142 (518)	0.722
Smoking during pregnancy (yes), n (%)	186 (23.3)	47 (26.3)	0.408
Gestational diabetes, n (%)	9 (1.1)	1 (0.6)	0.528
Gestational hypertensive disorders, n (%)	62 (7.6)	8 (4.5)	0.141
Paternal characteristics			
Age, mean (SD), years	33.9 (5.1)	32.9 (5.3)	0.014
Education (higher education), n (%)	472 (64.2)	80 (51.3)	0.010
Body mass index, mean (SD), kg/m^2	25.2 (3.2)	25.6 (3.5)	0.142
Birth and infant characteristics			
Boys, n (%)	439 (52.0)	99 (52.7)	0.861
Gestational age at birth, median (95% range), weeks	40.3 (35.9-42.4)	40.1 (33.7-42.1)	0.278
Birth weight, mean (SD), g	3515 (537)	3478 (601)	0.403
Caesarean delivery, n (%)	119 (14.9)	23 (13.8)	0.705
Breastfeeding duration, mean (SD), months	4.4 (3.9)	3.6 (3.8)	0.030
Introduction of solids foods (before 6 months), n (%)	600 (80.2)	112 (89.6)	0.041

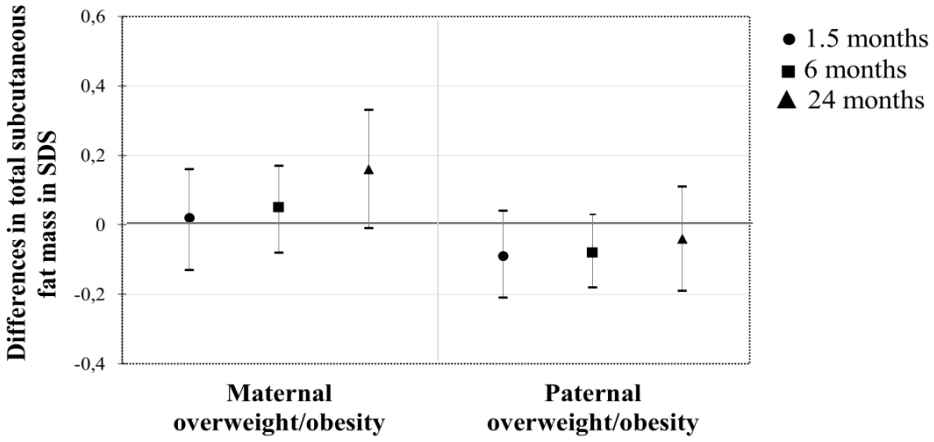
[1]Values are observed data and represent means (SD), medians (95% range) or numbers of subjects (valid %). Differences were tested using Student's t-tests and Mann-Whitney tests for normally and non-normally distributed variables, respectively and χ^2-test for dichotomous variables. SD, standard deviation. IOM criteria, Institute of Medicine criteria.

Figure S2. Associations of parental pre-pregnancy overweight with infant subcutaneous fat mass measures[1-2]

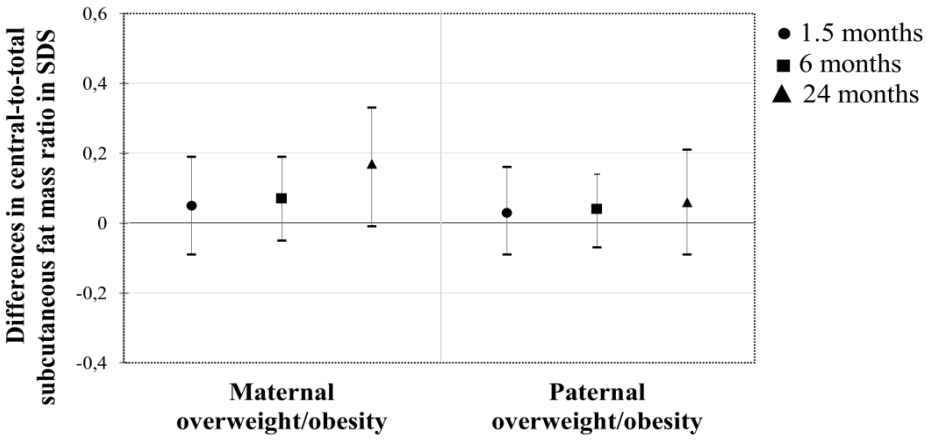
A. Body mass index



B. Total subcutaneous fat mass



C. Central-to-total subcutaneous fat mass ratio



[1]Values are regression coefficients (95% confidence interval) from repeated measurement linear regression models that reflect differences in subcutaneous fat mass measures in standard-deviation scores at 1.5, 6 and 24 months for maternal and paternal overweight/obesity as compared to the reference group (maternal and paternal underweight/normal weight). Total subcutaneous fat mass = biceps + triceps + suprailiacal + subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal + subscapular skinfold thicknesses)/total subcutaneous fat mass.

[2]Maternal model includes maternal age and educational level, parity, maternal total energy intake, smoking habits and weight gain until 30 weeks of gestation, gestational diabetes, gestational hypertensive disorders, child's sex and gestational age-adjusted birth weight standard-deviation scores, cesarean delivery, breastfeeding duration and timing of introduction of solid foods. Paternal model includes the same potential confounders as maternal model but paternal age and educational level instead of maternal age and educational level.

Table S2. Associations of maternal weight gain until 30 weeks of gestation with infant subcutaneous fat mass measures[1]

	Fat mass measures in standard deviation scores Difference (95% Confidence Interval)								
	1.5 months			6 months			24 months		
	Body mass index	Total subcutaneous fat mass	Central-to-total subcutaneous fat mass ratio	Body mass index	Total subcutaneous fat mass	Central-to-total subcutaneous fat mass ratio	Body mass index	Total subcutaneous fat mass	Central-to-total subcutaneous fat mass ratio
Weight gain until 30 weeks of gestation model									
n = 833	*n =721*	*n =687*	*n =687*	*n = 729*	*n = 723*	*n = 723*	*n =647*	*n =621*	*n =621*
Unadjusted	0.08 (0.01,0.16)*	0.01 (-0.07,0.08)	0 (-0.07,0.08)	0.08 (0.01,0.16)*	0.06 (-0.01,0.14)	0.02 (-0.06,0.09)	-0.01 (-0.09,0.06)	-0.05 (-0.13,0.02)	-0.07 (-0.14,0.01)
Adjusted	0.02 (-0.05,0.09)	-0.02 (-0.10,0.06)	0.01 (-0.07,0.09)	0.04 (-0.04,0.12)	0.07 (-0.01,0.14)	0.05 (-0.03,0.12)	-0.03 (-0.11,0.04)	-0.06 (-0.15,0.02)	-0.07 (-0.15,0.01)
Excessive gestational weight gain model¶									
n = 116	*n =104*	*n =95*	*n =95*	*n = 100*	*n = 98*	*n = 98*	*n =94*	*n =91*	*n =91*
Unadjusted	0.05 (-0.16,0.25)	-0.15 (-0.37,0.07)	0 (-0.22,0.21)	0.19 (-0.02,0.40)	0.17 (-0.04,0.38)	0.13 (-0.08,0.34)	0.06 (-0.16,0.28)	-0.02 (-0.24,0.20)	-0.17 (-0.39,0.06)
Adjusted	-0.03 (-0.22,0.16)	-0.17 (-0.39,0.05)	0.04 (-0.19,0.26)	0.10 (-0.12,0.31)	0.13 (-0.09,0.35)	0.19 (-0.03,0.40)	0.02 (-0.20,0.25)	-0.07 (-0.30,0.17)	-0.20 (-0.43,0.04)

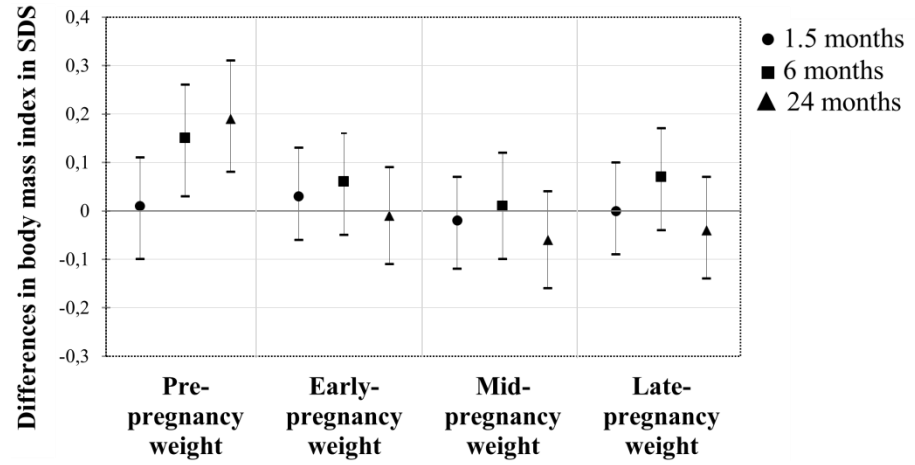
[1]Values are regression coefficients (95% confidence interval) from linear regression models that reflect differences in subcutaneous fat mass measures in standard-deviation scores at 1.5, 6 and 24 months per standard-deviation scores change in maternal weight gain until 30 weeks of gestation or for maternal excessive weight gain as compared to the reference group (insufficient and sufficient weight gain). Body mass index = weight/height^2. Total subcutaneous fat mass = biceps + triceps + suprailiacal + subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal + subscapular skinfold thicknesses)/total subcutaneous fat mass.

¶Adjusted for maternal age and educational level, parity, pre-pregnancy body mass index (for models with weight gain until 30 weeks of gestation as a continuous variable), maternal total energy intake, smoking habits during pregnancy, gestational diabetes, gestational hypertensive disorders, child's sex and gestational age-adjusted birth weight standard-deviation scores, cesarean delivery, breastfeeding duration and timing of introduction of solid foods (for 6 and 24 months outcomes).

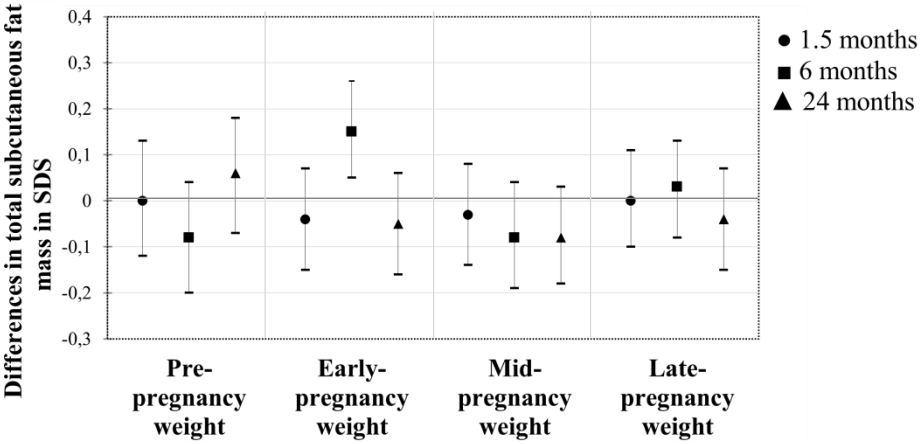
*P-value<0.05.

Figure S3. Associations of maternal pre-pregnancy weight and early-, mid- and late-pregnancy weight with infant subcutaneous fat mass measures from conditional analyses[1-2]

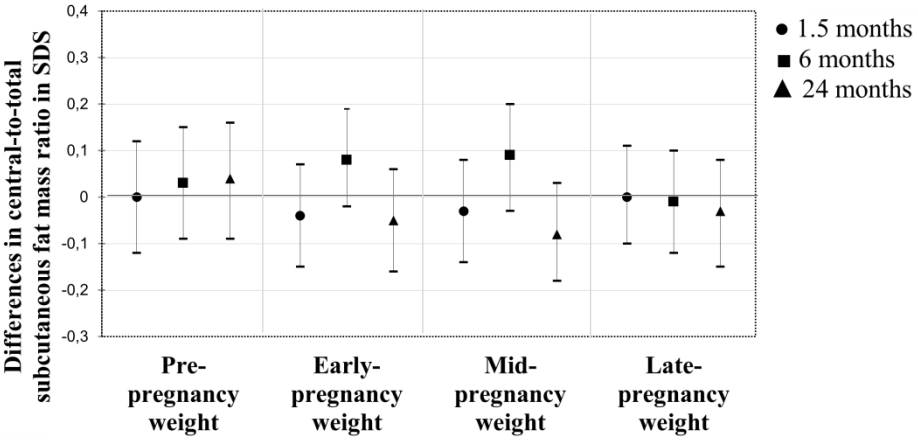
A. Body mass index



B. Total subcutaneous fat mass



C. Central-to-total subcutaneous fat mass ratio



[1]Values are regression coefficients (95% confidence interval) from linear regression models that reflect differences in subcutaneous fat mass measures in standard-deviation scores at 1.5, 6 and 24 months per standard-deviation scores change in maternal pre-pregnancy weight and per standard-deviation scores change in standardized residual change in maternal early-, mid- and late-pregnancy weight from conditional regression analyses. Total subcutaneous fat mass = biceps + triceps + suprailiacal + subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal + subscapular skinfold thicknesses)/total subcutaneous fat mass.

[2]Models were adjusted for maternal age and educational level, parity, height at intake, maternal total energy intake, smoking habits during pregnancy, gestational diabetes, gestational hypertensive disorders, child's sex and gestational age-adjusted birth weight standard-deviation scores, cesarean delivery, breastfeeding duration and timing of introduction of solid foods (for 6 and 24 months outcomes).

CHAPTER 2.3

Maternal prenatal exposures and fat mass in children: intrauterine programming or birth weight effects?

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Abstract

Background and aims: It remains unknown whether the effects of prenatal exposures on child´s adiposity reflect entirely intrauterine programming. We aimed to assess the effects of maternal gestational weight gain, diabetes and smoking on child´s body fat patterns, disentangling the direct (through intrauterine programming) and indirect (through birth weight) effects.

Methods and results: We included 4747 singleton 7-year-old children from the Generation XXI birth cohort (Porto, Portugal). At birth, maternal and newborn´s characteristics were obtained. Anthropometrics were measured at 7 years old and body fat patterns were identified by principal component analysis. Path analysis was used to quantify direct, indirect and total effects of gestational weight gain, diabetes and smoking on body fat patterns. Pattern 1 was characterized by strong factor loadings with body mass index, fat mass index and waist-to-height ratio (fat quantity) and pattern 2 with waist-to-hip ratio, waist-to-thigh ratio, and waist-to-weight ratio (fat distribution). The positive total effect of maternal gestational weight gain and diabetes on child's fat quantity was mainly through a direct pathway, responsible for 91.7% and 83.7% of total effects, respectively (β=0.022; 95% Confidence Interval (CI): 0.017, 0.027; β=0.041; 95% CI: -0.011, 0.093). No effects on fat distribution were found. Maternal prenatal smoking had a positive direct effect on patterns 1 and 2, explaining 94.9% and 76.1% of total effects, respectively.

Conclusion: The effects of maternal gestational weight gain, diabetes and smoking on child´s fat quantity seem to be mainly through intrauterine programming. Maternal smoking also showed a positive direct effect on child's fat distribution.

Introduction

Intrauterine programming has been pointed out, along with genetic predisposition, as a major cause of childhood obesity.[1] A large body of evidence has focused on adiposity programming by maternal under- or over-nutrition, weight gain, diabetes and smoking during pregnancy.[2] These exposures are likely to affect the development of adipocytes and their capacity to expand or contract, the appetite control system and the energy metabolism in later life.[3] However, it remains unknown whether the observed effects in these previous studies reflect entirely intrauterine programming. Confounding by lifestyles or genetics and the possibility of pathways mediated by other exposures might still be an issue.

Previous studies have addressed the effect of prenatal exposures on the risk of childhood obesity with and without adjustment for birth weight.[4-7] Since birth weight may be a mediator in these associations, the adjustment for birth weight might be inappropriate. First, the effect estimate obtained from a model adjusted for birth weight does no longer correspond to the total effect. Secondly, the approach of simply adjusting for mediators in the regression models is prone to bias and may produce flawed conclusions.[8] To our knowledge, no study has discussed separately the intrauterine programming effects from those mediated by birth weight. Assessing these effects separately will help to get further insight into the paths and mechanisms involved and their specific contributions to the associations between prenatal exposures and adiposity in later life.

Most studies looking at the relation between prenatal exposures and later adiposity have used proxies and have relied on single measures for defining adiposity, which might have limited their ability to detect associations.[9] The combination of anthropometric indices according to their inter-correlations into robust and uncorrelated patterns of body fat could be a more accurate and yet simple approach of evaluating childhood adiposity.

The present study aimed to assess the effects of prenatal exposures (gestational weight gain, diabetes and smoking) on body fat patterns of 7-year-old children. A path analysis helped to understand whether these effects are mainly explained by a direct (through intrauterine programming) or indirect pathway (through birth weight).

Methods

Study population

This study included participants from Generation XXI – a population-based birth cohort assembled during 2005-2006 at all public maternity units of Porto, Portugal. Of the invited

mothers, 91.4% accepted to participate, corresponding to 8647 newborns. A detailed description of the cohort methodology was previously reported.[10] At the age of 7 years (2012-2014), 6889 children were re-evaluated (79.7% of the entire cohort), of which 5849 provided data by face-to-face interviews.

For the definition of body fat patterns, of 5849 children who attended the face-to-face interviews at 7 years old, we excluded 130 children with missing information on anthropometrics and/or tetra-polar bioelectric impedance, yielding a total of 5719 children (48.5% females). Further, we excluded 212 twins and 760 children who lacked data on selected variables of interest. The final study sample included 4747 children (48.6% females). The comparison between participants (n=4747) and non-participants (excluding twins, n=3604) showed that mothers in this study were slightly older [mean (standard deviation, SD)=29.6 (5.30) vs. 28.1 (5.94) years old] and higher educated [mean (SD)=11.1 (4.27) vs. 9.5 (4.04) years] than mothers who were not included in this study. However, the magnitude of the differences was not high (Cohen's effect size values[11] of 0.27 and 0.38 for maternal age and education, respectively), suggesting that the differences were mostly due to the large sample size rather than due to large differences between participants' characteristics.

Data collection

Baseline evaluation

Data on maternal characteristics were collected in face-to-face interviews, within 72 hours after delivery, during the hospital stay. Maternal age and educational level at birth were recorded as completed years of aging and schooling. Information on prenatal smoking habits was collected and mothers were grouped into never or ever smokers during pregnancy. Pre-pregnancy weight was obtained through recall. Maternal height was measured by interviewers, and, when not possible, was abstracted from the national identity card. Pre-pregnancy body mass index (kg/m^2, BMI) was calculated. Maternal gestational weight gain was calculated as the difference between the final pre-delivery weight, reported by mothers, and the pre-pregnancy weight. Personal history of diabetes mellitus was considered present when participants reported a medical diagnosis of this condition before the current pregnancy. Gestational diabetes was considered present when recorded on obstetrical records as a diagnosis during the current pregnancy. Mothers were grouped into never or ever had a diagnosis of type 1 or type 2 diabetes mellitus or gestational diabetes. Clinical records were reviewed at birth to retrieve data on gestational age and birth weight. Gestational age was estimated by ultrasound and, when it was missing, it was estimated based on the last

menstrual period. The z-scores of birth weight for gestational age were calculated according to a sex-specific, population-based Canadian reference.[12]

Re-evaluation at 7 years old

Anthropometrics were obtained by trained personnel with children in underwear and barefoot, according to standard procedures. Body weight and height were measured using a digital scale (TANITA®) and a wall stadiometer (SECA®), respectively. Waist circumference was measured at the umbilicus level, with abdomen relaxed and hip circumference was measured at the level of the greatest posterior protuberance of the buttocks. Thigh circumference was measured at the position around the mid-thigh and perpendicular to the long axis of the thigh, with the leg slightly flexed. All anthropometrics were measured to the nearest 0.1 kg or cm. Body mass index was calculated (kg/m^2). The waist-to-height, waist-to-hip and waist-to-thigh ratios were calculated as waist circumference divided by height, hip circumference and thigh circumference, respectively. The relationship between waist circumference and weight was assessed using a log-log regression analysis. Log-waist circumference was regressed on log-weight. The gradient of the regression line was approximately 0.5, corresponding to the value by which weight should be raised in order to calculate a measure uncorrelated with it. Waist-to-weight ratio was calculated as waist circumference divided by the square root of weight.

Bioelectric impedance analysis was performed using a tetra-polar device (BIA 101 Anniversary, Akern, Florence, Italy). Four surface electrodes were placed on the right wrist, ankle, hand and foot with the child lying horizontally. Measurements were performed at least 30 minutes after the last meal. Fat free mass was determined using Schaefer et al. equation[13] and fat mass was derived accordingly. Fat mass was divided by the squared height to obtain the fat mass index,[14] in which fat mass was effectively uncorrelated with height, as confirmed by a log-log regression analysis.

Ethics

All phases of the study complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki. The study was approved by the University of Porto Medical School/ S. João Hospital Centre ethics committee and a signed informed consent according Helsinki was required for all participants.

Statistical analysis

Principal component analysis was applied to BMI, waist-to-height ratio, waist-to-hip ratio, waist-to-thigh ratio, waist-to-weight ratio, and fat mass index to identify uncorrelated patterns of body fat. The number of factors was decided using the Kaiser's criterion, by which factors with an eigenvalue ≥1.0 are retained. Varimax rotation was performed to simplify the interpretation of the factor loadings structure. The interpretation of the factors was based on those measures with factor loadings higher than 0.30, considering 0.30-0.70 as moderate and ≥0.70 as strong factor loadings. The scores that were entered in all subsequent analyses were calculated using the regression method with standardized scores. Analyses were conducted using the R® software version 3.0.1.

Path analysis determines whether data fit well within a prespecified causal model and allows the study of direct and indirect effects with multiple independent and dependent variables.[15] Path analysis was used to quantify direct, indirect (by multiplication of path regression coefficients involved) and total effects (by summing direct and indirect effects) of prenatal exposures on body fat patterns, taking into consideration the relationships depicted in the causal diagram presented as **Figure 1**. The path analysis used linear regression models for continuous outcomes and probit regression models for categorical outcomes and results were presented as regression coefficients (β) and corresponding 95% confidence intervals (95% CI). The adjustment sets for each regression model are presented in the footnotes of Figure 1. Covariates were included in the analyses when they changed the effect estimates substantially (>10%): maternal pre-pregnancy body mass index, age and educational level at birth. Other covariates such as parity, mode of delivery, child's sex, breastfeeding duration, physical exercise and fruit/vegetables intake were tested but did not fulfill the criterion for being considered confounders and therefore were not included in the regression models. Models were fitted, simultaneously for all body fat patterns identified, with Mplus software version 5.2 (Muthén & Muthén, Los Angeles, CA, USA). A Comparative Fit Index (CFI) and a Tucker-Lewis Index (TLI)≥0.90 and a Root Mean Square Error of Approximation (RMSEA) close to zero were used as criteria to support the goodness of fit.[16] No significant interactions between prenatal exposures and child's sex in the associations with body fat patterns were found.

Results

Characteristics of participants are shown in **Table 1**. Two body fat patterns, similar for both sexes, were identified: a pattern 1 characterized by strong factor loadings with BMI, fat mass index and waist-to-height ratio (fat quantity) and a pattern 2 characterized by strong factor

loadings with waist-to-hip ratio, waist-to-thigh ratio and waist-to-weight ratio (fat distribution) (**Table 2**). Both patterns explained 88.3% of the total variance (45.3% was explained by pattern 1 and 43.0% by pattern 2).

Figure 1 shows the theoretical causal diagram for the effects of prenatal exposures on body fat patterns, in which the direct effects correspond to the programming effects and the indirect effects correspond to the effects mediated by other exposures. Figure 1 also shows the effect estimates for each path. **Table 3** presents the estimates of direct, indirect and total effects of prenatal exposures on the body fat patterns. The overall fit of the model was good: CFI=0.993, TLI=0.954 and RMSEA=0.023. Maternal gestational weight gain had a positive total effect on pattern 1 (β=0.024; 95% CI: 0.019, 0.028), mainly due to a direct pathway that was responsible for 91.7% of the total effect (β=0.022; 95% CI: 0.017, 0.027). We observed a positive total effect of borderline statistical significance of maternal diabetes on pattern 1 (β=0.049; 95% CI: -0.003, 0.101), mainly due to a direct pathway that was responsible for 83.7% of the total effect (β=0.041; 95% CI: -0.011, 0.093). The indirect effects through birth weight of these prenatal exposures on both patterns were statistically significant but close to 0. After excluding the mothers with type 1 and type 2 diabetes mellitus from the analyses, similar results were observed for the associations of maternal gestational diabetes with both body fat patterns at 7 years old (results not shown). Maternal smoking during pregnancy had a positive total effect on both body fat patterns (pattern 1: β=0.178; 95% CI: 0.113, 0.243; pattern 2: β=0.109; 95% CI: 0.038, 0.180), mostly due to a direct effect that explained 94.9% and 76.1% of the total effect on patterns 1 and 2, respectively. A negative indirect effect through birth weight on pattern 1 (β=-0.024; 95% CI: -0.034, -0.014) and a positive indirect effect through birth weight on pattern 2 (β=0.032; 95% CI: 0.020, 0.043) were also found, i.e., prenatal smoking was negatively associated with birth weight (β=-0.275; 95% CI: -0.333, -0.216) which was positively related to pattern 1 (β=0.087; 95% CI: 0.055, 0.120) and negatively related to pattern 2 (β=-0.115; 95% CI: -0.150, -0.080) (Table 3 and Figure 1). We observed similar results in the principal component analysis and path analysis when we used the age- and sex-adjusted BMI standard deviation scores based on the World Health Organization Child Growth Standards (results not shown).

Discussion

The effects of maternal weight gain, diabetes and smoking during pregnancy on body fat quantity of 7-year-old children seem to be mainly through their intrauterine programing effects. These effects on child´s adiposity prevailed over the indirect effects through birth

weight. Maternal smoking during pregnancy also showed a positive direct effect on body fat distribution of children.

A previous meta-analysis showed that offspring of women with excessive gestational weight gain were at an increased risk of obesity, compared with offspring of women with adequate gestational weight gain.[6] Our study showed that this effect of maternal gestational weight gain on child's body fat quantity is mainly through intrauterine programming effects instead of indirect effects through birth weight. Higher maternal weight gain in pregnancy might program higher adiposity in the offspring through fetal over-nutrition that has been associated with a permanent increase in the capacity of adipocytes to store lipids,[17] and with excessive appetite postnatally.[18] Previous studies found that higher maternal gestational weight gain was associated with a central fat distribution in the offspring, mainly assessed by waist circumference.[19-21] As waist circumference and BMI are strongly correlated,[22] these associations could be reflecting the well-established positive effect of maternal gestational weight gain on fat quantity. In this study, no effects of maternal gestational weight gain on body fat distribution were found.

The effect of exposure to a diabetic intrauterine environment on child´s adiposity has raised some controversy. A systematic review has yielded inconclusive results, but overall the associations were not statistically significant in 8 studies from a total of 12 included studies.[5] Additionally, a meta-analysis has reported an association between maternal diabetes and increased offspring BMI that was no longer significant after adjustment for maternal pre-pregnancy BMI.[7] In the present study, maternal diabetes tended to be positively associated with child's body fat quantity after taking maternal pre-pregnancy BMI into consideration. The intrauterine programming effects prevailed over the indirect effects through birth weight. Hyperglycemia is thought to induce excessive appetite postnatally,[23] which might explain these results. Few studies have addressed the effect of maternal diabetes on child´s body fat distribution, reporting, on one hand, a positive association with trunk fat from dual energy x-ray absorptiometry (DXA)[24] but, on the other hand, no association with visceral or subcutaneous abdominal fat obtained by magnetic resonance imaging.[25] In this study, no effects of maternal diabetes on body fat distribution were found.

A previous meta-analysis has suggested that maternal prenatal smoking leads to offspring obesity.[4] Thus far, some studies have reported positive associations whereas other studies have reported no associations between maternal prenatal smoking and child's central fat.[26, 27] Our study showed that maternal smoking during pregnancy was associated with higher body fat quantity and a central fat distribution in childhood. These effects were mainly

through intrauterine programming. The mechanisms by which maternal prenatal smoking may program child´s adiposity involve an effect of cigarette smoke constituents, such as nicotine, which readily cross the placenta towards the fetus leading to permanent changes in the regulation of food intake and energy expenditure, such as increased appetite and decreased mobilization of fat from adipose tissue later in life.[28, 29] Maternal smoking during pregnancy may also mimic fetal under-nutrition by reducing blood supply to the fetus via the vasoconstrictive effects of nicotine[2] or by reducing the mother´s food intake.[30] Fetal under-nutrition has been associated with a reduced storage capacity of adipocytes, which may favor visceral fat deposition in the presence of a positive energy balance postnatally.[31] This could be one of the mechanisms involved in the higher accumulation of central fat in offspring of smoking mothers in our study. Moreover, fetal under-nutrition has been associated with reduced levels or a reduced effect of leptin during the postnatal period, thereby inducing excessive appetite.[32] A process favoring a better metabolic efficiency might also occur to enable fetal survival in a limited energy environment, which might lead to an excessive fat storage when enough food is available.[1] Even though weak, indirect effects through birth weight of maternal prenatal smoking on child´s fat quantity and distribution were also observed in our study. Maternal smoking during pregnancy seems to lead to a child with low birth weight that subsequently maintains this low weight and tends to have a central fat distribution at 7 years old. Smoking delays fetus growth due to the high levels of carboxyhemoglobin and other toxic substances in blood, the vasoconstrictive effects of nicotine and fetal under-nutrition.[2, 3] The maintenance of a low weight during childhood by low birth weight newborns could be explained, at least in part, by parental child-feeding practices, although this was not addressed in our study. However, we have previously shown in 4- and 7-year-old children of the Generation XXI birth cohort that a lower BMI leads to a greater parental use of pressure to eat that subsequently leads to a lower BMI.[33] Yet unknown mechanisms could be involved in the association of birth weight with body fat distribution.

Some limitations and strengths should be considered. Pre-pregnancy weight and final pre-delivery weight were both self-reported. Overall, weight tends to be underreported by women,[34] which might have led to an underestimation of pre-pregnancy BMI. The attendance of prenatal visits could have made pregnant women more aware of their weight status, minimizing the error of self-reported final pre-delivery weight. Similarly, data on diabetes mellitus relied on self-reporting. If misclassification of women occurred, the association between maternal diabetes and offspring adiposity could be attenuated. Moreover, treatment of diabetes (based on diet or insulin), by contributing to a better glycemic control, may

reduce the risk of long-term adverse outcomes for the offspring, thus complicating the detection of associations in observational studies like ours. Information on maternal smoking habits during pregnancy also relied on self-reporting, without biochemical validation, which may result in misclassification due to a reluctance to disclose a known adverse pregnancy behavior. However, any under-reporting would likely bias results towards the null, underestimating the associations. We measured fat mass at 7 years old using BIA which is a valid method to assess whole body composition.[35] We relied on anthropometrics to assess body fat distribution which might have greater measurement error, and be less accurate but on the other hand be easier and cheaper to obtain in large epidemiological studies as compared to imaging techniques of body composition.[36] In this study, we considered that the direct effects reflected the intrauterine programming effects. We tried to perform a comprehensive analysis, by including the most important paths described to date, and by testing several covariates as confounders. However, we cannot exclude the possibility of existing other paths not considered in this study and also residual confounding by lifestyles or genetics that might have biased our estimates for the intrauterine programming effects. This seems unlikely and the bias might be only limited. The major strength of this study is the comprehensive assessment of the effects of prenatal exposures, by quantifying their direct and indirect effects, on uncorrelated and more robust measures of fat quantity and distribution in a large population-based sample of 7-year-old children.

Conclusion

This study showed that the effects of maternal weight gain, diabetes and smoking during pregnancy on body fat quantity of 7-year-old children seem to be mainly through intrauterine programming. Maternal smoking during pregnancy also showed a positive direct effect on body fat distribution of children. Considering the lasting and lifelong effects of intrauterine programming, primary prevention strategies for obesity, as early as during prenatal care, are of utmost importance. Although the indirect effects through birth weight seem to be weak, this study reinforces the need for careful interpretation of findings adjusted and not adjusted for birth weight in studies addressing the associations between prenatal exposures and adiposity in later life.

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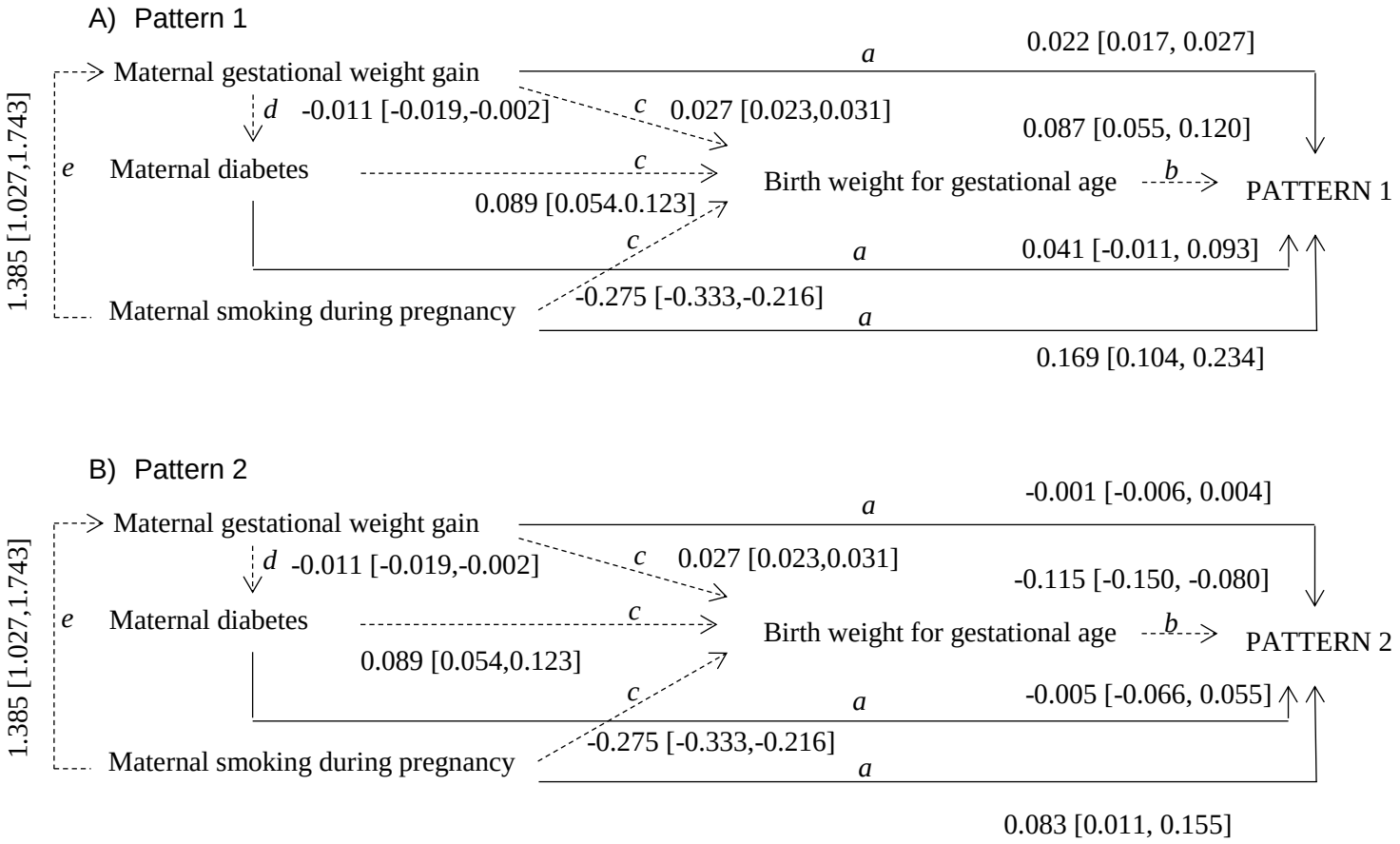
Figure 1. Causal diagram for the effects of prenatal exposures on body fat patterns identified by principal component analysis at 7-year-old children[1-2]



[1]Direct effects correspond to the intrauterine programming effects (solid arrows) and the indirect effects correspond to the effects mediated by other exposures (dashed arrows).
[2]Adjustment sets for each regression model:
[a]adjusted for the other two prenatal exposures, birth weight for gestational age, maternal pre-pregnancy body mass index, age and educational level at birth.
[b]adjusted for the prenatal exposures, maternal pre-pregnancy body mass index, age and educational level at birth.
[c]adjusted for the other two prenatal exposures, and maternal pre-pregnancy body mass index.
[d]adjusted for maternal pre-pregnancy body mass index and age at birth.
[e]adjusted for maternal pre-pregnancy body mass index, age and educational level at birth.

Table 1. Characteristics of study participants (n=4747)[1]

Child´s characteristics	**Mean (SD)**
Birth weight for gestational age, z-scores	-0.3 (0.85)
Body mass index at 7 y, kg/m^2	17.1 (2.51)
Fat mass index at 7 y, kg/m^2	3.2 (2.33)
Waist-to-height ratio at 7 y	0.5 (0.05)
Waist-to-hip ratio at 7 y	0.9 (0.05)
Waist-to-thigh ratio at 7 y	1.6 (0.10)
Waist-to-weight ratio at 7 y, cm/√kg	10.2 (0.48)
Maternal characteristics	
Age at birth, years	29.6 (5.30)
Educational level at birth, years	11.1 (4.27)
Pre-pregnancy body mass index, kg/m^2	23.8 (4.16)
Gestational weight gain, kg	13.7 (5.77)
	n (%)
Diabetes (type 1 or type 2 diabetes mellitus or gestational diabetes)	327 (6.9)
Smokers during pregnancy	992 (20.9)

[1]SD, standard deviation

Table 2. Factor loadings, obtained from principal component analysis, for anthropometric and bioelectric impedance measures (n=5719)

	Factor loadings	
	Pattern 1[a]	Pattern 2[b]
Body mass index, kg/m^2	0.980	0.051
Fat mass index, kg/m^2	0.937	0.059
Waist-to-height ratio	0.873	0.454
Waist-to-hip ratio	0.277	0.877
Waist-to-thigh ratio	-0.044	0.893
Waist-to-weight ratio, cm/√kg	0.202	0.895
Variance explained	45.3%	43.0%
Cumulative variance explained	45.3%	88.3%

[a]Representing fat quantity; [b]Representing fat distribution.

Table 3. Estimates of direct, indirect and total effects of prenatal exposures on body fat patterns of 7-year-old children, calculated by path analysis (n=4747)[1]

	Pattern 1		Pattern 2	
	β [95% CI]	% of total effect	β [95% CI]	% of total effect
Maternal gestational weight gain				
Direct effect	**0.022 [0.017,0.027]**	91.7%	-0.001 [-0.006,0.004]	25%
Indirect effects	**0.002 [0.001,0.003]**	8.3%	**-0.003 [-0.004,-0.002]**	75%
Indirect effect through birth weight	**0.002 [0.001,0.003]**	100%[a]	**-0.003 [-0.004,-0.002]**	100%[a]
Total effect	**0.024 [0.019,0.028]**		-0.004 [-0.009,0.001]	
Maternal diabetes				
Direct effect	0.041 [-0.011,0.093]	83.7%	-0.005 [-0.066,0.055]	33.3%
Indirect effects	**0.008 [0.004,0.012]**	16.3%	**-0.010 [-0.015,-0.005]**	66.7%
Indirect effect through birth weight	**0.008 [0.004,0.012]**	100%[a]	**-0.010 [-0.015,-0.005]**	100%[a]
Total effect	0.049 [-0.003,0.101]		-0.015 [-0.076,0.045]	
Maternal smoking during pregnancy				
Direct effect	**0.169 [0.104,0.234]**	94.9%	**0.083 [0.011,0.155]**	76.1%
Indirect effects	0.009 [-0.006,0.024]	5.1%	**0.026 [0.012,0.040]**	23.9%
Indirect effect through birth weight	**-0.024 [-0.034,-0.014]**	—[b]	**0.032 [0.020,0.043]**	—[b]
Total effect	**0.178 [0.113,0.243]**		**0.109 [0.038,0.180]**	

[1]β, regression coefficient; CI, confidence interval. Statistically significant results are shown in bold.
[a]Results expressed in % of indirect effects;
[b]Results not expressed in % of indirect effects since effects of opposite direction contribute to the total of indirect effects.

CHAPTER 2.4

Maternal obesity, excessive gestational weight gain and abdominal and organ fat in children

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Submitted.

Abstract

Background: Maternal obesity and excessive gestational weight gain are associated with an increased risk of obesity in the offspring.

Objective: To examine the associations of maternal pre-pregnancy body mass index (BMI) and gestational weight gain with general, abdominal, pericardial and liver fat measures in children aged 10 years.

Design: In a population-based prospective cohort study from early pregnancy onwards among 2,354 mothers and their children, we obtained information about maternal pre-pregnancy BMI and gestational weight gain. We measured offspring BMI, fat mass index (total fat mass/height^4) by dual-energy X-ray absorptiometry and subcutaneous fat index (subcutaneous fat mass/height^4), visceral fat index (visceral fat mass/height^3), pericardial fat index (pericardial fat mass/height^3) and liver fat fraction by Magnetic Resonance Imaging (MRI) at 10 years.

Results: A 1-standard deviation score (SDS) higher maternal BMI was associated with higher childhood BMI (difference 0.32 (95% Confidence Interval (CI) 0.28, 0.36) SDS), fat mass index (difference 0.28 (95% CI 0.24, 0.31) SDS), subcutaneous fat index (difference 0.26 (95% CI 0.22, 0.30) SDS), visceral fat index (difference 0.24 (95% CI 0.20, 0.28) SDS), pericardial fat index (difference 0.12 (95% CI 0.08, 0.16) SDS) and liver fat fraction (difference 0.15 (95% CI 0.11, 0.19) SDS). After conditioning each MRI measure of adiposity on BMI at 10 years, higher maternal BMI remained associated with higher childhood subcutaneous and visceral fat indices. Maximum gestational weight gain was not consistently associated with organ specific fat measures.

Conclusions: Higher maternal BMI, but not gestational weight gain, was associated with higher abdominal, pericardial and liver fat measures. The associations with abdominal subcutaneous and visceral fat were independent of BMI. Our results suggest that promoting a healthy BMI in women of reproductive age may be of greater importance for childhood organ fat than influencing gestational weight gain.

Introduction

Maternal obesity is associated with several short- and long-term adverse health effects, including an increased risk of obesity in the offspring.[1-5] It has been hypothesized that maternal obesity is related to an increased placental transfer of nutrients to the fetus, which might affect the development of adipocytes, the appetite control system, and the energy metabolism.[6] However, these associations might also be explained by shared family-based lifestyle or genetic factors. Although many studies reported the associations between maternal and offspring obesity, it remains unclear whether maternal obesity also affects body fat distribution in the offspring. Information about body fat distribution is important since, as compared to body mass index (BMI), body fat distribution, and more specifically excess visceral, heart and liver fat, may be better indicators of cardiometabolic health.[7-9] Previous studies have reported that higher maternal BMI is associated with higher abdominal and liver fat in newborns.[10, 11] Maternal pre-pregnancy obesity was also associated with higher visceral fat mass in Greek schoolchildren.[12] Next to maternal obesity, paternal obesity and excessive gestational weight gain may also affect childhood adiposity.[13-18] To date, results from studies focused on the associations of paternal BMI and gestational weight gain with body fat distribution remain scarce and not consistent.[16-18]

We examined, in a population-based prospective cohort study among 2,354 mothers, fathers and their children, the associations of parental pre-pregnancy BMI and gestational weight gain with offspring BMI, fat mass index measured by dual-energy X-ray absorptiometry (DXA) and subcutaneous fat index, visceral fat index, pericardial fat index and liver fat fraction measured by Magnetic Resonance Imaging (MRI) at 10 years. We explored whether any association with organ specific fat measures reflects specific accumulation, or just reflects general adiposity.

Subjects and methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands.[19] The study was approved by the Medical Ethical Committee of the Erasmus MC, Rotterdam (MEC 198.782/2001/31). Written informed consent was obtained from parents.[19] Pregnant women were enrolled between 2001 and 2005. Of all the eligible children in the study area, 61% participated at birth in the study. In total, 5,706 mothers and their singleton children attended

the study visit at 10 years, of whom information about pre-pregnancy BMI was available in 4,298 subjects. Further, we excluded children without any organ specific fat measures assessed by MRI (n=1,944). Thus, the population for analysis was 2,354 mothers and their children (**Supplemental Figure 1**).

Parental anthropometrics

Maternal and paternal height and weight were measured at enrolment. Information about maternal weight just before pregnancy was obtained by questionnaire. We calculated maternal and paternal BMI (kg/m^2). In our population for analysis, 56.4% of all women were enrolled before a gestational age of 14 weeks. Correlation of pre-pregnancy weight, obtained by questionnaire, and weight measured at enrolment was 0.96 (p-value<0.01). In this study, maternal pre-pregnancy BMI was used and categorized into underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), and obesity (≥30.0 kg/m^2). For the parental BMI comparison analyses, pre-pregnancy maternal and paternal BMI were categorized into normal weight (18.5-24.9 kg/m^2) and overweight/obesity (≥ 25.0 kg/m^2). As previously described, we measured maternal weight at early, mid and late pregnancy (median 13.2 weeks of gestation (95% range 9.8, 18.9), median 30.1 weeks of gestation (95% range 20.5, 31.4) and median 39.0 weeks of gestation (95% range 32.8, 42.0), respectively).[17] Information about maximum weight during pregnancy was assessed by questionnaire 2 months after delivery. Maximum weight from questionnaire and weight measured at late pregnancy were strongly correlated (r=0.99, p-value<0.01). We calculated maximum weight gain during pregnancy as the difference between maximum weight and pre-pregnancy weight. Further, we divided maximum weight gain by gestational age at birth to obtain the maximum weight gain per week. Maximum gestational weight gain was also classified as insufficient, sufficient and excessive weight gain in relation to maternal pre-pregnancy BMI according to the Institute of Medicine guidelines.[20]

Measures of adiposity at 10 years

We measured child's height and weight without shoes and heavy clothing and calculated BMI (kg/m^2). We calculated sex- and age- adjusted standard deviation scores (SDS) of childhood BMI based on Dutch reference growth charts (Growth Analyzer 4.0, Dutch Growth Research Foundation).[21] We measured total body fat mass using a DXA scanner (iDXA, GE-Lunar, 2008, Madison, WI, USA, enCORE software v.12.6), according to standard

procedures.[22] Previous studies have validated DXA against computed tomography for body fat assessment.[23-25]

Measures of organ fat at 10 years were obtained from MRI scans.[19] MRI has been described as an accurate and reproducible technique and considered the gold standard for the measurement of intra-abdominal and organ fat deposition.[24, 26-28] All children were scanned using a 3.0 Tesla MRI (MR 750w, GE Healthcare, Milwaukee, WI, USA) for body fat imaging using standard imaging and positioning protocols. They wore light clothing without metal objects while undergoing the body scan.[29] Abdominal fat scans were acquired in three separate acquisitions using a 3-point Dixon technique for fat and water separation (IDEAL sequence). The three acquisitions were centralized on the liver, on the umbilicus (abdominal fat scan), and on the top of the femur bone (hip scan), respectively. Obtained fat scans were subsequently analyzed by the Precision Image Analysis company (PIA, Kirkland, Washington, United States). Subcutaneous, visceral, pericardial and liver fat were quantified using the sliceOmatic (TomoVision, Magog, Canada) software package. All extraneous structures and any image artifacts were removed manually.[26] Total subcutaneous and visceral volumes were generated by summing the volumes of three scans. Subcutaneous and visceral fat masses were obtained by multiplying the total volumes by the specific gravity of adipose tissue, 0.9 g/ml. Pericardial fat included both epicardial- and paracardial fat directly attached to the pericardium. Pericardial fat volume was quantified using the summation of discs method and was subsequently multiplied by the specific gravity of adipose tissue, 0.9 g/ml. Liver fat fraction was determined using the IDEAL IQ sequence.[30] Four samples of at least 4 cm^2 were taken from the central portion of the hepatic volume. Subsequently, the mean signal intensities were averaged to generate an overall mean liver fat fraction estimation.

To create measures of general and organ fat independent of height at 10 years, we estimated the optimal adjustment by log-log regression analyses and subsequently we divided total and subcutaneous fat mass by $height^4$ (fat mass index and subcutaneous fat index) and visceral and pericardial fat mass by $height^3$ (visceral and pericardial fat indices) (More details given in **Supplemental Methods**).[31, 32]

Covariates

Information on maternal and paternal age, educational level, and ethnicity, and maternal parity and smoking habits was obtained by questionnaires during pregnancy. Information on child's sex was obtained from medical records. Information on breastfeeding duration and timing of introduction of solid foods was obtained by questionnaires in infancy, and

information on the average television watching time was obtained by questionnaires at the age of 10 years.

Statistical analysis

First, we used linear regression models to examine the associations of maternal and paternal pre-pregnancy BMI and maximum gestational weight gain, continuously and using clinical categories, with measures of adiposity (BMI, fat mass index, subcutaneous, visceral and pericardial fat indices and liver fat fraction) at 10 years. Second, we examined the independent associations of maternal pre-, early, mid, and late pregnancy weight with the childhood outcomes using conditional linear regression analyses to account for the correlations between the weight measurements.[33] For these models, we obtained standardized residuals for each weight from the regression of a maternal weight at a specific time point on prior maternal weights. These variables correspond to the difference between the actual weight and the expected weight based on prior weights and thus are statistically independent from each other and can be included simultaneously in the regression models.[33] Third, we used conditional regression analyses to assess whether the associations of maternal and paternal pre-pregnancy BMI and gestational weight gain with measures of organ fat at 10 years were independent of BMI at 10 years. We used as outcomes the standardized residuals for each measure of organ fat at 10 years obtained from the regression of those outcomes on BMI.[33] For all analyses, we used a basic model including child´s sex and age at outcome measurements, and a confounder model, which additionally included covariates. We included covariates in the models if they were strongly associated with parental anthropometrics and childhood adiposity in our study, or if they changed the effect estimates substantially (>10%). We log-transformed the non-normally distributed childhood DXA and MRI adiposity measures. We constructed SDS [(observed value - mean)/SD] of the sample distribution for all continuous exposures and DXA and MRI outcomes to enable comparisons of effect sizes. We tested for statistical interactions between maternal BMI and gestational weight gain, maternal BMI and paternal BMI and between both maternal BMI and gestational weight gain with child´s sex, but none of these were consistently significant. Since the maximum gestational weight gain was self-reported, sensitivity analyses using weight gain measured until late pregnancy were performed. Missing values in covariates (ranging from 0 to 28%) were multiple-imputed by using Markov chain Monte Carlo approach. Five imputed datasets were created and analyzed together. All statistical analyses were performed using the

Statistical Package of Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Subject characteristics

Table 1 shows the subject characteristics. Non-response analyses showed that parents of children with MRI follow-up data available were slightly older and had a higher educational level, and mothers were more likely to be non-smokers (p-values<0.05). No differences were observed for maternal BMI and gestational weight gain and paternal BMI (**Supplemental Table 1**). **Supplemental Table 2** shows that the correlation coefficients of BMI and fat mass index with subcutaneous and visceral fat indices are moderate to strong and higher than the correlation coefficients with pericardial fat index and liver fat fraction.

Maternal and paternal BMI and childhood organ fat measures

Table 2 shows that a 1-SDS higher maternal BMI was associated with higher childhood BMI (difference 0.32 (95% Confidence Interval (CI) 0.28, 0.36) SDS), fat mass index (difference 0.28 (95% CI 0.24, 0.31) SDS), subcutaneous fat index (difference 0.26 (95% CI 0.22, 0.30) SDS), visceral fat index (difference 0.24 (95% CI 0.20, 0.28) SDS), pericardial fat index (difference 0.12 (95% CI 0.08, 0.16) SDS) and liver fat fraction (difference 0.15 (95% CI 0.11, 0.19) SDS). As compared to maternal normal weight, maternal underweight was associated with lower fat measures whereas maternal overweight and obesity were associated with higher fat measures in childhood (p-values<0.05). After conditioning each MRI measure of adiposity on BMI at 10 years, higher maternal BMI remained associated with higher childhood subcutaneous and visceral fat indices (p-values<0.05).

Figure 1A shows that, as compared to normal weight parents, those in which only mothers or only fathers were overweight/obese had children with higher levels of all adiposity measures at the age of 10 years (p-values<0.05). The associations tended to be stronger when only mothers rather than only fathers were overweight/obese. The strongest associations were observed for children in which both parents were overweight/obese. After conditioning each MRI measure of child's adiposity on BMI at 10 years (**Figure 1B**), no significant associations were observed for couples in which only mothers or only fathers were overweight/obese. Those couples in which both parents were overweight/obese had children with higher subcutaneous, visceral and pericardial fat indices (p-values<0.05).

Maternal gestational weight gain and childhood organ fat measures

Table 3 shows that a 1-SDS higher maximum weight gain per week was only associated with higher childhood BMI (difference 0.08 (95% CI 0.03, 0.13) SDS). Excessive weight gain, as compared to sufficient weight gain, was associated with higher childhood BMI, fat mass index and subcutaneous and visceral fat indices (p-values<0.05). After conditioning each MRI measure of adiposity on BMI at 10 years, no consistent associations were observed. Similar results were observed when using maternal weight gain measured until late pregnancy (**Supplemental Table 5**). **Figure 2A** shows that independent from weights in other periods, higher pre-pregnancy weight was associated with higher levels of all adiposity measures (p-values<0.05). Higher early pregnancy weight was associated with higher BMI and fat mass index, but not with organ fat measures at 10 years. No associations were observed for mid and late pregnancy weight. After conditioning each MRI measure of adiposity on BMI at 10 years (**Figure 2B**), higher pre-pregnancy weight remained associated with higher subcutaneous and visceral fat indices. No associations were observed for early, mid and late pregnancy weight.

Discussion

We observed, in this population-based prospective cohort study, that higher maternal pre-pregnancy BMI was associated with higher BMI, fat mass index, subcutaneous, visceral and pericardial fat indices and liver fat fraction at 10 years. The associations of maternal BMI with offspring subcutaneous and visceral fat indices seemed to be independent of offspring BMI. Total and period-specific gestational weight gain were not consistently associated with organ fat measures.

Interpretation of main findings

Maternal obesity is a major public health concern.[34] A meta-analysis of published studies showed an increased risk of overweight in offspring of mothers with overweight and obesity, as compared to offspring of mothers with normal weight.[5] In the same cohort as the current study, we have previously reported that maternal overweight and obesity were strongly associated with increased risks of overweight and obesity in the offspring aged 4 and 6 years.[18, 35] In the present study, maternal overweight and obesity were associated with higher BMI and fat mass index at 10 years.

Large cohort studies such as the Framingham Heart Study and the Jackson Heart Study have reported that excess visceral and ectopic fat deposition is related to various cardiometabolic abnormalities in adults.[36-42] In 105 healthy mother-newborn pairs, higher maternal BMI was associated with higher infant abdominal fat, independently of weight, and higher intrahepatocellular lipid content.[10] In another study among 25 newborns, infants born to obese mothers with gestational diabetes had higher intrahepatocellular fat compared with infants born to normal weight mothers.[11] Maternal pre-pregnancy obesity was also associated with higher visceral fat mass levels in 1,228 Greek children aged 9-13 years.[12] In the present study, higher maternal BMI was associated with higher subcutaneous, visceral and pericardial fat indices and liver fat fraction at 10 years. The associations of maternal BMI with offspring subcutaneous and visceral fat indices seemed to be independent of offspring BMI. This means that higher maternal BMI is associated with a specific accumulation of fat in abdominal depots that is not a result of general adiposity. We did not observe differences in the results when we conditioned on fat mass index instead of BMI. These results are not in line with those of our previous study in 6-year-old children suggesting that higher maternal pre-pregnancy BMI was not associated with subcutaneous and preperitoneal abdominal fat measured by ultrasound, independently of child´s BMI.[18] The differences in results may be due to different ages or different imaging methods.

Previously, we reported that higher paternal BMI was associated with higher BMI but was not associated with subcutaneous and preperitoneal abdominal fat at the age of 6 years, independently of child's BMI.[18] In the present study, paternal overweight was associated with higher BMI, fat mass index and organ fat measures in children aged 10 years. The associations observed with MRI adiposity measures were not independent of BMI at 10 years. Our results suggest that both maternal and paternal BMI before pregnancy may be risk factors for offspring cardiometabolic health by influencing general and organ fat accumulation in later life. Previous studies comparing the associations of maternal and paternal BMI with childhood BMI and total fat mass have shown conflicting results.[18, 43-46] A recent study using genetic variants in a Mendelian randomization approach found little evidence to support strong causal intrauterine effects of maternal BMI on offspring adiposity.[47] Although we observed a tendency for stronger associations of maternal BMI, as compared to paternal BMI, with general and abdominal fat measures, the differences between the maternal and paternal effect estimates were not statistically significant. These findings may suggest that the associations of maternal BMI with offspring adiposity might be

explained by shared family-based lifestyle and genetic characteristics rather than by intrauterine programming.[48]

Next to maternal obesity, excessive gestational weight gain also seems to be associated with an increased risk of childhood overweight.[13-15] In our study, excessive weight gain was associated with higher BMI and fat mass index. A previous study among 313 mother-child pairs reported that higher maternal BMI was associated with higher childhood subcutaneous and visceral fat, particularly among mothers with excessive gestational weight gain.[16] However, in the same cohort as the current study, maternal weight gain in early, mid and late pregnancy was not associated with childhood subcutaneous and preperitoneal abdominal fat mass levels at 6 years, independently of BMI.[17] In our study, total and period-specific weight gain was not consistently associated with any MRI adiposity measures. Thus, gestational weight gain, contrary to BMI before pregnancy, seems to have a limited influence on offspring organ fat in later life.

The mechanisms by which maternal adiposity during pregnancy affects offspring organ fat accumulation are not fully known yet. Maternal over-nutrition may affect the development of adipocytes and their capacity to expand or contract, the appetite control system and the energy metabolism in later life,[6] which might lead to increased body fat in the offspring. Maternal over-nutrition might also lead to accumulation of fat in the liver and other developing organs of the fetus, especially during early and mid pregnancy due to the absence of adipose tissue.[49] The postnatal persistence of increased fat in these depots might be related to reduced fatty acid oxidation, changes in lipogenesis and lipoprotein export.[49]

Our study shows that higher maternal pre-pregnancy BMI, as opposed to gestational weight gain, is related to higher organ fat measures, which have important adverse cardiometabolic health consequences. Future preventive strategies focused on promoting a healthy weight in women of reproductive age before pregnancy are needed to improve cardiometabolic health of the offspring.

Methodological considerations

Strengths of this study were the large sample size, prospective design and data available on multiple maternal weight measurements throughout pregnancy and detailed childhood adiposity measures. Of the 4,298 mothers and their singleton children with information on pre-pregnancy BMI available, 2,354 had information on MRI adiposity measures at 10 years. The non-response could lead to biased effect estimates if the associations of maternal BMI and gestational weigh gain with childhood adiposity measures differ between mothers and

children included and not included in the present analyses. However, this seems unlikely since participants and non-participants did not differ regarding maternal BMI and weight gain during pregnancy. We relied on self-reported pre-pregnancy weight and maximum weight during pregnancy. Women tend to underestimate their weight on self-report,[50] which might have led to an underestimation of observed effects for maternal BMI. Since pre-pregnancy weight and maximum gestational weight are both self-reported and probably underestimated, the influence on maximum weight gain is likely to be minimal, which is confirmed by the fact that similar results were observed for weight gain measured until late pregnancy. Finally, although we adjusted for a large number of potential confounders, residual confounding due to lifestyle-related characteristics such as parental and child nutritional intake and physical activity might still be present in the observed associations.

Conclusions

Our study suggests that higher maternal BMI, but not gestational weight gain, is associated with organ fat accumulation, especially abdominal fat, in the offspring. Our findings emphasize the importance of promoting a healthy BMI in women who are planning to become pregnant rather that influencing weigh gain during pregnancy.

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Table 1. Characteristics of mothers, fathers and their children (n=2,354)[1]

Characteristics	Value
Maternal characteristics	
Age, mean (SD), years	31.0 (4.8)
Education, n (%)	
Low	160 (6.9)
Medium	935 (40.5)
High	1,211 (52.5)
Ethnicity, n (%)	
European	1,532 (65.3)
Non-European	815 (34.7)
Parity, n (%)	
Nulliparous	1,419 (60.3)
Multiparous	934 (39.7)
Pre-pregnancy body mass index, median (95% range), kg/m^2	22.5 (18.0, 34.9)
Pre-pregnancy body mass index clinical categories, n (%)	
Underweight	97 (4.1)
Normal weight	1,639 (69.6)
Overweight	451 (19.2)
Obesity	167 (7.1)
Maximum gestational weight gain, mean (SD), kg	14.8 (5.8)
Gestational weight gain clinical categories (IOM criteria), n (%)	
Insufficient gestational weight gain	299 (20.5)
Sufficient gestational weight gain	505 (34.5)
Excessive gestational weight gain	658 (45.0)
Weight in early pregnancy, mean (SD), kg	69.0 (12.9)

Weight in mid pregnancy, mean (SD), kg	76.0 (12.6)
Weight in late pregnancy, mean (SD), kg	81.6 (12.5)
Smoking during pregnancy, n (%)	
Yes	504 (22.3)
No	1,757 (77.7)
Paternal characteristics	
Age, mean (SD), years	33.6 (5.3)
Education, n (%)	
Low	83 (4.9)
Medium	652 (38.6)
High	953 (56.5)
Ethnicity, n (%)	
European	1,383 (74.4)
Non-European	477 (25.6)
Body mass index, mean (SD), kg/m^2	25.3 (3.3)
Body mass index clinical categories, n (%)	
Underweight	9 (0.5)
Normal weight	945 (50.3)
Overweight	771 (41.1)
Obesity	152 (8.1)
Birth and infant characteristics	
Child's sex, n (%)	
Boys	1,151 (48.9)
Girls	1,203 (51.1)
Breastfeeding duration, median (95% range), months	3.5 (0.0, 12.0)
Introduction of solid foods, n (%)	
< 3 months	123 (7.0)

3-6 months	1,435 (81.7)
> 6 months	199 (11.3)
Childhood characteristics	
Age, mean (SD), years	9.8 (0.3)
Television watching time, n (%)	
< 2 hours/day	1,342 (70.0)
≥ 2 hours/day	575 (30.0)
Body mass index, mean (SD), kg/m^2	17.5 (2.6)
Total fat mass, median (95% range), g	8,451 (4,549, 21,235)
Subcutaneous fat mass, median (95% range), g	1,297 (603, 5,226)
Visceral fat mass, median (95% range), g	365 (163, 1,004)
Pericardial fat mass, median (95% range), g	10.6 (4.6, 22.6)
Liver fat fraction, median (95% range), %	2.0 (1.2, 5.2)

[1]Values are observed data and represent means (SD), medians (95% range) or numbers of subjects (valid %). IOM, Institute of Medicine; SD, standard deviation.

Table 2. Maternal body mass index and childhood general and organ fat measures[1]

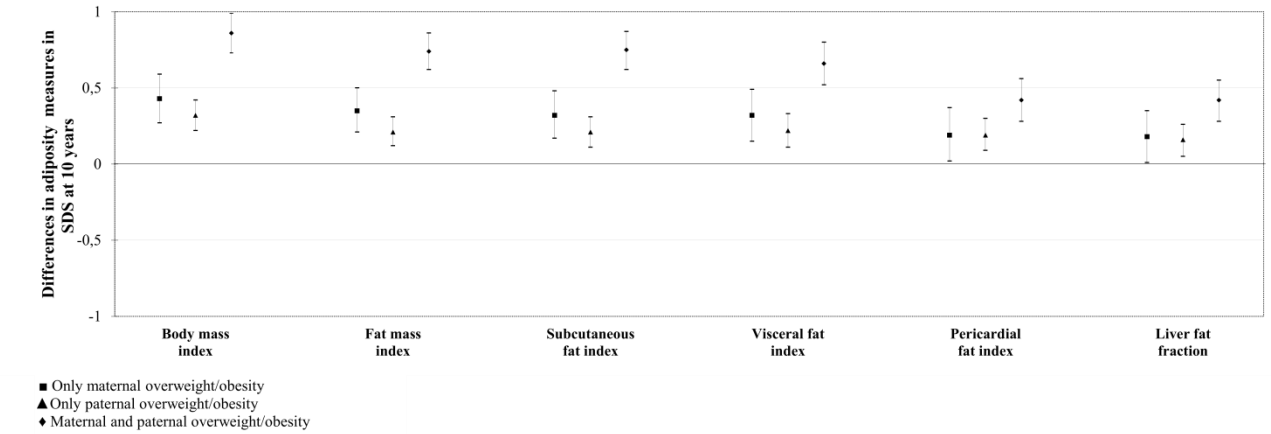
	Measures of adiposity at 10 years in SDS[2]					
	Body mass index (n=2,354)	**Fat mass index** (n=2,339)	**Subcutaneous fat index** (n=2,049)	**Visceral fat index** (n=2,052)	**Pericardial fat index** (n=2,123)	**Liver fat fraction** (n=2,319)
BMI (kg/m^2 in SDS)	0.32 (0.28, 0.36)*	0.28 (0.24, 0.31)*	0.26 (0.22, 0.30)*	0.24 (0.20, 0.28)*	0.12 (0.08, 0.16)**	0.15 (0.11, 0.19)*
Underweight (<18.5 kg/m^2)	-0.49 (-0.69, -0.29)*	-0.32 (-0.50, -0.14)*	-0.31 (-0.50, -0.12)*	-0.37 (-0.58, -0.17)*	-0.26 (-0.47, -0.05)**	-0.17 (-0.37, 0.04)
Normal weight (18.5 – 24.9 kg/m^2)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Overweight (25.0 – 29.9 kg/m^2)	0.46 (0.36, 0.56)*	0.39 (0.30, 0.48)*	0.40 (0.30, 0.50)*	0.35 (0.24, 0.45)*	0.15 (0.04, 0.26)*	0.19 (0.09, 0.30)*
Obesity (≥30.0 kg/m^2)	0.88 (0.73, 1.04)*	0.81 (0.66, 0.95)*	0.76 (0.61, 0.92)*	0.69 (0.52, 0.86)*	0.42 (0.24, 0.59)*	0.45 (0.28, 0.61)*
	MRI measures of adiposity at 10 years in SDS conditional on body mass index[3]					
			Subcutaneous fat index (n=2,049)	**Visceral fat index** (n=2,052)	**Pericardial fat index** (n=2,123)	**Liver fat fraction** (n=2,319)
BMI (kg/m^2 in SDS)			0.05 (0.01, 0.09)**	0.07 (0.03, 0.11)*	0.02 (-0.02, 0.07)	0.03 (-0.01, 0.07)
Underweight (<18.5 kg/m^2)			0.09 (-0.09, 0.28)	-0.12 (-0.33, 0.10)	-0.10 (-0.31, 0.11)	0.02 (-0.19, 0.23)
Normal weight (18.5 – 24.9 kg/m^2)			*Reference*	*Reference*	*Reference*	*Reference*
Overweight (25.0 – 29.9 kg/m^2)			0.12 (0.03, 0.22)**	0.13 (0.02, 0.24)**	0.02 (-0.09, 0.13)	0.02 (-0.09, 0.13)
Obesity (≥30.0 kg/m^2)			0.26 (0.10, 0.41)*	0.24 (0.07, 0.42)*	0.16 (-0.01, 0.34)	0.12 (-0.04, 0.29)

[1]Estimates are based on multiple imputed data. Model includes child´s sex and age at outcome measurements (except for sex- and age-adjusted body mass index SDS), maternal age, educational level, ethnicity, parity, and smoking habits during pregnancy, and child's breastfeeding duration, timing of introduction of solid foods and television watching time. Results from the basic model are given in **Supplemental Table 3**. **P-value<0.05, *P-value<0.01. [2]Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal pre-pregnancy body mass index or for body mass index clinical groups as compared to the reference group (normal weight). [3]Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in the standardized residuals of the childhood outcomes (obtained by conditional regression analyses on body mass index at 10 years) per SDS

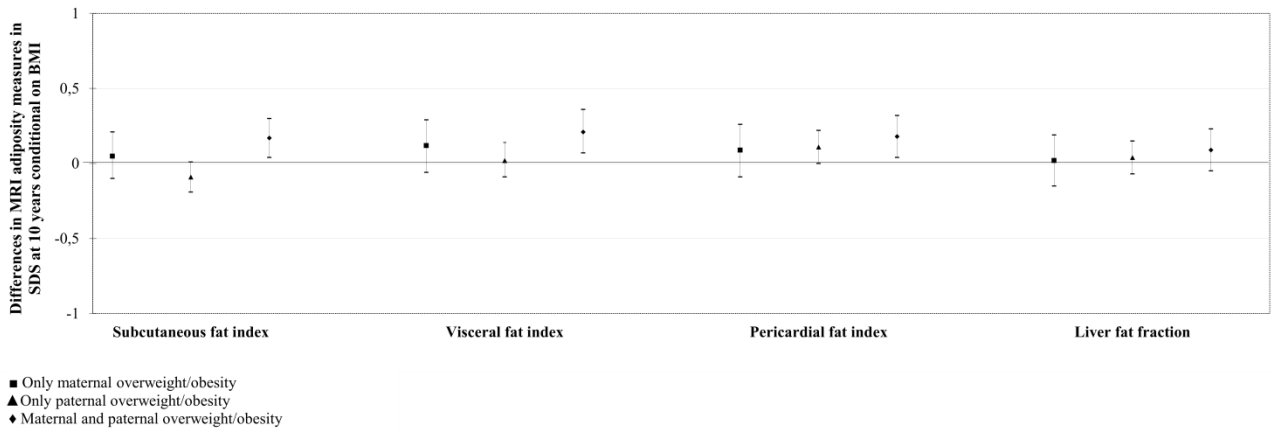
change in maternal pre-pregnancy body mass index or for body mass index clinical groups as compared to the reference group (normal weight). SDS, standard deviation scores.

Figure 1. Parental body mass index and childhood general and organ fat measures (n=1,795)[1]

A.



B.



[1]Estimates are based on multiple imputed data. Model includes child´s sex and age at outcome measurements (except for sex- and age-adjusted body mass index SDS), parental age, educational level, and ethnicity, parity, maternal smoking habits during pregnancy, breastfeeding duration, and timing of introduction of solid foods. Results from the basic model are given in **Supplemental Figure 2**. Values in A are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood outcomes in SDS for parental body mass index clinical groups as compared to the reference group (maternal and paternal normal weight). Values in B are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in the standardized residuals of the childhood outcomes (obtained by conditional regression analyses on body mass index at 10 years) for parental body mass index clinical groups as compared to the reference group (maternal and paternal normal weight). SDS, standard deviation scores.

Table 3. Maternal gestational weight gain and childhood general and organ fat measures[1]

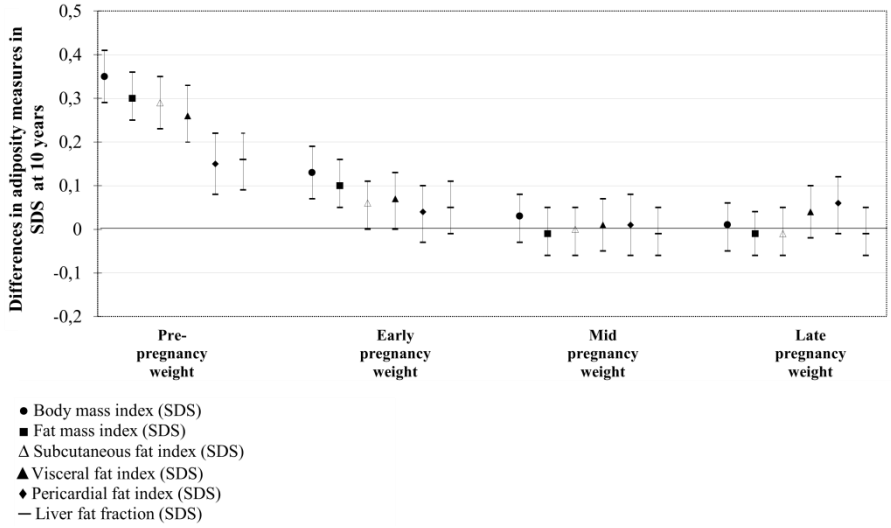
	Measures of adiposity at 10 years in SDS[2]					
	Body mass index (n=1,462)	**Fat mass index** (n=1,451)	**Subcutaneous fat index** (n=1,287)	**Visceral fat index** (n=1,288)	**Pericardial fat index** (n=1,336)	**Liver fat fraction** (n=1,444)
Maximum weight gain per week (kg in SDS)	0.08 (0.03, 0.13)*	0.02 (-0.03, 0.06)	0.01 (-0.04, 0.05)	0.03 (-0.02, 0.08)	0.02 (-0.03, 0.08)	0.00 (-0.05, 0.05)
Insufficient weight gain	-0.09 (-0.23, 0.05)	0.01 (-0.12, 0.14)	-0.01 (-0.14, 0.13)	-0.03 (-0.17, 0.12)	0.04 (-0.11, 0.20)	0.05 (-0.09, 0.19)
Sufficient weight gain	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Excessive weight gain	0.19 (0.07, 0.30)*	0.14 (0.04, 0.25)*	0.12 (0.01, 0.23)**	0.16 (0.04, 0.28)**	0.09 (-0.03, 0.22)	0.06 (-0.05, 0.18)

	MRI measures of adiposity at 10 years in SDS conditional on body mass index[3]			
	Subcutaneous fat index (n=1,287)	**Visceral fat index** (n=1,288)	**Pericardial fat index** (n=1,336)	**Liver fat fraction** (n=1,444)
Maximum weight gain per week (kg in SDS)	-0.09 (-0.13, -0.04)*	-0.02 (-0.07, 0.04)	0.00 (-0.06, 0.05)	-0.04 (-0.09, 0.02)
Insufficient weight gain	0.11 (-0.02, 0.23)	0.04 (-0.10, 0.19)	0.08 (-0.07, 0.23)	0.10 (-0.04, 0.24)
Sufficient weight gain	*Reference*	*Reference*	*Reference*	*Reference*
Excessive weight gain	-0.03 (-0.13, 0.08)	0.07 (-0.05, 0.19)	0.04 (-0.09, 0.16)	-0.01 (-0.12, 0.11)

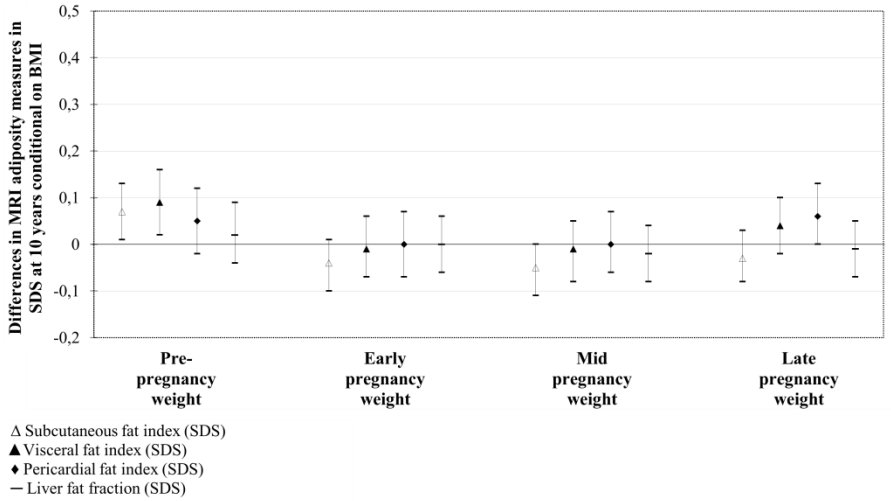
[1]Estimates are based on multiple imputed data. Model includes child´s sex and age at outcome measurements (except for sex- and age- adjusted body mass index SDS), maternal age, educational level, ethnicity, parity, smoking habits during pregnancy, and child's breastfeeding duration, timing of introduction of solid foods and television watching time. Models for maximum weight gain per week were additionally adjusted for pre-pregnancy body mass index. Results from the basic model are given in **Supplemental Table 4**. **P-value<0.05, *P-value<0.01. [2]Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal maximum weight gain per week or for IOM weight gain clinical groups as compared to the reference group (sufficient weight gain). [3]Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in the standardized residuals of the childhood outcomes (obtained by conditional regression analyses on body mass index at 10 years) per SDS change in maternal maximum weight gain per week or for IOM weight gain clinical groups as compared to the reference group (sufficient weight gain). SDS, standard deviation scores.

Figure 2. Maternal pre-, early, mid, and late pregnancy weight with childhood general and organ fat measures (n=1,121)[1]

A.



B.

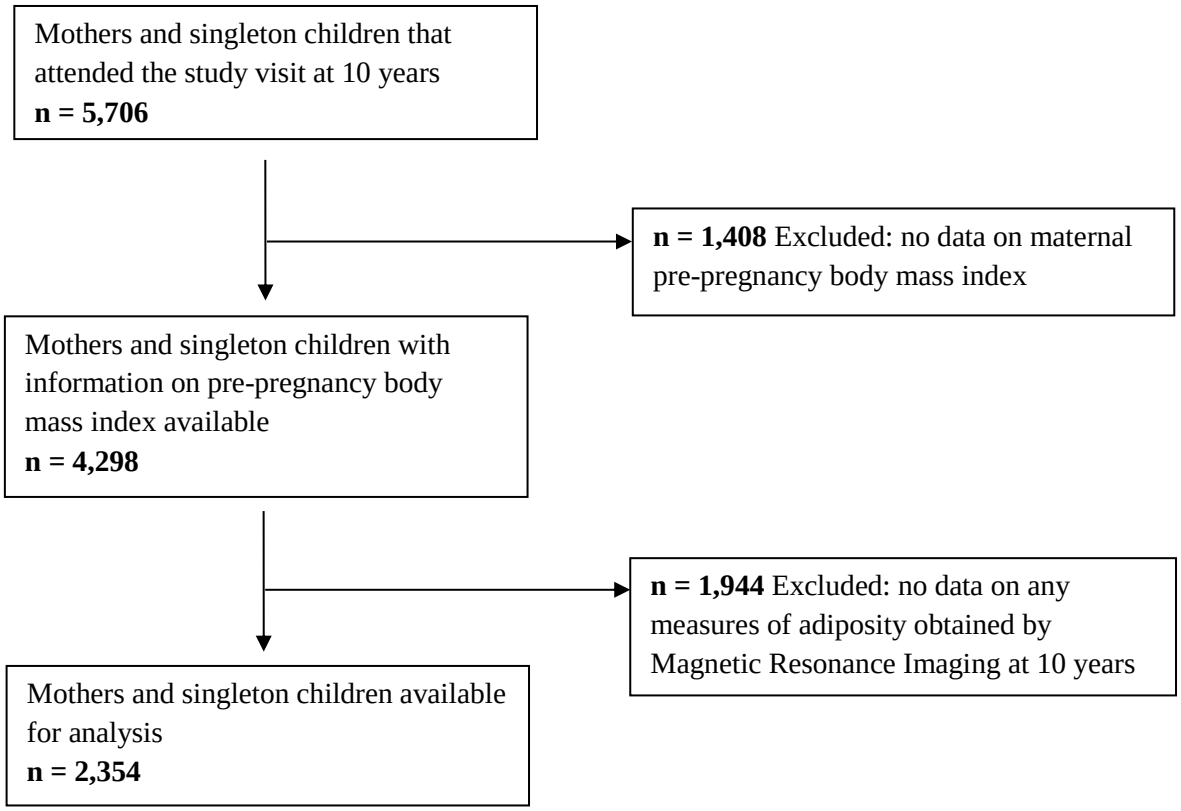


[1]Estimates are based on multiple imputed data. Model includes child´s sex and age at outcome measurements (except for sex- and age-adjusted body mass index SDS), maternal age, educational level, and ethnicity, parity, height at intake, smoking habits during pregnancy, breastfeeding duration, and timing of introduction of solid foods. Results from the basic model are given in **Supplemental Figure 3**. Values in A are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal pre-pregnancy weight and per change in standardized residuals of maternal early, mid, and late pregnancy weight obtained from conditional regression analyses. Values in B are regression coefficients (95% Confidence Intervals) from linear regression models that

reflect differences in the standardized residuals of the childhood outcomes (obtained by conditional regression analyses on body mass index at 10 years) per SDS change in maternal pre-pregnancy weight and per change in standardized residuals of maternal early, mid, and late pregnancy weight obtained from conditional regression analyses. SDS, standard deviation scores.

Supplementary materials

Supplemental Figure 1. Selection of study participants



Supplemental Methods: Log-log regression analyses

To create measures of adiposity independent of height at 10 years, we estimated the optimal adjustment by log-log regression analyses.[1] Total fat mass, subcutaneous fat mass, visceral fat mass and pericardial fat mass and height were log-transformed, using natural logs. Log-adiposity measures were regressed on log-height. The regression slope corresponds to the power by which height should be raised in order to calculate an index uncorrelated with height. Thus, we divided total fat mass by height^4, subcutaneous fat mass by height^4, visceral fat mass by height^3, and pericardial fat mass by height^3.

References

1. Wells JC, Cole TJ, ALSPAC study team. Adjustment of fat-free mass and fat mass for height in children aged 8 y. Int J Obes Relat Metab Disord. 2002;26:947-952.

Supplemental Table 1. Comparison of subject characteristics between participants and non-participants.[1]

Characteristics	Participants (n=2,354)	Non-participants (n=1,944)	P-value
Maternal characteristics			
Age, mean (SD), years	31.0 (4.8)	30.6 (5.1)	0.01
Education (higher education), n (%)	1211 (52.5)	887 (46.9)	<0.01
Ethnicity (European), n (%)	1532 (65.3)	1256 (64.7)	0.72
Parity (nulliparous), n (%)	1419 (60.3)	1123 (57.9)	0.10
Pre-pregnancy body mass index, median (95% range), kg/m^2	22.5 (18.0, 34.9)	22.6 (18.2, 34.2)	0.90
Obesity, n (%)	167 (7.1)	164 (8.4)	0.22
Maximum gestational weight gain, mean (SD), kg	14.8 (5.8)	14.9 (5.5)	0.62
Excessive gestational weight gain (IOM criteria), n (%)	658 (45.0)	416 (42.9)	0.16
Weight in early pregnancy, mean (SD), kg	69.0 (12.9)	68.7 (12.0)	0.47
Weight in mid pregnancy, mean (SD), kg	76.0 (12.6)	75.5 (12.5)	0.17
Weight in late pregnancy, mean (SD), kg	81.6 (12.5)	80.1 (11.8)	0.01
Smoking during pregnancy (yes), n (%)	504 (22.3)	473 (25.5)	0.02

Paternal characteristics			
Age, mean (SD), years	33.6 (5.3)	33.1 (5.7)	0.02
Education (higher education), n (%)	953 (56.5)	713 (52.5)	0.02
Ethnicity (European), n (%)	1383 (74.4)	1089 (72.9)	0.36
Body mass index, mean (SD), kg/m^2	25.3 (3.3)	25.2 (3.4)	0.33
Birth and infant characteristics			
Boys, n (%)	1151 (48.9)	986 (50.7)	0.23
Breastfeeding duration, median (95% range), months	3.5 (0.0, 12.0)	3.5 (0.0, 12.0)	0.58
Introduction of solid foods (> 6 months), n (%)	199 (11.3)	99 (9.8)	0.44
Childhood characteristics			
Age, mean (SD), years	9.8 (0.3)	9.8 (0.4)	0.02
Television watching time (≥ 2 hours/day), n (%)	575 (30.0)	477 (32.8)	0.09
Body mass index, mean (SD), kg/m^2	17.5 (2.6)	17.7 (2.9)	0.04
Total fat mass, median (95% range), g	8,451 (4,549, 21,235)	8,557 (4,505, 23,454)	0.26

[1]Values are observed data and represent means (SD), medians (95% range) or numbers of subjects (valid %). Differences were tested using Student's t-tests and Mann-Whitney tests for normally and non-normally distributed variables, respectively and χ^2-test for dichotomous variables. IOM, Institute of Medicine; SD, standard deviation.

Supplemental Table 2. Correlation coefficients between all measures of adiposity at 10 years (n=2,354)[1]

Measures of adiposity	Body mass index	Fat mass index	Subcutaneous fat index	Visceral fat index	Pericardial fat index	Liver fat fraction
Body mass index	1	0.81*	0.77*	0.58*	0.31*	0.38*
Fat mass index	0.81*	1	0.95*	0.69*	0.33*	0.42*
Subcutaneous fat index	0.77*	0.95*	1	0.74*	0.35*	0.45*
Visceral fat index	0.58*	0.69*	0.74*	1	0.47*	0.39*
Pericardial fat index	0.31*	0.33*	0.35*	0.47*	1	0.18*
Liver fat fraction	0.38*	0.42*	0.45*	0.39*	0.18*	1

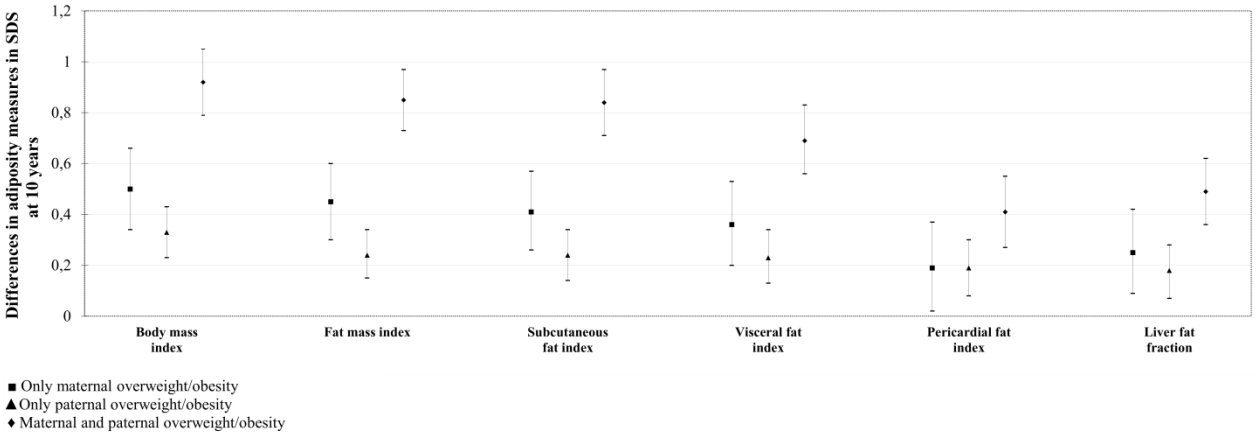
[1]Values are Spearman correlation coefficients. *P-value<0.01

Supplemental Table 3. Maternal body mass index and childhood general and organ fat measures[1]

	Measures of adiposity at 10 years in SDS					
	Body mass index (n=2,354)	**Fat mass index** (n=2,339)	**Subcutaneous fat index** (n=2,049)	**Visceral fat index** (n=2,052)	**Pericardial fat index** (n=2,123)	**Liver fat fraction** (n=2,319)
BMI (kg/m^2 in SDS)	0.35 (0.31, 0.39)*	0.32 (0.28, 0.36)*	0.30 (0.26, 0.34)*	0.25 (0.21, 0.29)*	0.12 (0.08, 0.16)*	0.18 (0.14, 0.22)*
Underweight (<18.5 kg/m^2)	-0.44 (-0.64, -0.24)*	-0.27 (-0.45, -0.08)*	-0.25 (-0.44, -0.05)**	-0.35 (-0.56, -0.15)*	-0.27 (-0.48, -0.06)**	-0.15 (-0.36, 0.05)
Normal weight (18.5 – 24.9 kg/m^2)	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Overweight (25.0 – 29.9 kg/m^2)	0.53 (0.43, 0.63)*	0.48 (0.38, 0.57)*	0.47 (0.37, 0.57)*	0.36 (0.26, 0.47)*	0.15 (0.04, 0.26)*	0.25 (0.15, 0.35)*
Obesity (≥30.0 kg/m^2)	0.99 (0.83, 1.14)*	0.95 (0.81, 1.10)*	0.89 (0.73, 1.05)*	0.72 (0.55, 0.88)*	0.42 (0.25, 0.60)*	0.53 (0.37, 0.69)*

[1]Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal pre-pregnancy body mass index or for body mass index clinical groups as compared to the reference group (normal weight). Model includes child´s sex and age at outcome measurements (except for sex- and age-adjusted body mass index SDS). **P-value<0.05, *P-value<0.01. SDS, standard deviation scores.

Supplemental Figure 2. Parental body mass index and childhood general and organ fat measures (n=1,795)[1]



[1]Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood outcomes in SDS for parental body mass index clinical groups as compared to the reference group (maternal and paternal normal weight). Model includes child´s sex and age at outcome measurements (except for sex- and age-adjusted body mass index SDS). SDS, standard deviation scores.

Supplemental Table 4. Maternal gestational weight gain and childhood general and organ fat measures[1]

	Measures of adiposity at 10 years in SDS					
	Body mass index (n=1,462)	**Fat mass index** (n=1,451)	**Subcutaneous fat index** (n=1,287)	**Visceral fat index** (n=1,288)	**Pericardial fat index** (n=1,336)	**Liver fat fraction** (n=1,444)
Maximum weight gain per week (kg in SDS)	0.02 (-0.03, 0.07)	-0.03 (-0.08, 0.01)	-0.04 (-0.09, 0.01)	0.00 (-0.05, 0.06)	0.01 (-0.04, 0.07)	-0.02 (-0.07, 0.03)
Insufficient weight gain	-0.06 (-0.21, 0.08)	0.06 (-0.07, 0.19)	0.04 (-0.10, 0.17)	0.00 (-0.15, 0.15)	0.04 (-0.11, 0.19)	0.08 (-0.06, 0.22)
Sufficient weight gain	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Excessive weight gain	0.21 (0.10, 0.33)*	0.18 (0.07, 0.29)*	0.15 (0.04, 0.26)**	0.20 (0.08, 0.32)*	0.11 (-0.01, 0.23)	0.10 (-0.02, 0.21)

[1]Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal maximum weight gain per week or for IOM weight gain clinical groups as compared to the reference group (sufficient weight gain). Model includes child´s sex and age at outcome measurements (except for sex- and age-adjusted body mass index SDS). **P-value<0.05, *P-value<0.01. SDS, standard deviation scores.

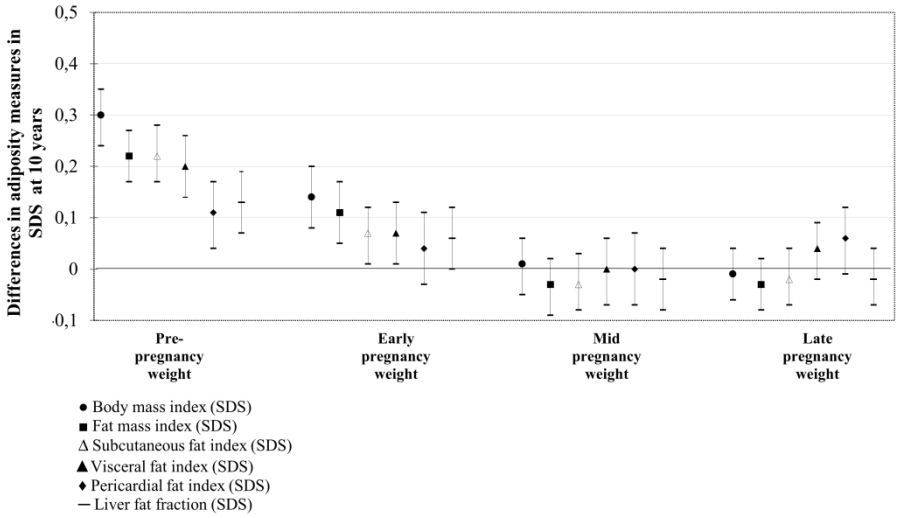
Supplemental Table 5. Maternal weight gain until late pregnancy and childhood general and organ fat measures[1]

	Measures of adiposity at 10 years in SDS[2]					
	Body mass index (n=1,316)	**Fat mass index** (n=1,306)	**Subcutaneous fat index** (n=1,157)	**Visceral fat index** (n=1,158)	**Pericardial fat index** (n=1,207)	**Liver fat fraction** (n=1,301)
Weight gain until late pregnancy per week (kg in SDS)	0.08 (0.03, 0.13)*	0.02 (-0.03, 0.07)	0.01 (-0.04, 0.06)	0.04 (-0.02, 0.09)	0.02 (-0.04, 0.08)	0.00 (-0.05, 0.06)
Insufficient weight gain	-0.13 (-0.28, 0.02)	-0.05 (-0.19, 0.08)	-0.02 (-0.16, 0.12)	-0.04 (-0.19, 0.12)	0.06 (-0.11, 0.22)	0.04 (-0.11, 0.18)
Sufficient weight gain	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*	*Reference*
Excessive weight gain	0.18 (0.06, 0.30)*	0.14 (0.03, 0.25)**	0.14 (0.02, 0.25)**	0.19 (0.06, 0.31)*	0.12 (-0.01, 0.25)	0.07 (-0.05, 0.19)
			MRI measures of adiposity at 10 years in SDS conditional on body mass index[3]			
			Subcutaneous fat index (n=1,157)	**Visceral fat index** (n=1,158)	**Pericardial fat index** (n=1,207)	**Liver fat fraction** (n=1,301)
Weight gain until late pregnancy per week (kg in SDS)			-0.09 (-0.14, -0.04)*	-0.01 (-0.07, 0.05)	0.00 (-0.06, 0.06)	-0.03 (-0.08, 0.02)
Insufficient weight gain			0.13 (0.00, 0.27)	0.06 (-0.10, 0.21)	0.11 (-0.06, 0.27)	0.10 (-0.05, 0.25)
Sufficient weight gain			*Reference*	*Reference*	*Reference*	*Reference*
Excessive weight gain			0.01 (-0.10, 0.12)	0.11 (-0.01, 0.24)	0.07 (-0.06, 0.20)	0.01 (-0.11, 0.13)

[1]Estimates are based on multiple imputed data. Model includes child´s sex and age at outcome measurements (except for sex- and age-adjusted body mass index SDS), maternal age, educational level, ethnicity, parity, smoking habits during pregnancy, and child's breastfeeding duration, timing of introduction of solid foods and television watching time. Models for weight gain until late pregnancy per week were additionally adjusted for pre-pregnancy body mass index. **P-value<0.05, *P-value<0.01. [2]Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal weight gain until late pregnancy per week or for IOM weight gain clinical groups as compared to the reference group (sufficient weight gain). [3]Values are regression

coefficients (95% Confidence Intervals) from linear regression models that reflect differences in the standardized residuals of the childhood outcomes (obtained by conditional regression analyses on body mass index at 10 years) per SDS change in maternal weight gain until late pregnancy per week or for IOM weight gain clinical groups as compared to the reference group (sufficient weight gain). SDS, standard deviation scores.

Supplemental Figure 3. Maternal pre-, early, mid, and late pregnancy weight with childhood general and organ fat measures (n=1,121)[1]



[1] Values are regression coefficients (95% Confidence Intervals) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal pre-pregnancy weight and per change in standardized residuals of maternal early, mid, and late pregnancy weight obtained from conditional regression analyses. Model includes child´s sex and age at outcome measurements (except for sex- and age-adjusted body mass index SDS). SDS, standard deviation scores.

CHAPTER 3

Infant adiposity

CHAPTER 3.1

Anthropometric indices based on waist circumference as measures of total and abdominal adiposity in children

Susana Santos, Milton Severo, Carla Lopes, Andreia Oliveira

Submitted.

Abstract

Background: We compared the accuracy of body fat patterns and single measures in assessing body fat and clarified the use of indices based on waist circumference as measures of adiposity in children.

Methods: This study included 2531 7-year-old children from the Generation XXI birth cohort (Porto, Portugal). Anthropometrics were obtained by trained personnel and body fat patterns were identified by principal component analysis. Whole-body dual energy X-ray absorptiometry (DXA) scans were performed. Pearson´s correlation coefficients of the scores obtained by principal component analysis and the single measures with DXA-fat mass index and -central fat were obtained.

Results: Principal component analysis identified two body fat patterns, similar by sex and explaining 88.3% of total variance. Pattern 1 (body mass index, fat mass index from tetra-polar bioelectric impedance and waist-to-height ratio) showed stronger correlations with DXA-fat mass index ($r=0.85$, $p<0.001$) and pattern 2 (waist-to-hip, waist-to-thigh and waist-to-weight ratios) showed stronger correlations with DXA-central fat ($r=0.35$, $p<0.001$). As compared to single measures, body fat patterns showed similar correlations with DXA-derived measures.

Conclusions: As compared to single anthropometric indices, body fat patterns seem to add little value for estimating body fat in children. Waist-to-height ratio seems to be a proxy for total fat, while waist-to-hip, waist-to-thigh and waist-to-weight ratios seem to be proxies for central fat. Further studies should address the predictive ability of waist-to-weight ratio to identify children at risk of developing cardiometabolic disease, as this was the first time describing its potential usefulness.

Introduction

Epidemiologic studies on childhood obesity often use proxies and single measures for its definition, which might have limited their ability to detect associations.[1] The combination of anthropometric indices into robust body fat patterns could be a more accurate and yet simple approach of evaluating childhood obesity. However, no study has addressed whether body fat patterns are more accurate than single measures for assessing body fat in children. Another drawback of most previous studies is related to the use of waist circumference as proxy for central fat. Waist circumference has been more strongly correlated with body mass index (BMI) and total body fat than with abdominal visceral fat and thus should be considered a marker of total fat, rather than of central fat.[2, 3] This evidence raises doubts about the use of indices based on waist circumference, such as waist-to-height ratio, as proxies for central fat, but no study has addressed this to date.

We aimed to compare the accuracy of body fat patterns and single measures for assessing body fat and to clarify the use of indices based on waist circumference as measures of adiposity in children.

Methods

This study included participants from the population-based birth cohort Generation XXI that was assembled during 2005-2006 at all public maternity units of Porto, Portugal.[4] Of 5849 children who attended the face-to-face interviews at 7 years old, 5719 children had anthropometrics and tetra-polar bioelectric impedance available. A sub-sample of 2531 children had whole-body dual energy X-ray absorptiometry (DXA) measurements (47.6% females). As compared to children without DXA measurements (n=3188), those children with DXA measurements (n=2531) were slightly younger [mean (standard deviation, SD)=6.7 (0.49) vs. 6.9 (0.36) years old] and had a lower BMI [mean (SD)=16.9 (2.45) vs. 17.1 (2.55) kg/m^2]. Although these differences were statistically significant, the magnitude of the differences was small. No differences were observed for child's sex and for the other anthropometric indices. All phases of the study complied with the Ethical Principles for Medical Research Involving Human Subjects expressed in the Declaration of Helsinki. The study was approved by the University of Porto Medical School/ S. João Hospital Centre Ethics Committee and a signed informed consent according Helsinki was required for all participants.

Anthropometrics were obtained by trained personnel with children in underwear and barefoot, according to standard procedures.[5] Body weight was measured to the nearest 0.1 kg

using a digital scale (TANITA®) and height was measured to the nearest 0.1 cm using a wall stadiometer (SECA®). Waist circumference was measured at the umbilicus level, with abdomen relaxed and hip circumference at the level of the greatest posterior protuberance of the buttocks; both were measured to the nearest 0.1 cm, with the child in a standing position, arms at the sides and feet positioned together. Thigh circumference was measured to the nearest 0.1cm around the mid-thigh and perpendicular to the long axis of the thigh, with the leg slightly flexed. The inter-observer variability in anthropometric measurements was assessed comparing a subset of measurements made by each interviewer. The inter-observer variability adjusted for child's age was close to zero for all anthropometrics, showing no differences between interviewers.

Body mass index was calculated as the value of weight (kg) over the squared height (m). Waist-to-height ratio, waist-to-hip ratio and waist-to-thigh ratio were calculated as waist circumference divided by height, hip and thigh circumferences, respectively. The relationship between waist circumference and weight was assessed using a log-log regression analysis, which gave a slope of 0.5, corresponding to the value by which weight should be raised in order to calculate a measure uncorrelated with it. Waist-to-weight ratio was calculated, for the first time in the literature, as waist circumference/weight$^{0.5}$.

Tetra-polar bioelectric impedance analysis was performed (BIA 101 Anniversary, Akern, Florence, Italy). Fat free mass was determined using Schaefer et al. equation and fat mass was derived accordingly.[6] Fat mass/height^2 was calculated to obtain the fat mass index (BIA-FMI), in which fat mass was effectively uncorrelated with height.

Whole-body DXA scans were performed (QDR 4500A; Hologic, Bedford, MA, USA). Fat mass/height^4 was calculated and was effectively uncorrelated with height. Central fat was assessed as trunk fat. Fat mass obtained from bioelectric impedance and DXA was compared. Pearson´s correlation coefficient was strong ($r=0.89$, $p<0.001$) and an intraclass correlation coefficient using an absolute agreement definition of 58.7% was obtained through a two-way mixed effects model, showing reasonable accuracy of bioelectric impedance analysis.

Principal component analysis was applied to BMI, waist-to-height ratio, waist-to-hip ratio, waist-to-thigh ratio, waist-to-weight ratio, and BIA-FMI. Factors with eigenvalues ≥1.0 were retained and varimax rotation was performed. Factor loadings>0.30 were used in the interpretation of factors. The scores were calculated using the regression method with standardized scores. Pearson´s correlation coefficients of the scores obtained by principal component analysis and the single measures with DXA-FMI and -central fat were obtained.

Analyses were conducted using R® version 3.0.1 and SPSS 21.0 (SPSS Inc. 2004, Chicago, IL, USA).

Results

Characteristics of participants are shown in **Table 1**. Two independent body fat patterns, which explained 88.3% of total variance, were identified: a pattern 1 characterized by BMI, BIA-FMI and waist-to-height ratio and a pattern 2 characterized by waist-to-hip, waist-to-thigh and waist-to-weight ratios (**Table 2**). No sex differences in the body fat patterns were observed (data not shown). Pattern 1 (*Fat quantity*) presented a stronger correlation with DXA-FMI ($r=0.85$, $p<0.001$) while pattern 2 (*Fat distribution*) presented a stronger correlation with DXA-central fat ($r=0.35$, $p<0.001$) (**Table 3**). The magnitude of the correlations with DXA-derived measures was similar for body fat patterns and single measures. Waist-to-height ratio presented a stronger correlation with DXA-FMI ($r=0.82$, $p<0.001$) while waist-to-thigh ratio presented a stronger correlation with DXA-central fat ($r=0.30$, $p<0.001$). Waist-to-hip and waist-to-weight ratios were similarly correlated with both DXA-derived measures. No sex differences in the associations of body fat patterns and single measures with DXA-derived measures were observed (data not shown).

Discussion

Pattern 1 combines well-known measures of total adiposity (BMI and BIA-FMI) and pattern 2 combines measures of regional adiposity (waist-to-hip ratio and waist-to-thigh ratio) and seem to represent fat quantity and distribution, respectively. This is corroborated by the stronger correlations between pattern 1 and DXA-FMI and between pattern 2 and DXA-central fat. Nonetheless, both body fat patterns showed similar correlations with DXA-derived measures as compared to single measures. Body fat patterns seem to add little value for estimating body fat in children. Fat distribution, either using patterns or single measures, seems to be poorer measured as compared to fat quantity.

Anthropometric indices based on waist circumference may represent both fat quantity and distribution, which may raise controversial findings in population-based settings. In our study, waist-to-height ratio was stronger correlated with DXA-FMI while waist-to-thigh ratio was stronger correlated with DXA-central fat and thus seem to be proxies for total and central fat, respectively. Although waist-to-hip ratio and waist-to-weight ratio were similarly correlated with both DXA-derived measures, their correlations with DXA-FMI were much weaker as compared to BMI, BIA-FMI and waist-to-height ratio while their correlations with

DXA-central fat were similar as compared to waist-to-thigh ratio and thus might be reasonable proxies for central fat. Waist-to-thigh ratio seems to be the measure of central fat less correlated with total fat. Waist circumference showed similar findings as compared to waist-to-height ratio (data not shown).

Measurements of waist circumference require adjustment for body height or weight if comparisons between individuals, or within individuals over time, are to be meaningful. Previous studies showed that shorter subjects had a higher cardiometabolic risk than taller subjects with similar waist circumference, which contributed to the definition of waist-to-height ratio as another proxy for central adiposity.[7] The correction of waist circumference for height allows that a single cut-off level may be used in different ethnic, age and sex groups[8] while waist circumference requires sex- and population-specific cut-off levels to account for the effect of height on metabolic risk within different populations.[9] However, adjustment of waist circumference for height seems not enough to distinguish children according to their fat distribution. Two hypothetical children of identical height might have different waist circumference due to different body weights. Thus, waist-to-height ratio remains influenced by body weight and, like waist circumference, is a better index of total adiposity rather than of central adiposity, as corroborated by our findings. Previous studies have discussed whether waist circumference adjusted for BMI is a better predictor of visceral fat in adults as compared to waist circumference and have reported contradictory results.[10, 11] In our sample of 7-year-old children, height explains 3.9% while weight explains 83.4% of the variance of waist circumference, and thus adjustment for weight seems to be appropriate in order to obtain an accurate measure of central adiposity. Waist circumference can be adjusted for weight as waist circumference/weight^{p}, where p is the appropriate power by which to raise weight in order to obtain an index uncorrelated with it. As compared to waist-to-hip ratio and waist-to-thigh ratio, the other measures of fat distribution in our study, waist-to-weight ratio might be easier to obtain and less likely to measurement error in most age groups. This was the first time suggesting waist-to-weight ratio as a proxy for central fat, and thus further studies are needed to address its predictive ability to identify children at risk of developing cardiometabolic disease.

Some strengths and limitations should be considered. Major strengths of this study are the population-based design with detailed childhood body fat measurements available. We relied on anthropometric measurements, which might have greater measurement error and be less accurate, but on the other hand be easier and cheaper to obtain in large epidemiological studies as compared to imaging techniques of body composition.[12] In this study, we have also

used DXA, which is an imaging technique that quantifies total and regional body fat content with high precision.[12] Of the 5719 children with anthropometrics and tetra-polar bioelectric impedance available, 2531 children had DXA measurements. However, the non-response is unlikely to lead to biased effect estimates since children with and without DXA measurements did not differ regarding sex, age and the anthropometric indices.

In conclusion, this study suggests that body fat patterns, as compared to single anthropometric indices, add little value for estimating body fat quantity and distribution in children. Waist-to-height ratio seems to be a proxy for total fat (and not for central fat, as suggested in previous studies), while waist-to-hip, waist-to-thigh and waist-to-weight ratios seem to be proxies for central fat. Waist-to-weight ratio is a promising proxy for abdominal fat distribution, with population-based advantages over other measures, that warrants further research.

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Table 1 Characteristics of study participants (n=2531)[1]

Child´s characteristics	Mean (SD)
Age, years	6.7 (0.49)
Body mass index, kg/m^2	16.9 (2.45)
BIA-fat mass index, kg/m^2	3.1 (2.27)
Waist-to-height ratio	0.5 (0.05)
Waist-to-hip ratio	0.9 (0.04)
Waist-to-thigh ratio	1.6 (0.10)
Waist-to-weight ratio, cm/√kg	10.2 (0.47)
DXA-fat mass index, kg/m^4	3.7 (1.32)
DXA-central fat, kg	3.3 (1.71)

[1]BIA, bioelectric impedance analysis; DXA, dual energy X-ray absorptiometry; SD, standard deviation.

Table 2 Factor loadings, obtained from principal component analysis, for anthropometric and bioelectric impedance measures (n=5719)[1]

	Factor loadings	
	Pattern 1	Pattern 2
Body mass index, kg/m^2	**0.98**	0.05
BIA-fat mass index, kg/m^2	**0.94**	0.06
Waist-to-height ratio	**0.87**	0.45
Waist-to-hip ratio	0.28	**0.88**
Waist-to-thigh ratio	-0.04	**0.89**
Waist-to-weight ratio, cm/√kg	0.20	**0.90**
Variance explained	45.3%	43.0%
Cumulative variance explained	45.3%	88.3%

[1]BIA, bioelectric impedance analysis.

Table 3 Pearson´s correlation coefficients of body fat patterns and single measures with DXA-derived measures in children (n=2531)[1]

	DXA-fat mass index, kg/m^4	DXA-central fat, kg[a]
Pattern 1 - *Fat quantity*	0.85 (p<0.001)	0 (p=0.879)
Pattern 2 - *Fat distribution*	0.10 (p<0.001)	0.35 (p<0.001)
Body mass index, kg/m^2	0.83 (p<0.001)	0.08 (p<0.001)
BIA-fat mass index, kg/m^2	0.80 (p<0.001)	-0.02 (p=0.305)
Waist-to-height ratio	0.82 (p<0.001)	0.28 (p<0.001)
Waist-to-hip ratio	0.27 (p<0.001)	0.31 (p<0.001)
Waist-to-thigh ratio	0.02 (p=0.462)	0.30 (p<0.001)
Waist-to-weight ratio, cm/√kg	0.35 (p<0.001)	0.33 (p<0.001)

[1]BIA, bioelectric impedance analysis; DXA, dual energy X-ray absorptiometry.

[a]adjusted for total fat mass.

CHAPTER 3.2

Subcutaneous fat mass in infancy and total and abdominal fat mass at school-age

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Abstract

Background: Skinfold thickness enables the measurement of overall and regional subcutaneous fatness in infancy and may be associated with total and abdominal body fat in later childhood. We examined the associations of subcutaneous fat in infancy with total and abdominal fat at school-age.

Methods: In a population-based prospective cohort study among 821 children, we calculated total subcutaneous fat (sum of biceps, triceps, suprailiacal and subscapular skinfold thicknesses) and central-to-total subcutaneous fat ratio (sum of suprailiacal and subscapular skinfold thicknesses/total subcutaneous fat) at 1.5 and 24 months. At 6 years, we measured fat mass index (total fat/height^3), central-to-total fat ratio (trunk fat/total fat) and android-to-gynoid fat ratio (android fat/gynoid fat) by dual-energy X-ray absorptiometry and preperitoneal fat mass area by abdominal ultrasound.

Results: Central-to-total subcutaneous fat ratio at 1.5 months was positively associated with fat mass index and central-to-total fat ratio at 6 years, whereas both total and central-to-total subcutaneous fat ratio at 24 months were positively associated with all childhood adiposity measures. A 1-standard-deviation scores higher total subcutaneous fat at 24 months was associated with an increased risk of childhood overweight (Odds Ratio 1.70 [95% Confidence Interval 1.36, 2.12]). These associations were weaker than those for body mass index and stronger among girls than boys.

Conclusions: Subcutaneous fat in infancy is positively associated with total and abdominal fat at school-age. Our results also suggest that skinfold thicknesses add little value to estimate later body fat, as compared to body mass index.

Introduction

Infancy seems to be a critical period for the development of obesity.[1] An accumulating body of evidence has suggested that nutrition and growth in infancy are related to the risk of obesity in later life.[2, 3] Also, infant growth patterns seem to be related not only to body mass index, but also to an adverse body fat distribution.[4, 5] Several studies have shown that compared to body mass index, body fat distribution plays a greater role in the development of obesity-related complications, such as cardiovascular disease and type 2 diabetes.[6] Although previous studies have suggested that central fat mass tracks moderately from mid-childhood into adulthood,[7-10] it is not known whether body fat distribution measures in infancy are associated with similar measures in later childhood.

Skinfold thickness is a valid measurement of subcutaneous fat mass that enables assessment of overall and regional fatness in infancy.[11] Previously, we reported the tracking of subcutaneous fat mass measured by skinfold thickness during the first 2 years of life.[12] Assessing the associations of these specific fat mass measures during infancy with fat mass measures during childhood helps to further understand the stability of body fat across childhood. Also, skinfold thickness measurements in infancy may be associated with an adverse body fat pattern in later childhood.

Therefore, we examined, in a population-based prospective cohort study among 821 children, the associations of infant subcutaneous fat mass measures with total and abdominal fat mass measures and with the risk of overweight at school-age.

Methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards among 9778 mothers and their children living in Rotterdam, the Netherlands.[13] The study was approved by the local Medical Ethical Committee. Written informed consent was obtained from all mothers. Additional detailed assessments of fetal and postnatal growth and development were conducted in a subgroup of Dutch mothers and their children from late pregnancy onwards. Of all approached women, 80% agreed to participate. Of the total of 1205 singleton children participating in the subgroup study, 965 children had body mass index or skinfold thicknesses measured at the age of 1.5 or 24 months. Of the group of 965 children, 821 children had follow-up measurements at the age of 6 years (Flow chart is given in **Supplemental Figure S1**).

Body fat measurements during infancy

We measured weight to the nearest gram in naked infants at the age of 1.5 months by using an electronic infant scale and at 24 months by using a mechanical personal scale (SECA, Almere, the Netherlands). Body length at the age of 1.5 months was measured in supine position to the nearest millimeter by using a neonatometer and body height at 24 months was measured in standing position by using a Harpenden stadiometer (Holtain Limited, Dyfed, UK). Body mass index (kg/m^2) was calculated.

We measured skinfold thicknesses at the ages of 1.5 and 24 months on the left side of the body at the biceps, triceps, suprailiacal and subscapular area by using a skinfold caliper (Slim Guide, Creative Health Products) according to standard procedures.[12] We calculated total subcutaneous fat mass from the sum of all four skinfold thicknesses, and central subcutaneous fat mass from the sum of suprailiacal and subscapular skinfold thicknesses.[14, 15] To create total subcutaneous fat mass independent of length or height and central subcutaneous fat mass independent of total subcutaneous fat mass, we estimated the optimal adjustment by log-log regression analyses.[16] Details of these regressions are given in the **Supplemental Methods**. Total subcutaneous fat mass was only weakly correlated with length or height, and was not adjusted for it whereas a central-to-total subcutaneous fat mass ratio was calculated as central divided by total subcutaneous fat mass.

Body fat measurements at school-age

Measurements were performed in a dedicated research center by a well-trained staff.[4] We measured height to the nearest millimeter by using a Harpenden stadiometer (Holtain Limited, Dyfed, UK) and weight to the nearest gram by using a mechanical personal scale (SECA, Almere, the Netherlands) in standing position without shoes and heavy clothing. We calculated body mass index (kg/m^2), and defined overweight and obesity as described by Cole et al.[17]

We measured total and regional body fat mass using a DXA scanner (iDXA, GE-Lunar, 2008, Madison, WI, USA, enCORE software v.12.6), according to standard procedures.[4] Previous studies have validated DXA against computed tomography for body fat assessment.[18-20] We divided total fat mass by height^3 in order to obtain a fat mass index uncorrelated with height, as confirmed by a log-log regression analysis.[16, 21] We assessed central fat mass as fat mass of the trunk, and divided by total fat mass in order to obtain a central-to-total fat mass ratio effectively uncorrelated with total fat mass. We calculated the

ratio of android and gynoid fat mass, which reflects the relation between fat mass in the abdomen (android) and hip (gynoid) regions.

Abdominal preperitoneal fat mass, as a proxy for visceral fat mass, was measured by abdominal ultrasound (GE LOGIQ E9, Milwaukee, WI, USA), as described previously.[22] Briefly, a linear (L12-5 MHz) transducer was placed perpendicular to the skin surface on the median upper abdomen.[23] We scanned longitudinally from the xiphoid process to the navel along the midline (linea alba). Preperitoneal fat mass area was measured as the area of 2 cm length along the midline starting from the reference point in direction of the navel.

Covariates

Information on maternal age, educational level, parity, pre-pregnancy weight and smoking habits during pregnancy was assessed using self-reported questionnaires during pregnancy. We measured maternal height at enrolment, and calculated pre-pregnancy body mass index (kg/m^2). Information about child's sex, gestational age and weight at birth was obtained from medical records. Information about breastfeeding duration, timing of introduction of solid foods and average television watching time at 6 years old was obtained by questionnaires.

Statistical analysis

We examined differences between boys and girls for maternal and child's characteristics with Student's t-tests and Mann-Whitney tests for normally and non-normally distributed variables, respectively and with χ^2-test for dichotomous variables. We used Pearson's or Spearman's rank correlation coefficients to estimate correlations of subcutaneous fat mass measures at 1.5 or 24 months with total and abdominal fat mass measures at 6 years. We assessed the associations of infant subcutaneous fat mass measures with childhood total and abdominal fat mass measures using linear regression models and with the risk of childhood overweight using logistic regression models. These regression models were adjusted for maternal age, educational level, parity, pre-pregnancy body mass index and smoking habits during pregnancy, and child's gestational age-adjusted birth weight SDS, breastfeeding duration, timing of introduction of solid foods, and TV watching time. We included covariates in the models when they changed the effect estimates substantially (>10%), or when they were strongly associated with body fat mass in our or previous studies. Since we observed statistically significant interactions between infant subcutaneous fat mass measures and child's sex in the association with childhood total and abdominal fat mass measures, we performed all analyses for the total group and for boys and girls separately. No significant

interactions were observed with body mass index categories at 1.5 or 24 months. We constructed SDS [(observed value - mean)/SD] for all continuous body fat measures at each age to take into account the expected changes in body composition and fat distribution across ages and also to enable comparisons in effect size for different exposure and outcome measures. Missing values in covariates (ranging from 0 to 15%) were multiple-imputed, by using Markov chain Monte Carlo approach. Five imputed datasets were created and analyzed together. We performed statistical analyses using the Statistical Package of Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Subject characteristics

Tables 1 and **2** show the subject characteristics. Boys had higher body mass index, whereas girls had higher central-to-total subcutaneous fat mass ratio at 1.5 months and a higher total subcutaneous fat mass and central-to-total subcutaneous fat mass ratio at 24 months ($p<0.05$). At 6 years, girls had higher fat mass index, central-to-total fat mass ratio, and abdominal preperitoneal fat mass area than boys ($p<0.05$). **Supplemental Table S2** gives all subcutaneous fat mass measures at 1.5 and 24 months. Non-response analyses showed that as compared to children who did not participate in the follow-up studies, those who did participate were born with a higher weight and gestational age at birth and were breastfed for a longer period ($p<0.05$). (**Supplemental Table S3**).

Body fat from infancy to childhood

The crude correlations of infant subcutaneous fat mass measures at 1.5 or 24 months with total and abdominal fat mass measures at 6 years old for the total group and by sex are shown in **Supplemental Tables S4** and **S5**, respectively.

A 1-standard-deviation scores (SDS) higher body mass index at 1.5 months was only associated with a 0.16 [95% Confidence Interval (CI) 0.09, 0.24] SDS increase in body mass index at 6 years (**Table 3**). A 1-SDS higher central-to-total subcutaneous fat mass ratio at 1.5 months was associated with a 0.12 [95% CI 0.05, 0.19] SDS increase in fat mass index and a 0.11 [95% CI 0.04, 0.18] SDS increase in central-to-total fat mass ratio at 6 years. No other associations of fat mass at 1.5 months with fat outcomes at 6 years were observed. Also, no sex differences were observed.

A 1-SDS higher body mass index at 24 months was associated with an increase of 0.48 [95% CI 0.42, 0.54] SDS in body mass index, 0.33 [95% CI 0.26, 0.40] SDS in fat mass index, 0.18 [95% CI 0.11, 0.26] SDS in central-to-total fat mass ratio, and 0.16 [95% CI 0.08, 0.23] SDS in android-to-gynoid fat mass ratio (**Table 4**). A 1-SDS higher total subcutaneous fat mass at 24 months was associated with an increase of 0.30 [95% CI 0.23, 0.37] SDS in body mass index, 0.36 [95% CI 0.29, 0.43] SDS in fat mass index, 0.28 [95% CI 0.20, 0.35] SDS in central-to-total fat mass ratio, 0.23 [95% CI 0.16, 0.31] SDS in android-to-gynoid fat mass ratio and 0.19 [95% CI 0.12, 0.26] SDS in preperitoneal fat mass area. The associations of central-to-total subcutaneous fat mass ratio at 24 months with fat mass measures at 6 years were also significant but weaker. The associations tended to be stronger among girls than among boys.

Infant body fat and risk of childhood overweight

A 1-SDS higher body mass index at 1.5 months was associated with an increased risk of overweight at 6 years (Odds Ratio (OR) 1.40 [95% CI 1.08, 1.81]) (**Figure 1**), whereas a 1-SDS higher central-to-total subcutaneous fat mass ratio at 1.5 months was associated with an increased risk of overweight at 6 years among girls only (OR 1.61 [95% CI 1.09, 2.38]). A 1-SDS higher body mass index and total subcutaneous fat mass at 24 months were associated with increased risks of childhood overweight (OR 2.76 [95% CI 2.07, 3.69] and OR 1.70 [95% CI 1.36, 2.12]), respectively). We did not observe associations for central-to-total subcutaneous fat mass ratio at 24 months with the risk of childhood overweight. Stronger associations were present among girls than among boys.

Comment

We observed that infant subcutaneous fat mass measures, calculated from skinfold thickness, are associated with total and abdominal fat mass at school-age. The effect estimates were stronger for body mass index and total subcutaneous fat mass than for central-to-total subcutaneous fat mass ratio, and for girls compared to boys. Also, the effect estimates were stronger for 24 months than for 1.5 months fat mass measures.

Interpretation of main findings

Body mass index tends to track from infancy onwards. A previous review among 21 studies has shown that large body size (weight or body mass index) in 0- to 4-year-old children was related to large body size at primary school age.[24] A meta-regression analysis among 48

cohort studies has shown a high degree of tracking for body mass index among children until 10 years of age. The tracking estimates gradually decreased over follow-up time and were not influenced by body mass index at first measurement or sex.[25] In our study, we observed a moderate tracking of body mass index from infancy to childhood. Stronger associations were observed from 24 months onwards than from 1.5 months onwards, in line with the stronger tracking reported in the meta-regression for shorter follow-up periods. We did not observe differences in results when we used ponderal index (weight/height^3) at 1.5 months (data not shown). We observed slightly stronger effect estimates among girls compared to boys.

Body mass index provides limited information about body fat distribution. Android-to-gynoid fat mass ratio as well as preperitoneal fat mass have previously been associated with an adverse cardiovascular risk profile in childhood and adulthood, independently of body mass index.[26, 27] Previous studies have suggested that total and central fat mass track moderately from childhood into adulthood,[7-10, 28-30] although with a lower tracking as compared to body mass index. Since we used different body fat mass measures in infancy and at school-age, we could not directly estimate tracking coefficients. However, we observed that total subcutaneous fat mass at 24 months was positively associated with fat mass index at school-age, suggesting tracking of total fat mass from infancy into school-age. Higher total and central-to-total subcutaneous fat mass ratio in infancy were associated with higher central-to-total and android-to-gynoid fat mass ratios measured by DXA and preperitoneal fat mass area measured by abdominal ultrasound at 6 years old. Our results suggest that besides higher body mass index, higher total subcutaneous fat mass and central-to-total subcutaneous fat mass ratio in infancy relate to an adverse body fat profile at school-age.

We observed stronger effect estimates for infant total subcutaneous fat mass than for central-to-total subcutaneous fat mass ratio, in the associations with similar measures at school-age, though both showed weaker effect estimates compared to the tracking of body mass index from infancy to childhood. The latter may be explained by the differences in methodology to assess the fat mass components in infancy and childhood. In infancy, skinfold thickness measurements reflect the subcutaneous depots only, whereas in childhood DXA comprise both subcutaneous and intra-abdominal depots.[11] However, during the first 4 months of life approximately 90% of body fat is located subcutaneously,[31] and preperitoneal fat mass seems to increase only from the second year of life onwards.[32] Finally, skinfold thickness measurements may be more liable to measurement error than body mass index,[33, 34] which may also lead to an underestimation of the effect estimates for subcutaneous fat measures from infancy onwards. We also observed stronger effect estimates among girls than

boys. Sex-specific adiposity differences have been attributed to sex hormones. During infancy, testosterone concentrations increase during the first week of life before decreasing around 6 months of life among boys, whereas oestrogen increases shortly after birth and remains raised until 2-3 years among girls.[35] The stronger associations that we observed among girls may be partly a result of a more stable body fat development during infancy driven by less hormonal fluctuations. Further studies are needed to explore the mechanisms underlying the observed sex differences in body fat development. The stronger associations observed for 24 months than for 1.5 months could be due to the shorter interval between 24 months and 6 years old, as previously reported for body mass index.[25] Also, 1.5 months might be more reflective of fetal growth patterns which seemed to be less associated, as compared to postnatal growth, with later abdominal adiposity in our previous study.[5]

Whether skinfold thickness measurements during infancy are useful in clinical practice is not known. Our results suggest that compared to body mass index, subcutaneous fat mass measures in infancy add little value to estimate total or abdominal fat mass at school-age. Given the challenges of obtaining precise and reliable skinfold thickness measurements, the additional clinical value of skinfold thicknesses compared to body mass index may be only limited.

Methodological considerations

Major strengths of this study are the population-based prospective design with detailed infant and childhood body fat measurements available. Of the 965 singleton children with information on body fat mass measures at the age of 1.5 or 24 months, 85% (821) participated in the adiposity follow-up study at 6 years old. The non-response could lead to biased effect estimates if the associations of interest would differ between children included and not included in the analyses. Children included in the analyses were born with a higher weight and gestational age and were breastfed for a longer period compared to those not included. It is difficult to speculate if these differences might have influenced our effect estimates. However, this seems unlikely since children that did not participate in the follow-up studies did not differ from those who did participate regarding infant body mass index and subcutaneous fat mass measures. Our sample was ethnically homogeneous, which may limit the generalizability of our results to other ethnic groups.[25] We used skinfold thickness which is a valid measurement of subcutaneous fat in children but in extremely overweight children the measurement error is larger.[15] The inter- and intra-observer measurement error is also larger as compared to other anthropometric measurements.[33, 34] We did not have available

skinfold thickness measurements from the lower limbs such as thigh or calf, which could have improved our estimates of subcutaneous fat mass. We obtained detailed measures of adiposity at school-age by using DXA that quantifies body fat content with high precision and abdominal ultrasound which is a valid method to assess abdominal preperitoneal fat mass area.[23] Finally, although we adjusted for a large number of potential confounders, residual confounding in the observed associations might still occur, as in any observational study.

Conclusion

Subcutaneous fat mass measures in infancy are positively associated with total and abdominal fat mass at school-age. The effect estimates were stronger for body mass index and total subcutaneous fat mass than for central-to-total subcutaneous fat mass ratio, and for girls compared to boys. Our results suggest that skinfold thickness measurements in infancy add little value to estimate body fat in later childhood, as compared to body mass index.

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Table 1. Characteristics of study participants[1]

	Total group (n = 821)	Boys (n = 412)	Girls (n = 409)	P-value
Maternal characteristics				
Age (years), mean (SD)	32.0 (3.9)	31.8 (3.9)	32.1 (3.8)	0.346
Highest completed education, n (%)				
Primary school	10 (1.2)	3 (0.7)	7 (1.7)	0.428
Secondary school	265 (32.3)	135 (32.8)	130 (31.8)	
Higher education	546 (66.5)	274 (66.5)	272 (66.5)	
Parity, n (%) primiparae	522 (63.6)	262 (63.6)	260 (63.6)	0.995
Pre-pregnancy body mass index (kg/m^2), mean (SD)	23.5 (4.0)	23.4 (4.0)	23.7 (4.0)	0.208
Smoking habits during pregnancy, n (%) yes	175 (21.3)	87 (21.1)	88 (21.5)	0.776
Child's characteristics				
Sex, %		412 (50.2)	409 (49.8)	
Birth weight (g), mean (SD)	3533 (522)	3588 (503)	3477 (536)	0.002
Gestational age at birth (weeks), median (95% range)	40.3 (36.3-42.4)	40.3 (36.5-42.4)	40.3 (36.0-42.4)	0.710
Breastfeeding duration (months), mean (SD)	4.8 (3.8)	4.7 (3.7)	4.9 (3.9)	0.442
Introduction of solid foods, n (%)				
<3 months	47 (5.7)	23 (5.6)	24 (5.9)	0.885
3 to 6 months	631 (76.9)	316 (76.7)	315 (77.0)	
>6 months	143 (17.4)	73 (17.7)	70 (17.1)	
TV watching time, n (%) $\geq$ 2 hours/day	76 (9.3)	45 (10.9)	31 (7.6)	0.131

[1]Values are expressed as means (SD), medians (95% range) or numbers of subjects (%). The values represent the pooled results after multiple imputation. Observed data are given in **Supplemental Table S1**. SD, standard deviation.

Table 2. Body fat at 1.5 and 24 months by skinfold thicknesses and at 6 years old by dual-energy X-ray absorptiometry and abdominal ultrasound[1]

	Total group	Boys	Girls	P-value
1.5 months	**n = 742**	**n = 372**	**n = 370**	
Age (months), mean (SD)	1.6 (0.4)	1.6 (0.4)	1.6 (0.5)	0.340
Body mass index (kg/m^2), mean (SD)	15.1 (1.4)	15.3 (1.5)	15.0 (1.3)	0.001
Total subcutaneous fat mass (mm), mean (SD)	23.9 (7.1)	23.8 (7.0)	24.0 (7.1)	0.705
Central-to-total subcutaneous fat mass ratio, mean (SD)	0.50 (0.05)	0.49 (0.05)	0.51 (0.05)	<0.001
24 months	**n = 746**	**n = 381**	**n = 365**	
Age (months), mean (SD)	25.2 (1.1)	25.3 (1.1)	25.2 (1.1)	0.408
Body mass index (kg/m^2), mean (SD)	15.9 (1.3)	16.0 (1.3)	15.9 (1.3)	0.145
Total subcutaneous fat mass (mm), mean (SD)	27.4 (7.5)	26.7 (7.2)	28.1 (7.7)	0.012
Central-to-total subcutaneous fat mass ratio, mean (SD)	0.43 (0.06)	0.42 (0.06)	0.44 (0.06)	0.001
6 years	**n = 821**	**n = 412**	**n = 409**	
Age (years), mean (SD)	6.0 (0.3)	6.0 (0.3)	6.0 (0.2)	0.450
Body mass index (kg/m^2), mean (SD)	15.9 (1.4)	15.9 (1.3)	15.9 (1.5)	0.955
Overweight and obese (IOTF), n (%)	88 (10.7)	34 (8.3)	54 (13.2)	0.023
Fat mass index (kg/m^3), mean (SD)	3.2 (0.8)	2.9 (0.7)	3.4 (0.9)	<0.001
Central-to-total fat mass ratio, mean (SD)	0.33 (0.04)	0.32 (0.03)	0.34 (0.04)	<0.001
Android-to-gynoid fat mass ratio, mean (SD)	0.24 (0.05)	0.24 (0.05)	0.25 (0.06)	0.089
Preperitoneal fat mass area (cm^2), median (95% range)	0.4 (0.2-0.9)	0.3 (0.2-0.7)	0.4 (0.2-1.0)	<0.001

[1]Values are expressed as means (SD), medians (95% range) or numbers of subjects (valid %). Body mass index = weight/height^2. Total subcutaneous fat mass = biceps + triceps + suprailiacal + subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal + subscapular skinfold thicknesses)/total subcutaneous fat mass. Fat mass index = total fat mass/height^3. Central-to-total fat mass ratio = trunk fat mass/total fat mass. Android-to-gynoid fat mass ratio = android fat mass/gynoid fat mass. IOTF, International Obesity Task Force. SD, standard deviation.

Table 3. Associations of subcutaneous fat mass measures at 1.5 months with total and abdominal fat mass at 6 years old[1-3]

	Fat mass measures at 6 years in standard-deviation scores Difference [95% Confidence Interval]				
Fat mass measures at 1.5 months	Body mass index	Fat mass index	Central-to-total fat mass ratio	Android-to-gynoid fat mass ratio	Preperitoneal fat mass area
Total group					
Body mass index	0.16 [0.09,0.24]**	0.05 [-0.03,0.12]	-0.03 [-0.11,0.05]	0.04 [-0.04,0.12]	0.05 [-0.03,0.13]
Total subcutaneous fat mass	0.06 [-0.02,0.13]	0.05 [-0.02,0.13]	0.01 [-0.07,0.09]	0.01 [-0.07,0.09]	0.05 [-0.02,0.12]
Central-to-total subcutaneous fat mass ratio	0.04 [-0.02,0.12]	0.12 [0.05,0.19]**	0.11 [0.04,0.18]**	0.07 [0.00,0.14]	0.07 [-0.01,0.14]
Boys					
Body mass index	0.25 [0.14,0.36]**	0.21 [0.09,0.33]**	0.10 [-0.02,0.22]	0.13 [0.02,0.25]*	0.20 [0.07,0.33]**
Total subcutaneous fat mass	0.12 [0.01,0.23]*	0.07 [-0.04,0.19]	0.09 [-0.03,0.20]	0.03 [-0.08,0.14]	0.14 [0.02,0.26]*
Central-to-total subcutaneous fat mass ratio	0.03 [-0.08,0.13]	0.07 [-0.03,0.18]	0.06 [-0.05,0.16]	0.07 [-0.04,0.17]	0.01 [-0.11,0.13]
Girls					
Body mass index	0.08 [-0.02,0.18]	0.02 [-0.08,0.12]	-0.06 [-0.16,0.05]	-0.03 [-0.14,0.08]	0.02 [-0.09,0.12]
Total subcutaneous fat mass	-0.01 [-0.11,0.09]	0.01 [-0.10,0.11]	-0.08 [-0.19,0.03]	-0.02 [-0.13,0.09]	-0.03 [-0.13,0.06]
Central-to-total subcutaneous fat mass ratio	0.08 [-0.01,0.18]	0.10 [0.01,0.20]*	0.09 [-0.01,0.19]	0.06 [-0.04,0.17]	0.06 [-0.03,0.16]

[1]Values are standardized regression coefficients [95% confidence interval] and represent the difference in standard-deviation scores for fat mass measures at 6 years per 1-standard-deviation scores increase in body mass index and subcutaneous fat mass measures at 1.5 months. Body mass index = weight/height^2. Total subcutaneous fat mass = biceps + triceps + suprailiacal + subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal + subscapular skinfold thicknesses)/total subcutaneous fat mass. Fat mass index = total fat mass/height^3. Central-to-total fat mass ratio = trunk fat mass/total fat mass. Android-to-gynoid fat mass ratio = android fat mass/gynoid fat mass.

[2]Models are adjusted for maternal age, educational level, parity, pre-pregnancy body mass index, smoking habits during pregnancy, and child's gestational age-adjusted birth weight standard-deviation scores, breastfeeding duration, timing of introduction of solid foods, and TV watching time.

[3]P-value for interaction of child´s sex with fat mass measures at 1.5 months >0.05.

*P-value<0.05; **P-value<0.01.

Table 4. Associations of subcutaneous fat mass measures at 24 months with total and abdominal fat mass at 6 years old[1-2]

	Fat mass measures at 6 years in standard-deviation scores Difference [95% Confidence Interval]				
Fat mass measures at 24 months	Body mass index	Fat mass index	Central-to-total fat mass ratio	Android-to-gynoid fat mass ratio	Preperitoneal fat mass area
Total group					
Body mass index	0.48 [0.42,0.54]**†	0.33 [0.26,0.40]**†	0.18 [0.11,0.26]**†	0.16 [0.08,0.23]**†	0.07 [0.00,0.14]†
Total subcutaneous fat mass	0.30 [0.23,0.37]**	0.36 [0.29,0.43]**†	0.28 [0.20,0.35]**	0.23 [0.16,0.31]**†	0.19 [0.12,0.26]**
Central-to-total subcutaneous fat mass ratio	0.09 [0.01,0.16]*†	0.14 [0.06,0.22]**†	0.12 [0.04,0.20]**†	0.10 [0.03,0.18]**†	0.10 [0.02,0.17]*
Boys					
Body mass index	0.46 [0.36,0.55]**	0.35 [0.25,0.45]**	0.20 [0.09,0.30]**	0.10 [-0.01,0.21]	0.01 [-0.12,0.13]
Total subcutaneous fat mass	0.26 [0.15,0.36]**	0.33 [0.22,0.43]**	0.22 [0.11,0.32]**	0.12 [0.01,0.23]*	0.14 [0.01,0.27]*
Central-to-total subcutaneous fat mass ratio	-0.01 [-0.12,0.10]	-0.02 [-0.13,0.10]	0.00 [-0.11,0.11]	0.01 [-0.10,0.12]	0.11 [-0.02,0.24]
Girls					
Body mass index	0.50 [0.41,0.59]**	0.43 [0.33,0.52]**	0.25 [0.14,0.36]**	0.22 [0.11,0.32]**	0.15 [0.05,0.25]**
Total subcutaneous fat mass	0.34 [0.24,0.44]**	0.38 [0.28,0.47]**	0.30 [0.20,0.41]**	0.31 [0.20,0.41]**	0.21 [0.12,0.29]**
Central-to-total subcutaneous fat mass ratio	0.15 [0.05,0.26]**	0.18 [0.07,0.28]**	0.14 [0.04,0.25]**	0.14 [0.03,0.25]*	0.05 [-0.05,0.15]

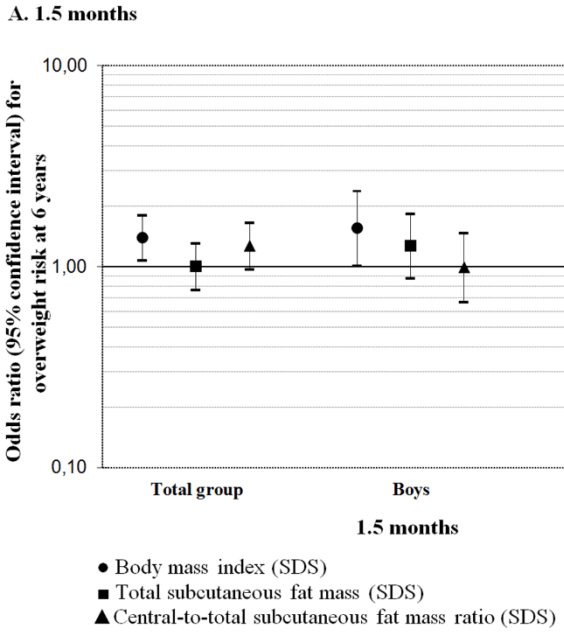
[1]Values are standardized regression coefficients [95% confidence interval] and represent the difference in standard-deviation scores for fat mass measures at 6 years per 1-standard-deviation scores increase in body mass index and subcutaneous fat mass measures at 24 months. Body mass index = weight/height^2. Total subcutaneous fat mass = biceps + triceps + suprailiacal + subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal + subscapular skinfold thicknesses)/total subcutaneous fat mass. Fat mass index = total fat mass/height^3. Central-to-total fat mass ratio = trunk fat mass/total fat mass. Android-to-gynoid fat mass ratio = android fat mass/gynoid fat mass.

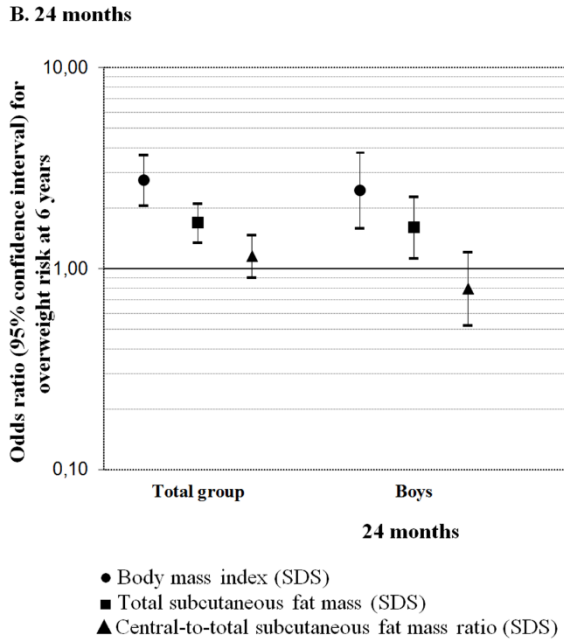
[2]Models are adjusted for maternal age, educational level, parity, pre-pregnancy body mass index, smoking habits during pregnancy, and child's gestational age-adjusted birth weight standard-deviation scores, breastfeeding duration, timing of introduction of solid foods, and TV watching time.

[†]P-value for interaction of child´s sex with fat mass measures at 24 months ≤0.05.

*P-value<0.05; **P-value<0.01.

Figure 1. Associations of subcutaneous fat mass measures at 1.5 or 24 months with risk of overweight at 6 years old[1-3]





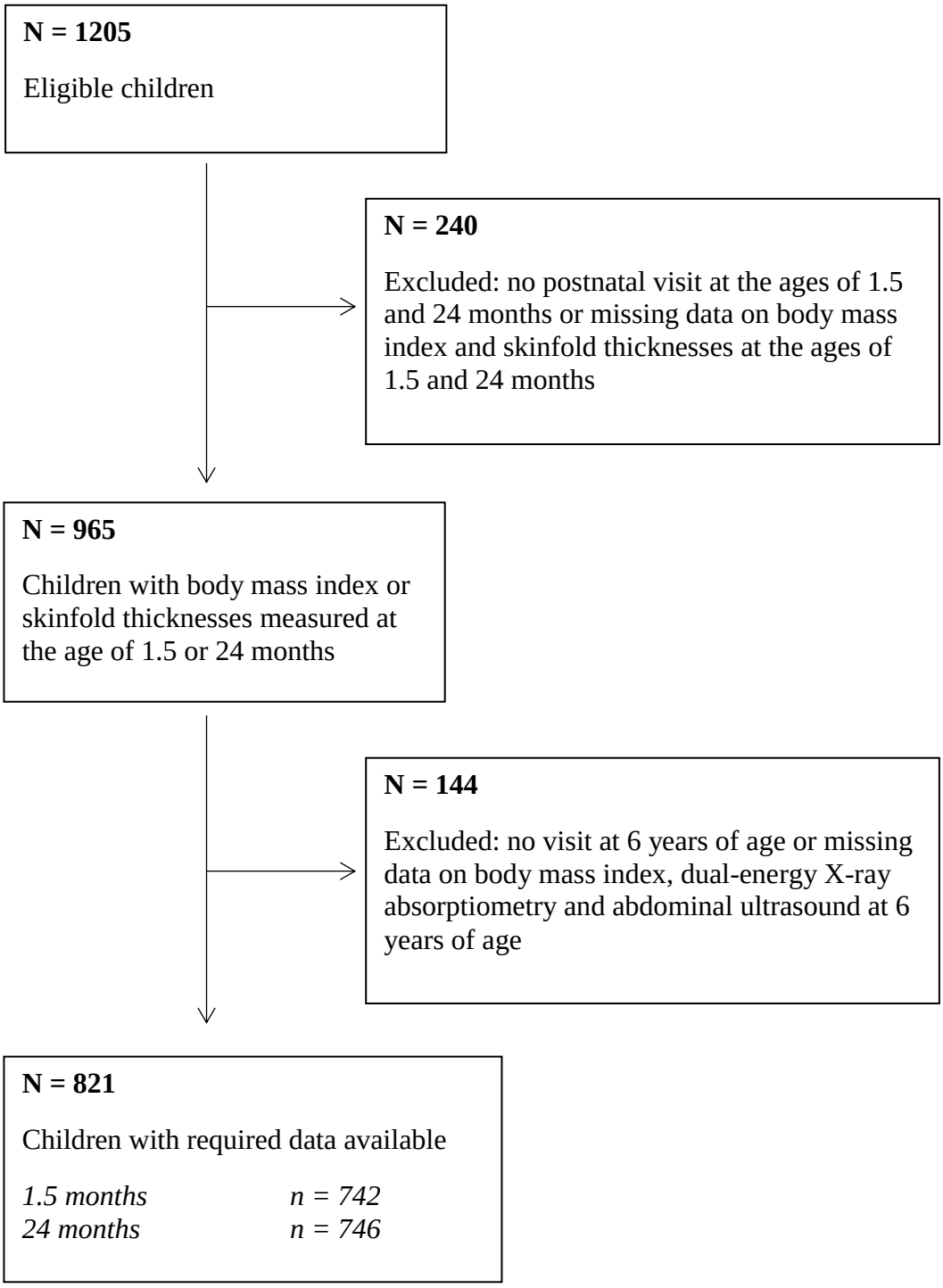
[1]Values are odds ratios (95% Confidence Interval) on a logarithmic scale and represent the risk of overweight at 6 years per 1-standard-deviation scores increase in body mass index and subcutaneous fat mass measures at 1.5 or 24 months. Body mass index = weight/height^2. Total subcutaneous fat mass = biceps + triceps + suprailiacal + subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal + subscapular skinfold thicknesses)/total subcutaneous fat mass. SDS, standard-deviation scores.

[2]Models are adjusted for maternal age, educational level, parity, pre-pregnancy body mass index, smoking habits during pregnancy, and child's gestational age-adjusted birth weight standard-deviation scores, breastfeeding duration, timing of introduction of solid foods, and TV watching time.

[3]P-value for interaction of child´s sex with total subcutaneous fat mass and central-to-total subcutaneous fat mass ratio at 1.5 months ≤0.05. P-value for interaction of child´s sex with body mass index at 24 months <0.05.

Supplementary materials

Figure S1. Flow chart of participants in study



Supplemental Methods: Log-log regression analyses

Measurements of body fat quantity and distribution require appropriate adjustment for body size or total fat mass, respectively, in order to undertake informative comparisons between children and within children over time. The relationships between total subcutaneous fat mass and length or height, and between central subcutaneous fat mass and total subcutaneous fat mass were assessed using log-log regression analyses. Total and central subcutaneous fat mass measures as well as length or height were all log-transformed. Log-total subcutaneous fat mass was regressed on log-length or height. The regression slope corresponds to the power P by which length or height should be raised in order to calculate an index uncorrelated with length or height (total subcutaneous fat mass/length or height^{P}). A similar calculation was undertaken for log-central and -total subcutaneous fat mass.[1]

References

1. Wells JC, Cole TJ, ALSPAC study team. Adjustment of fat-free mass and fat mass for height in children aged 8 y. Int J Obes Relat Metab Disord. 2002;26:947-952.

Table S1. Characteristics of study participants[1]

	Total group (n = 821)	Boys (n = 412)	Girls (n = 409)	P-value
Maternal characteristics				
Age (years), mean (SD)	32.0 (3.9)	31.8 (3.9)	32.1 (3.8)	0.346
Highest completed education, n (%)				
Primary school	10 (1.2)	3 (0.7)	7 (1.7)	0.429
Secondary school	265 (32.4)	135 (32.9)	130 (31.9)	
Higher education	542 (66.3)	272 (66.3)	270 (66.3)	
Parity, n (%) primiparae	522 (63.6)	262 (63.6)	260 (63.6)	0.995
Pre-pregnancy body mass index (kg/m^2), mean (SD)	23.6 (4.2)	23.4 (4.2)	23.8 (4.2)	0.159
Smoking habits during pregnancy, n (%) yes	157 (21.1)	76 (20.7)	81 (21.5)	0.781
Child's characteristics				
Sex, %		50.2	49.8	
Birth weight (g), mean (SD)	3533 (522)	3588 (503)	3477 (536)	0.002
Gestational age at birth (weeks), median (95% range)	40.3 (36.3-42.4)	40.3 (36.5-42.4)	40.3 (36.0-42.4)	0.710
Breastfeeding duration (months), mean (SD)	4.6 (3.9)	4.5 (3.8)	4.8 (4.0)	0.394
Introduction of solid foods, n (%)				
<3 months	41 (5.4)	20 (5.2)	21 (5.6)	0.857
3 to 6 months	578 (76.7)	290 (76.1)	288 (77.2)	
>6 months	135 (17.9)	71 (18.6)	64 (17.2)	
TV watching time, n (%) ≥ 2 hours/day	65 (8.7)	38 (10.2)	27 (7.3)	0.153

[1]Values are observed data and represent means (SD), medians (95% range) or numbers of subjects (valid %). Of the total group, data were missing on maternal highest completed education (n = 4), pre-pregnancy body mass index (n = 121), smoking habits during pregnancy (n = 78), and child's breastfeeding duration (n = 117), timing of introduction of solid foods (n = 67) and TV watching time (n = 77). SD, standard deviation.

Table S2. Subcutaneous fat mass (mm)[1]

	Total group	Boys	Girls	P-value
1.5 months	**n = 742**	**n = 372**	**n = 370**	
Triceps	6.6 (2.2)	6.6 (2.1)	6.6 (2.3)	0.746
Biceps	5.4 (2.0)	5.5 (2.0)	5.4 (2.1)	0.318
Suprailiacal	5.7 (2.0)	5.6 (2.0)	5.8 (2.1)	0.111
Subscapular	6.2 (1.8)	6.1 (1.8)	6.2 (1.8)	0.222
24 months	**n = 746**	**n = 381**	**n = 365**	
Triceps	8.9 (2.9)	8.9 (3.0)	8.9 (2.8)	0.722
Biceps	6.8 (2.4)	6.6 (2.3)	7.0 (2.4)	0.059
Suprailiacal	5.6 (2.2)	5.3 (2.0)	6.0 (2.3)	<0.001
Subscapular	6.1 (1.9)	5.8 (1.7)	6.3 (2.0)	<0.001

[1]Values are means (standard deviation).

Table S3. Comparison of maternal and child's characteristics between children included and not included in the analyses[1]

	Participants (n = 821)	Non-participants (n = 144)	P-value
Maternal characteristics			
Age (years), mean (SD)	32.0 (3.9)	30.8 (4.9)	0.008
Highest completed education, n (%)			
Primary school	10 (1.2)	8 (5.6)	<0.001
Secondary school	265 (32.4)	58 (40.8)	
Higher education	542 (66.3)	76 (53.5)	
Parity, n (%) primiparae	522 (63.6)	74 (51.4)	0.005
Pre-pregnancy body mass index (kg/m^2), mean (SD)	23.6 (4.2)	22.9 (3.4)	0.082
Smoking habits during pregnancy, n (%) yes	157 (21.1)	47 (34.6)	0.001
Child's characteristics			
Sex, % male	50.2	58.3	0.071
Birth weight (g), mean (SD)	3533 (522)	3402 (611)	0.017
Gestational age at birth (weeks), median (95% range)	40.3 (36.3-42.4)	39.9 (34.9-42.5)	0.001
Breastfeeding duration (months), mean (SD)	4.6 (3.9)	3.3 (3.6)	0.001
Introduction of solid foods, n (%)			
<3 months	41 (5.4)	7 (6.8)	0.322
3 to 6 months	578 (76.7)	72 (69.9)	
>6 months	135 (17.9)	24 (23.3)	
TV watching time, n (%) ≥ 2 hours/day	65 (8.7)	4 (7.0)	0.656
1.5 months			
Body mass index (kg/m^2), mean (SD)	15.1 (1.4)	15.2 (1.3)	0.553
Total subcutaneous fat mass (mm), mean (SD)	23.9 (7.1)	24.9 (9.2)	0.267
Central-to-total subcutaneous fat mass ratio, mean (SD)	0.50 (0.05)	0.50 (0.04)	0.304
24 months			
Body mass index (kg/m^2), mean (SD)	15.9 (1.3)	15.9 (1.2)	0.660
Total subcutaneous fat mass (mm), mean (SD)	27.4 (7.5)	27.0 (5.8)	0.712
Central-to-total subcutaneous fat mass ratio, mean (SD)	0.43 (0.06)	0.44 (0.07)	0.161

[1]Values are observed data and represent means (SD), medians (95% range) or numbers of subjects (valid %). Differences were tested using Student's t-tests and Mann-Whitney tests for normally and non-normally distributed variables, respectively and χ^2-test for dichotomous variables. SD, standard deviation.

Table S4. Correlation coefficients between body fat mass measures at 1.5 months and 6 years old[1]

Fat mass measures at 1.5 months	**Fat mass measures at 6 years**				
	Pearson correlation coefficients				*Spearman correlation coefficients*
	Body mass index	Fat mass index	Central-to-total fat mass ratio	Android-to- gynoid fat mass ratio	Preperitoneal fat mass area
Total group					
Body mass index	0.22**	0.08*	-0.01	0.05	0.08*
Total subcutaneous fat mass	0.08*	0.05	0.01	0.03	0.05
Central-to-total subcutaneous fat mass ratio	0.06	0.12**	0.11**	0.07	0.07
Boys					
Body mass index	0.29**	0.17**	0.06	0.12*	0.18**
Total subcutaneous fat mass	0.14**	0.07	0.06	0.06	0.08
Central-to-total subcutaneous fat mass ratio	0.05	0.09	0.07	0.08	0.01
Girls					
Body mass index	0.16**	0.09	-0.01	0.01	0.08
Total subcutaneous fat mass	0.02	0.03	-0.05	0.01	0.01
Central-to-total subcutaneous fat mass ratio	0.07	0.07	0.07	0.05	0.04

[1]Values are correlation coefficients between body fat mass measures standard-deviation scores using Pearson r tests for normally distributed variables and Spearman's rho tests for skewed variables. Body mass index = weight/height^2. Total subcutaneous fat mass = biceps + triceps + suprailiacal + subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal + subscapular skinfold thicknesses)/total subcutaneous fat mass. Fat mass index = total fat mass/height^3. Central-to-total fat mass ratio = trunk fat mass/total fat mass. Android-to-gynoid fat mass ratio = android fat mass/gynoid fat mass.
*P-value<0.05; **P-value<0.01.

Table S5. Correlation coefficients between body fat mass measures at 24 months and 6 years old[1]

	Fat mass measures at 6 years				
	Pearson correlation coefficients				*Spearman correlation coefficients*
Fat mass measures at 24 months	Body mass index	Fat mass index	Central-to-total fat mass ratio	Android-to- gynoid fat mass ratio	Preperitoneal fat mass area
Total group					
Body mass index	0.52**	0.36**	0.18**	0.17**	0.11**
Total subcutaneous fat mass	0.32**	0.37**	0.28**	0.24**	0.25**
Central-to-total subcutaneous fat mass ratio	0.11**	0.17**	0.14**	0.12**	0.13**
Boys					
Body mass index	0.47**	0.33**	0.14**	0.08	0.06
Total subcutaneous fat mass	0.26**	0.32**	0.20**	0.11	0.18**
Central-to-total subcutaneous fat mass ratio	-0.01	0.00	0.01	0.03	0.13*
Girls					
Body mass index	0.57**	0.48**	0.28**	0.25**	0.21**
Total subcutaneous fat mass	0.36**	0.39**	0.32**	0.32**	0.30**
Central-to-total subcutaneous fat mass ratio	0.21**	0.23**	0.18**	0.18**	0.08

[1]Values are correlation coefficients between body fat mass measures standard-deviation scores using Pearson r tests for normally distributed variables and Spearman's rho tests for skewed variables. Body mass index = weight/height^2. Total subcutaneous fat mass = biceps + triceps + suprailiacal + subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal + subscapular skinfold thicknesses)/total subcutaneous fat mass. Fat mass index = total fat mass/height^3. Central-to-total fat mass ratio = trunk fat mass/total fat mass. Android-to-gynoid fat mass ratio = android fat mass/gynoid fat mass.
*P-value<0.05; **P-value<0.01.

CHAPTER 3.3

Subcutaneous fat mass in infancy and cardiovascular risk factors at school-age

Susana Santos, Romy Gaillard, Andreia Oliveira, Henrique Barros, Albert Hofman, Oscar H. Franco, Vincent W.V. Jaddoe

Adapted from *Obesity (Silver Spring) 2016;24(2):424-429.*

Abstract

Objective: To examine the associations of infant subcutaneous fat with cardiovascular risk factors at school-age.

Methods: In a population-based prospective cohort study among 808 children, total subcutaneous fat (sum of biceps, triceps, suprailiacal and subscapular skinfold thicknesses) and central-to-total subcutaneous fat ratio (sum of suprailiacal and subscapular skinfold thicknesses/total subcutaneous fat) at 1.5 and 24 months were estimated. At 6 years, body mass index, blood pressure, cholesterol, triglycerides, and insulin levels were measured.

Results: Infant subcutaneous fat measures were not associated with childhood blood pressure, triglycerides or insulin levels. A 1-standard-deviation score (SDS) higher total subcutaneous fat at 1.5 months was, independently of body mass index, associated with lower low-density lipoprotein (LDL)-cholesterol levels at 6 years. In contrast, a 1-SDS higher total subcutaneous fat at 24 months was associated with higher total-cholesterol (difference 0.13 (95% Confidence Interval (CI) 0.03, 0.23) SDS) and LDL-cholesterol levels (difference 0.12 (95% CI 0.02, 0.21) SDS) at 6 years. There were no associations of central-to-total subcutaneous fat ratio with childhood cholesterol levels.

Conclusions: These results suggest that infant total subcutaneous fat is weakly associated with cholesterol levels at school-age. Further studies are needed to assess the long-term cardiometabolic consequences of infant body fat.

Introduction

Early infant and childhood growth rates are associated with cardiovascular disease risk in later life.[1] Results from longitudinal studies suggest that rapid weight gain in infancy or gain in body mass index in childhood are associated with an adverse cardiovascular risk profile in adulthood.[2-4] Weight or body mass index are suboptimal measures of fat mass development and provide no information about body fat distribution.[5] Several studies have shown that as compared to body mass index, body fat distribution plays a greater role in the development of risk factors for cardiovascular disease.[6] We have previously reported in a cross-sectional study among 6-year-old children that both general and abdominal fat mass measures are associated with cardiovascular risk factors, independently of body mass index.[7] Also, previous studies have shown that high total subcutaneous fat mass measured by the sum of skinfold thicknesses is associated with high blood pressure, an unfavorable blood lipids profile and high glucose and insulin levels in childhood.[8-12] Currently, it is not known whether, next to rapid weight gain, total and regional subcutaneous fat mass development in infancy is associated with cardiovascular risk factors in later life. Assessing the contribution of fatness in infancy to later cardiovascular risk status is particularly relevant to identify early critical periods of fat development that influence the risk for cardiovascular disease.

Therefore, we examined, in a population-based prospective cohort study among 808 children, the associations of infant subcutaneous fat mass measures with cardiovascular risk factors at school-age. We used skinfold thickness measurements at 1.5 and 24 months to estimate total subcutaneous fat mass and central-to-total subcutaneous fat mass ratio. Cardiovascular risk factors of interest include blood pressure, total-, high-density lipoprotein (HDL)-, and low-density lipoprotein (LDL)-cholesterol levels, triglycerides levels and insulin levels at 6 years.

Methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards among 9,778 mothers and their children living in Rotterdam, the Netherlands.[13, 14] The study protocol was approved by the local Medical Ethical Committee. Written informed consent was obtained from all mothers. Additional detailed

assessments of fetal and postnatal growth and development were conducted in a subgroup of Dutch mothers and their children from late pregnancy onwards. Of all approached women, 80% agreed to participate. Of the total of 1,205 singleton children participating in the subgroup study, 965 children had body mass index or skinfold thicknesses measured at the age of 1.5 or 24 months. Of these children, 808 children had cardiovascular risk factors measurements at the age of 6 years (Flow chart is given in **Supplemental Figure S1**). Missing measurements were due to loss to follow-up, crying behavior or non-consent for venous puncture at 6 years old.

Body fat measurements during infancy

We measured weight to the nearest gram in naked infants at the age of 1.5 months by using an electronic infant scale and at 24 months by using a mechanical personal scale (SECA, Almere, the Netherlands). Body length at the age of 1.5 months was measured in supine position to the nearest millimeter by using a neonatometer and body height at 24 months was measured in standing position by using a Harpenden stadiometer (Holtain Limited, Dyfed, UK). Body mass index (kg/m^2) was calculated.

We measured skinfold thicknesses at the ages of 1.5 and 24 months on the left side of the body at the biceps, triceps, suprailiacal and subscapular area by using a skinfold caliper (Slim Guide, Creative Health Products) according to standard procedures described in detail previously.[15] We calculated total subcutaneous fat mass from the sum of all four skinfold thicknesses, and central subcutaneous fat mass from the sum of suprailiacal and subscapular skinfold thicknesses.[16] Measurements of body fat quantity and distribution require appropriate adjustment for body size or total fat mass, respectively, in order to undertake informative comparisons between children and within children over time. To create total subcutaneous fat mass independent of length or height and central subcutaneous fat mass independent of total subcutaneous fat mass, we estimated the optimal adjustment by log-log regression analyses.[17] Details of these regressions are given in the **Supplemental Methods**. Based on these analyses, total subcutaneous fat mass was only weakly correlated with length at 1.5 months or height at 24 months, and was not adjusted for height whereas a central-to-total subcutaneous fat mass ratio was calculated as central divided by total subcutaneous fat mass.

Cardiovascular risk factors at school-age

Blood pressure was measured at the right brachial artery four times with one-minute intervals, using the validated automatic sphygmanometer Datascope Accutor Plus (Paramus, NJ).[18] We calculated the mean value for systolic and diastolic blood pressure using the last three blood pressure measurements of each participant. Thirty-minutes fasting blood samples were collected to measure total-, HDL-, and LDL-cholesterol, triglycerides, and insulin concentrations, using Cobas 8000 analyzer (Roche, Almere, the Netherlands). Quality control samples demonstrated intra- and interassay coefficients of variation ranging from 0.77 to 1.39%, and 0.87 to 2.40%, respectively.

Covariates

Information on maternal age, educational level, parity, pre-pregnancy weight and smoking habits during pregnancy was assessed using self-reported questionnaires during pregnancy. We measured maternal height at enrollment and calculated pre-pregnancy body mass index (kg/m^2). First trimester maternal nutritional information was obtained by food frequency questionnaire.[19] Gestational weight gain was calculated as the difference between maternal weight measured at 30 weeks of gestation and pre-pregnancy weight. Information about gestational diabetes and gestational hypertensive disorders, child's sex, gestational age and weight at birth was obtained from medical records. Information about breastfeeding duration, timing of introduction of solid foods and average TV watching time at 6 years old was obtained by questionnaires. At the age of 6 years, we measured child's height and weight in standing position without shoes and heavy clothing, and calculated body mass index (kg/m^2).

Statistical analysis

We assessed the associations of body mass index, total subcutaneous fat mass and central-to-total subcutaneous fat mass ratio at 1.5 and 24 months and the change between these ages, with cardiovascular risk factors (blood pressure, total-, HDL-, and LDL-cholesterol, triglycerides and insulin levels) at school-age using linear regression models. The regression models were adjusted for maternal age, educational level, parity, pre-pregnancy body mass index, maternal total energy intake, smoking habits and total weight gain during pregnancy, gestational diabetes, gestational hypertensive disorders, and child's sex, gestational age-adjusted birth weight standard-deviation scores (SDS), breastfeeding duration, timing of introduction of solid foods, and TV watching

time. We included covariates in the models when they were strongly associated with body fat mass and cardiovascular risk factors in our or previous studies, or when they changed the effect estimates substantially (>10%). Additionally, we adjusted these models for childhood body mass index to assess whether any association of infant fat mass measures with childhood cardiovascular risk factors was independent of body mass index at 6 years. For the models with body mass index as main exposure, we constructed a conditional body mass index variable at 6 years that was statistically independent of body mass index at 1.5 and 24 months, allowing simultaneous inclusion in multiple regression models.[20] Details of these models are given in the Supplemental Methods. For the models with subcutaneous fat mass measures as exposures, body mass index at 6 years was simultaneously included in the regression models since no collinearity was observed. We did not observe significant interactions between infant fat mass measures and sex, breastfeeding groups (never, ever) and body mass index categories at 1.5 or 24 months in the associations with childhood cardiovascular risk factors. For all analyses, we log-transformed not normally distributed cardiovascular risk factors (triglycerides and insulin levels). We constructed SDS [(observed value - mean)/SD] of the sample distribution for all variables to enable comparisons in effect size. Missing values in covariates were multiple-imputed, by using Markov chain Monte Carlo approach. Five imputed datasets were created and analyzed together. We performed statistical analyses using the Statistical Package of Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA).

Results

Subject characteristics

Tables 1 and **2** show the subject characteristics. Of all participating children, 50.1% were boys, the mean (SD) birth weight was 3535 (517) g and the median (95% range) gestational age at birth was 40.3 (36.4-42.4) weeks.

Non-response analyses showed that as compared to mothers who did not participate in the follow-up studies, those who did participate were slightly older, had a higher educational level and pre-pregnancy body mass index and were more likely to be nulliparous and non-smokers ($p<0.05$). Their children were born with a higher weight and gestational age at birth and were breastfed for a longer period ($p<0.05$) (**Supplemental Table S1**).

Infant body fat and cardiovascular risk factors at school-age

Table 3 shows that, after adjustment for potential confounders, body mass index at 1.5 and 24 months or its change over infancy was not associated with cardiovascular risk factors at school-age. Similarly, total subcutaneous fat mass and central-to-total subcutaneous fat mass ratio at 1.5 and 24 months or its change over infancy were not associated with childhood blood pressure and triglycerides or insulin levels. A 1-SDS higher total subcutaneous fat mass at 1.5 months was associated with lower LDL-cholesterol levels at the age of 6 years (difference -0.10 (95% Confidence Interval (CI) -0.20, -0.01) SDS), whereas 1-SDS higher total subcutaneous fat mass at 24 months was associated with higher childhood total- and LDL-cholesterol levels (differences 0.15 (95% CI 0.05, 0.24) SDS and 0.14 (95% CI 0.04, 0.23) SDS, respectively). Also, 1-SDS increase in total subcutaneous fat mass from 1.5 to 24 months was associated with higher childhood total- and LDL-cholesterol levels (differences 0.10 (95% CI 0.03, 0.18) SDS and 0.11 (95% CI 0.04, 0.19) SDS, respectively). These results were not materially affected by additional adjustment for childhood body mass index (Table 3). Results from the unadjusted analyses are given in **Supplemental Table S2**.

Discussion

We observed in this population-based prospective cohort study that infant subcutaneous fat mass measures at 1.5 and 24 months were not associated with childhood blood pressure and triglycerides or insulin levels, but were weakly associated with childhood cholesterol levels at 6 years, independently of body mass index.

Methodological considerations

Major strengths of this study are the population-based prospective design with detailed infant body fat and childhood cardiovascular risk factors measurements available. Some methodological issues need to be considered. Of the 965 singleton children with information on body fat mass measures at the age of 1.5 or 24 months, 84% (808) participated in the adiposity and cardiovascular follow-up study at 6 years old. The non-response could lead to biased effect estimates if the associations of infant subcutaneous fat mass with cardiovascular risk factors at school-age differ between children included and not included in the present analyses. However,

this seems unlikely since children that did not participate in the follow-up studies did not differ from those who did participate regarding body mass index and subcutaneous fat mass measures at 1.5 and 24 months. Our study group was ethnically homogeneous (Dutch only), which may limit the generalizability of our results to other ethnic groups.[21] We used skinfold thickness as a measure of subcutaneous fat mass and therefore we were not able to measure intra-abdominal depots. However, during the first months of life approximately 90% of body fat is located subcutaneously[22] and preperitoneal fat mass seems to increase only from the second year of life onwards.[23] Previous studies have shown that skinfold thickness is a valid measurement of fat mass in children, but in extremely overweight children the measurement error is larger.[24] The inter- and intra-observer measurement error is also larger as compared to other anthropometric measurements.[25, 26] The use of thirty-minutes fasting blood samples may have resulted in misclassification and thus may have led to underestimation of the observed associations. However, it has been shown in adults that non-fasting blood lipid levels can accurately predict increased risks of cardiovascular events in later life.[27] Finally, we adjusted for a large number of potential confounders but residual confounding in the observed associations might still occur, as in any observational study. For example, in our study, we were unable to adjust our results for detailed nutritional information during infancy and at school-age.

Interpretation of main findings

A high body mass index is an important risk factor for an adverse cardiovascular risk profile in late childhood.[7, 12, 28] Also, a high body mass index gain during childhood has been associated with cardiovascular risk in adolescence and adulthood.[3, 29] Few studies have assessed the relation of body mass index and cardiovascular risk in preschool children and have found weak or no associations with blood lipid and insulin levels.[30-32] Rapid weight gain in the first 3 months of life has been associated with increased adiposity and an unfavorable cardiovascular profile in later life.[2] However, it remains unknown whether gain in body mass index or ponderal index in infancy predisposes individuals to cardiovascular risk. A study among 4,601 UK subjects has shown that changes in ponderal index from 0 to 2 years were not associated with cardiovascular risk factors in adolescence.[29] In the present study, we observed no associations of body mass index at the ages of 1.5 and 24 months or its change in this period with cardiovascular risk

factors at school-age. Also, we did not observe differences in results when we used ponderal index calculated as weight/height^3 at 1.5 months (data not shown).[33]

Body mass index may not be an accurate measure of total fat mass and provides no information on body fat distribution.[5] An accumulating body of evidence has suggested that body fat distribution is more strongly associated with cardiovascular disease and type 2 diabetes than body mass index.[6] We have previously reported in a cross-sectional study among 6-year-old children that high fat mass percentage and android-to-gynoid fat mass ratio measured by dual-energy X-ray absorptiometry were associated with adverse levels of cardiovascular risk factors, independently of body mass index. The associations of these more detailed body fat mass measures with blood lipid levels tended to be stronger than the associations for body mass index.[7]

Previous studies have also shown that high total and central subcutaneous fat mass measures, estimated from skinfold thicknesses, are associated with an adverse cardiovascular risk profile, namely high blood pressure, an unfavorable blood lipids profile and high insulin levels, in late childhood.[8-10, 12, 34] Another study has shown that an increase in total subcutaneous fat mass from 8 to 18 years old was associated with an increase in total-cholesterol, LDL-cholesterol and triglycerides levels and a decrease in HDL-cholesterol levels.[11] Few studies have assessed the associations of subcutaneous fat mass measures with cardiovascular risk in preschool children.[31, 35] Those studies have found that total subcutaneous fat mass was positively but weakly related to fasting insulin in children aged 2 to 3 years[31] while no association was found with blood lipid levels in children aged 4 years.[35] It is not known whether total and regional subcutaneous fat mass in infancy is associated with cardiovascular risk factors in later life. We observed no associations of infant subcutaneous fat mass measures with childhood blood pressure and triglycerides or insulin levels. At 1.5 months, total subcutaneous fat mass was inversely associated with LDL-cholesterol at the age of 6 years old. We cannot explain this finding. Child's dietary intake and physical activity may have confounded this association, since we had only available information about breastfeeding, age at introduction of solid foods and TV watching time at 6 years. At 24 months, total subcutaneous fat mass, but not central-to-total subcutaneous fat mass ratio, was positively but also weakly associated with childhood total- and LDL-cholesterol, independently of body mass index at 6 years old. LDL-cholesterol is an

important determinant of cardiovascular risk and a causal agent in the atherothrombotic process.[36]

In line with the previous cross-sectional studies performed among preschool children,[31, 35] our study suggests that subcutaneous fat mass measures in infancy seem to be poor indicators of cardiovascular risk profile in later childhood. These findings may be partially explained by the fact that fat mass measures in infancy represent the accumulation of fat mass over a short period of time, which may not be long enough to influence cardiovascular risk profile. Further studies are needed to assess the long-term cardiovascular consequences of infant total and regional fat mass measures.

Conclusion

Our results suggest that infant subcutaneous fat mass measures do not affect childhood blood pressure and triglycerides or insulin levels, and are weakly associated with childhood total- and LDL-cholesterol levels at 6 years, independently of body mass index.

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Table 1. Characteristics of study participants[1]

	Total group (n = 808)
Maternal characteristics	
Age (years), mean (SD)	32.0 (3.8)
Highest completed education, n (%)	
Primary school	10 (1.2)
Secondary school	261 (32.5)
Higher education	533 (66.3)
Parity, n (%) nulliparous	512 (63.4)
Pre-pregnancy body mass index (kg/m^2), mean (SD)	23.6 (4.2)
Total energy intake (kcal), mean (SD)	2131 (499)
Total weight gain during pregnancy (kg), mean (SD)	10.2 (4.6)
Smoking habits during pregnancy, n (%)	
No	575 (78.7)
Yes	156 (21.3)
Gestational diabetes, n (%)	9 (1.1)
Gestational hypertensive disorders, n (%)	64 (8.1)
Child's characteristics	
Boys, n (%)	405 (50.1)
Birth weight (g), mean (SD)	3535 (517)
Gestational age at birth (weeks), median (95% range)	40.3 (36.4-42.4)
Breastfeeding duration (months), mean (SD)	4.7 (3.9)
Introduction of solid foods, n (%)	
<3 months	41 (5.5)
3 to 6 months	569 (76.6)
>6 months	133 (17.9)
TV watching time, n (%)	
< 2 hours/day	668 (91.3)
≥ 2 hours/day	64 (8.7)
Body mass index at 6 years (kg/m^2), mean (SD)	15.9 (1.4)
Overweight and obesity at 6 years (IOTF criteria), n (%)	87 (10.8)

[1]Values are observed data and represent means (SD), medians (95% range) or numbers of subjects (valid %). IOTF, International Obesity Task Force; SD, standard deviation.

Table 2. Body fat at 1.5 and 24 months by skinfold thicknesses and cardiovascular risk factors at 6 years old[1]

	Total group (n = 808)
Fat mass measures at 1.5 months	**n = 731**
Age (months), mean (SD)	1.6 (0.4)
Body mass index (kg/m^2), mean (SD)	15.2 (1.4)
Total subcutaneous fat mass (mm), mean (SD)	23.9 (7.0)
Central-to-total subcutaneous fat mass ratio, mean (SD)	0.50 (0.05)
Fat mass measures at 24 months	**n = 735**
Age (months), mean (SD)	25.2 (1.1)
Body mass index (kg/m^2), mean (SD)	16.0 (1.3)
Total subcutaneous fat mass (mm), mean (SD)	27.4 (7.5)
Central-to-total subcutaneous fat mass ratio, mean (SD)	0.43 (0.06)
Cardiovascular risk factors at 6 years	**n = 808**
Age (years), mean (SD)	6.0 (0.3)
Systolic blood pressure (mm/Hg), mean (SD)	102.4 (7.9)
Diastolic blood pressure (mm/Hg), mean (SD)	60.2 (6.4)
Total-cholesterol (mmol/l), mean (SD)	4.2 (0.6)
HDL-cholesterol (mmol/l), mean (SD)	1.3 (0.3)
LDL-cholesterol (mmol/l), mean (SD)	2.3 (0.6)
Triglycerides (mmol/l), median (95% range)	1.0 (0.4-2.2)
Insulin (U/I), median (95% range)	116 (18-384)

[1]Values are expressed as means (SD) or medians (95% range). Body mass index = weight/height^2. Total subcutaneous fat mass = biceps + triceps + suprailiacal + subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal + subscapular skinfold thicknesses)/total subcutaneous fat mass. HDL-cholesterol, high-density lipoprotein-cholesterol; LDL-cholesterol, low-density lipoprotein-cholesterol; SD, standard deviation.

Table 3. Associations of infant subcutaneous fat mass measures with cardiovascular risk factors at 6 years old[1-2]

Fat mass measures	Cardiovascular risk factors at 6 years in standard-deviation scores Difference (95% Confidence Interval)						
	Systolic blood pressure	Diastolic blood pressure	Total-cholesterol	HDL-cholesterol	LDL-cholesterol	Triglycerides	Insulin
1.5 months							
Body mass index							
Model 1	0.08 (-0.01,0.16)	0.04 (-0.04,0.12)	0.05 (-0.05,0.16)	0.04 (-0.06,0.14)	0.02 (-0.09,0.12)	0.03 (-0.07,0.13)	0.03 (-0.07,0.14)
Model 2	0.08 (0.01,0.16)*	0.04 (-0.04,0.12)	0.06 (-0.05,0.16)	0.04 (-0.06,0.14)	0.02 (-0.08,0.12)	0.03 (-0.07,0.13)	0.04 (-0.06,0.14)
Total subcutaneous fat mass							
Model 1	0.06 (-0.02,0.14)	-0.01 (-0.08,0.07)	-0.03 (-0.13,0.07)	0.05 (-0.05,0.15)	-0.10 (-0.20,-0.01)*	-0.03 (-0.13,0.06)	0.06 (-0.04,0.16)
Model 2	0.05 (-0.03,0.13)	-0.01 (-0.09,0.07)	-0.04 (-0.14,0.06)	0.05 (-0.05,0.15)	-0.11 (-0.21,-0.01)*	-0.04 (-0.14,0.06)	0.04 (-0.06,0.14)
Central-to-total subcutaneous fat mass ratio							
Model 1	-0.02 (-0.09,0.06)	-0.04 (-0.12,0.04)	0.06 (-0.04,0.15)	0.03 (-0.06,0.13)	0.02 (-0.07,0.12)	0.08 (-0.01,0.17)	0.03 (-0.07,0.12)
Model 2	-0.02 (-0.10,0.05)	-0.04 (-0.12,0.04)	0.05 (-0.04,0.14)	0.04 (-0.06,0.13)	0.02 (-0.07,0.11)	0.08 (-0.01,0.17)	0.02 (-0.07,0.11)
24 months							
Body mass index							
Model 1	-0.01 (-0.09,0.06)	-0.03 (-0.11,0.05)	-0.03 (-0.13,0.06)	-0.09 (-0.19,0.01)	0.01 (-0.09,0.10)	0.01 (-0.08,0.11)	-0.05 (-0.14,0.05)
Model 2	0.00 (-0.08,0.07)	-0.03 (-0.11,0.05)	-0.03 (-0.12,0.07)	-0.09 (-0.19,0.01)	0.01 (-0.08,0.10)	0.02 (-0.08,0.11)	-0.04 (-0.13,0.06)
Total subcutaneous fat mass							
Model 1	-0.01 (-0.09,0.06)	0.01 (-0.07,0.09)	0.15 (0.05,0.24)**	0.02 (-0.07,0.11)	0.14 (0.04,0.23)**	0.03 (-0.07,0.12)	0.02 (-0.08,0.12)
Model 2	-0.07 (-0.14,0.01)	-0.01 (-0.09,0.07)	0.13 (0.03,0.23)**	0.03 (-0.07,0.12)	0.12 (0.02,0.21)*	0.01 (-0.09,0.11)	-0.04 (-0.14,0.05)
Central-to-total subcutaneous fat mass ratio							
Model 1	0.02 (-0.06,0.10)	0.02 (-0.06,0.10)	0.05 (-0.05,0.15)	-0.08 (-0.18,0.01)	0.09 (-0.01,0.19)	0.03 (-0.07,0.13)	-0.02 (-0.12,0.08)
Model 2	0.01 (-0.07,0.08)	0.02 (-0.06,0.09)	0.04 (-0.07,0.14)	-0.08 (-0.18,0.02)	0.08 (-0.02,0.18)	0.02 (-0.08,0.12)	-0.05 (-0.16,0.05)
Change from 1.5 to 24 months							
Body mass index							
Model 1	-0.06 (-0.13,0.01)	-0.05 (-0.12,0.01)	-0.05 (-0.14,0.03)	-0.06 (-0.15,0.02)	-0.02 (-0.10,0.07)	-0.03 (-0.11,0.06)	-0.03 (-0.12,0.06)
Model 2	-0.07 (-0.14,-0.01)*	-0.06 (-0.12,0.01)	-0.06 (-0.14,0.03)	-0.06 (-0.15,0.02)	-0.02 (-0.10,0.07)	-0.03 (-0.11,0.06)	-0.04 (-0.12,0.05)
Total subcutaneous fat mass							
Model 1	-0.02 (-0.08,0.03)	0.01 (-0.05,0.07)	0.10 (0.03,0.18)**	-0.01 (-0.08,0.06)	0.11 (0.04,0.19)**	0.03 (-0.04,0.11)	0.00 (-0.08,0.08)
Model 2	-0.05 (-0.11,0.01)	0.01 (-0.05,0.07)	0.10 (0.02,0.17)*	-0.01 (-0.08,0.07)	0.11 (0.03,0.18)**	0.02 (-0.05,0.10)	-0.03 (-0.10,0.05)
Central-to-total subcutaneous fat mass ratio							
Model 1	0.01 (-0.04,0.07)	0.03 (-0.03,0.09)	-0.05 (-0.12,0.03)	-0.06 (-0.14,0.01)	0.02 (-0.06,0.09)	-0.05 (-0.12,0.03)	-0.01 (-0.09,0.07)
Model 2	0.01 (-0.05,0.07)	0.03 (-0.03,0.09)	-0.05 (-0.13,0.02)	-0.06 (-0.14,0.01)	0.01 (-0.07,0.09)	-0.05 (-0.13,0.02)	-0.03 (-0.10,0.05)

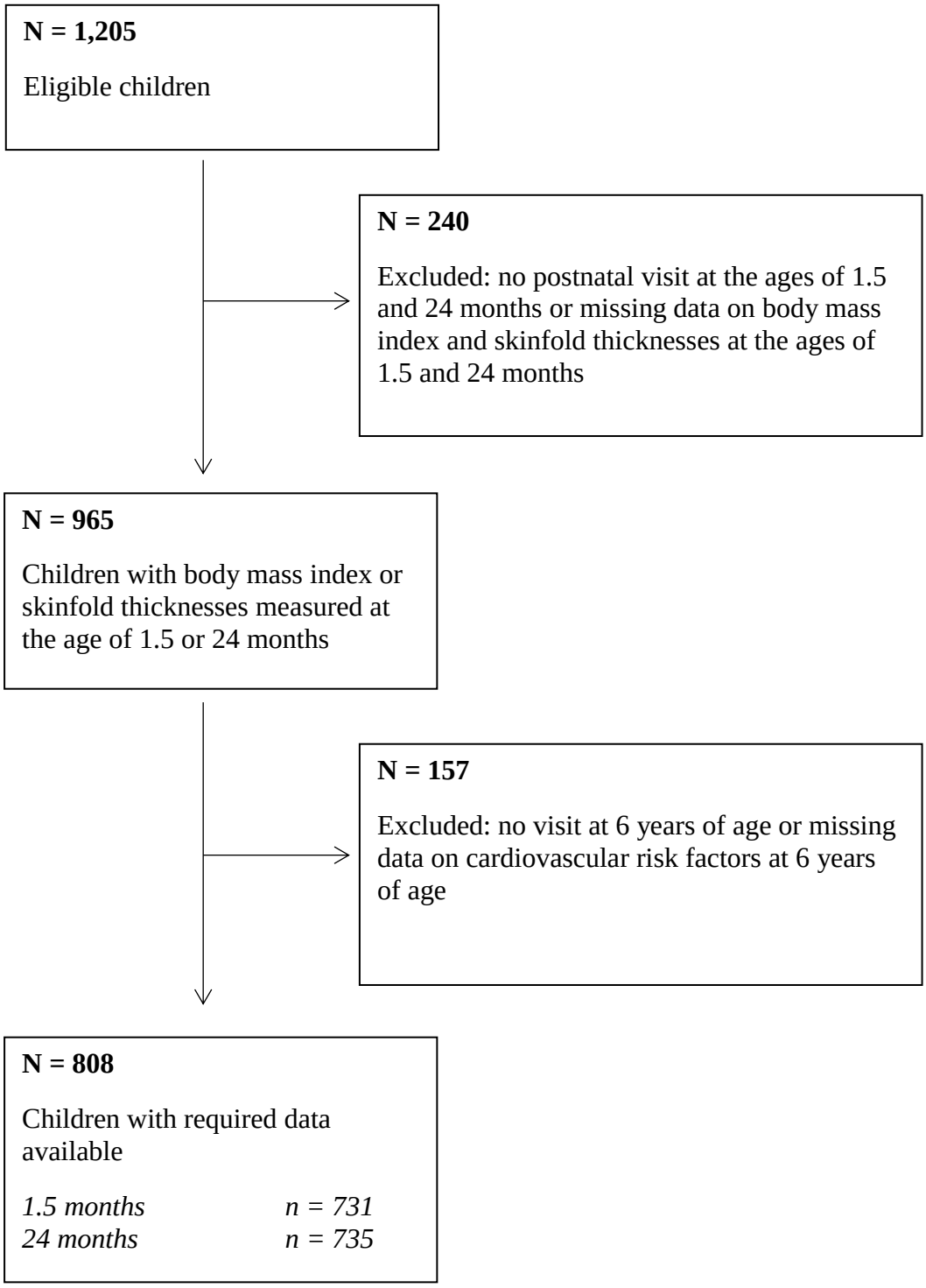
[1]Values are standardized regression coefficients (95% confidence interval) and represent the difference in standard-deviation scores for cardiovascular risk factors at 6 years per 1-standard-deviation score increase in body mass index and subcutaneous fat mass measures. Body mass index = weight/height^2. Total subcutaneous fat mass = biceps + triceps + suprailiacal + subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal + subscapular skinfold thicknesses)/total subcutaneous fat mass. HDL-cholesterol, high-density lipoprotein-cholesterol; LDL-cholesterol, low-density lipoprotein-cholesterol.

[2]Model 1 is adjusted for maternal age, educational level, parity, pre-pregnancy body mass index, maternal total energy intake, smoking habits and total weight gain during pregnancy, gestational diabetes, gestational hypertensive disorders and child's sex, gestational age-adjusted birth weight standard-deviation scores, breastfeeding duration, timing of introduction of solid foods, and TV watching time. Model 2 is additionally adjusted for body mass index standard-deviation scores at 6 years. Conditional analysis was used in body mass index models to allow the adjustment for body mass index at 6 years.

*P-value<0.05; **P-value<0.01.

Supplementary materials

Figure S1. Flow chart of participants in study



Supplemental Methods

Log-log regression analyses

The relationships between total subcutaneous fat mass and length or height, and between central subcutaneous fat mass and total subcutaneous fat mass were assessed using log-log regression analyses. Total and central subcutaneous fat mass measures as well as length or height were all log-transformed. Log-total subcutaneous fat mass was regressed on log-length or height. The regression slope corresponds to the power P by which length or height should be raised in order to calculate an index uncorrelated with length or height (total subcutaneous fat mass/length or height^{P}). A similar calculation was undertaken for log-central and -total subcutaneous fat mass.[1]

Conditional analysis

We performed conditional analysis to enable inclusion of body mass index measures at different ages in the same linear regression model, without problems regarding the correlations between the measures. First, we calculated the expected body mass index at the age of 6 years based on body mass index at 1.5 or 24 months by performing a linear regression model of body mass index at 1.5 or 24 months regressed on body mass index at 6 years. The standardized residuals obtained from these regression models correspond to the difference between the expected and the actual body mass index at 6 years and thus are entirely uncorrelated with body mass index at 1.5 or 24 months. Then, we added the standardized residuals to the models in order to assess the associations of infant body mass index with childhood cardiovascular risk factors, independently of body mass index at 6 years.[2]

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Table S1. Comparison of maternal and child's characteristics between children included and not included in the analyses[1]

	Participants (n = 808)	Non-participants (n = 157)	P-value
Maternal characteristics			
Age (years), mean (SD)	32.0 (3.8)	30.9 (4.9)	0.008
Highest completed education, n (%)			
Primary school	10 (1.2)	8 (5.2)	<0.001
Secondary school	261 (32.5)	62 (40.0)	
Higher education	533 (66.3)	85 (54.8)	
Parity, n (%) nulliparous	512 (63.4)	84 (53.5)	0.020
Pre-pregnancy body mass index (kg/m^2), mean (SD)	23.6 (4.2)	22.8 (3.4)	0.040
Total energy intake (kcal), mean (SD)	2131 (499)	2091 (533)	0.391
Total weight gain during pregnancy (kg), mean (SD)	10.2 (4.6)	10.4 (4.7)	0.477
Smoking habits during pregnancy, n (%)			
No	575 (78.7)	100 (67.6)	0.004
Yes	156 (21.3)	48 (32.4)	
Gestational diabetes, n (%)	9 (1.1)	2 (0.5)	0.318
Gestational hypertensive disorders, n (%)	64 (8.1)	19 (5.0)	0.056
Child's characteristics			
Boys, n (%)	405 (50.1)	91 (58.0)	0.072
Birth weight (g), mean (SD)	3535 (517)	3403 (624)	0.014
Gestational age at birth (weeks), median (95% range)	40.3 (36.4-42.4)	39.9 (34.8-42.4)	0.003
Breastfeeding duration (months), mean (SD)	4.7 (3.9)	3.3 (3.5)	<0.001
Introduction of solid foods, n (%)			
<3 months	41 (5.5)	7 (6.1)	0.417
3 to 6 months	569 (76.6)	81 (71.1)	
>6 months	133 (17.9)	26 (22.8)	
TV watching time, n (%)			
< 2 hours/day	668 (91.3)	64 (92.8)	0.672
≥ 2 hours/day	64 (8.7)	5 (7.2)	
1.5 months			
Body mass index (kg/m^2), mean (SD)	15.2 (1.4)	15.2 (1.3)	0.989
Total subcutaneous fat mass (mm), mean (SD)	23.9 (7.0)	24.9 (9.1)	0.235
Central-to-total subcutaneous fat mass ratio, mean (SD)	0.50 (0.05)	0.50 (0.04)	0.297
24 months			
Body mass index (kg/m^2), mean (SD)	16.0 (1.3)	15.9 (1.2)	0.504
Total subcutaneous fat mass (mm), mean (SD)	27.4 (7.5)	26.8 (6.0)	0.488
Central-to-total subcutaneous fat mass ratio, mean (SD)	0.43 (0.06)	0.44 (0.07)	0.123

[1]Values are observed data and represent means (SD), medians (95% range) or numbers of subjects (valid %). Differences were tested using Student's t-tests and Mann-Whitney tests for normally and non-normally distributed variables, respectively and χ^2-test for dichotomous variables. SD, standard deviation.

Table S2. Associations of infant subcutaneous fat mass measures with cardiovascular risk factors at 6 years old[1-2]

Fat mass measures	Cardiovascular risk factors at 6 years in standard-deviation scores Difference (95% Confidence Interval)						
	Systolic blood pressure	Diastolic blood pressure	Total-cholesterol	HDL-cholesterol	LDL-cholesterol	Triglycerides	Insulin
1.5 months							
Body mass index	0.03 (-0.04,0.11)	-0.01 (-0.08,0.06)	0.02 (-0.07, 0.11)	0.03 (-0.06,0.12)	-0.01 (-0.10,0.08)	0.01 (-0.08,0.10)	-0.01 (-0.10,0.08)
Total subcutaneous fat mass	0.03 (-0.04,0.11)	-0.02 (-0.10,0.05)	-0.05 (-0.14,0.05)	0.02 (-0.08,0.11)	-0.10 (-0.19,-0.01)*	-0.02 (-0.11,0.08)	0.05 (-0.04,0.15)
Central-to-total subcutaneous fat mass ratio	-0.01 (-0.09,0.06)	-0.03 (-0.10,0.05)	0.06 (-0.03,0.15)	0.02 (-0.06,0.12)	0.02 (-0.06,0.12)	0.09 (-0.01,0.18)	0.02 (-0.07,0.11)
24 months							
Body mass index	-0.03 (-0.10,0.05)	-0.06 (-0.14,0.02)	-0.05 (-0.15,0.04)	-0.10 (-0.19,-0.01)*	0.00 (-0.10,0.09)	-0.01 (-0.10,0.09)	-0.06 (-0.15,0.04)
Total subcutaneous fat mass	0.01 (-0.07,0.08)	0.02 (-0.06,0.09)	0.14 (0.05,0.24)**	0.01 (-0.08,0.10)	0.13 (0.04,0.22)**	0.03 (-0.06,0.12)	0.03 (-0.06,0.13)
Central-to-total subcutaneous fat mass ratio	0.04 (-0.04,0.11)	0.04 (-0.04,0.11)	0.05 (-0.04,0.15)	-0.10 (-0.19,-0.01)*	0.10 (0.01,0.20)*	0.04 (-0.06,0.14)	-0.01 (-0.11,0.09)
Change from 1.5 to 24 months							
Body mass index	-0.05 (-0.11,0.02)	-0.04 (-0.11,0.02)	-0.05 (-0.13,0.03)	-0.06 (-0.14,0.02)	-0.01 (-0.10,0.07)	-0.02 (-0.10,0.06)	-0.01 (-0.10,0.07)
Total subcutaneous fat mass	-0.01 (-0.06,0.05)	0.02 (-0.04,0.08)	0.10 (0.03,0.17)**	0.00 (-0.07,0.07)	0.10 (0.03,0.18)**	0.03 (-0.04,0.11)	0.01 (-0.06,0.09)
Central-to-total subcutaneous fat mass ratio	0.02 (-0.04,0.08)	0.04 (-0.02,0.10)	-0.04 (-0.12,0.03)	-0.06 (-0.13,0.01)	0.02 (-0.06,0.10)	-0.04 (-0.12,0.03)	-0.01 (-0.09,0.06)

[1]Values are standardized regression coefficients (95% confidence interval) and represent the difference in standard-deviation scores for cardiovascular risk factors at 6 years per 1-standard-deviation scores increase in body mass index and subcutaneous fat mass measures. Body mass index = weight/height^2. Total subcutaneous fat mass = biceps + triceps + suprailiacal + subscapular skinfold thicknesses. Central-to-total subcutaneous fat mass ratio = (suprailiacal + subscapular skinfold thicknesses)/total subcutaneous fat mass. HDL-cholesterol, high-density lipoprotein-cholesterol; LDL-cholesterol, low-density lipoprotein-cholesterol.
[2]Unadjusted model.
*P-value<0.05; **P-value<0.01.

CHAPTER 4

General discussion and conclusions

Introduction

Obesity is a major public health problem in adulthood,[1] and leads to increased risks of cardiometabolic diseases, musculoskeletal, respiratory and psychological disorders, and cancers.[2,3] Likewise, obesity is reaching alarming levels in women of reproductive age, infants and children,[4,5] which also has major public health implications.

Obesity at the start of pregnancy and excessive gestational weight gain may have short- and long-term consequences for both mother and child, such as an increased risk of pregnancy complications and childhood obesity.[6-13] A maternal obesogenic environment during pregnancy leads to fetal over-nutrition that might cause permanent changes in the development of adipocytes and in the appetite control system, resulting in larger susceptibility to develop obesity later in life.[14] Infancy, and especially nutrition and growth during this period, also seems to be critical for the development of obesity and cardiometabolic diseases.[15-19] Assessing the influence of maternal pre-pregnancy body mass index, gestational weight gain and infant growth and adiposity on detailed offspring fat measures and cardiovascular risk factors, already from early infancy onwards, is important to obtain a better understanding of the influence of maternal and infant adiposity on health outcomes in later life.

The general aim of this thesis was to clarify the adiposity and cardiovascular health related consequences of maternal and infant adiposity. This chapter provides a general discussion of the main findings of the studies in this thesis, discusses general methodological issues and provides suggestions for future research and implications for clinical practice and policy.

Interpretation of main findings

Maternal adiposity during pregnancy

Pre-pregnancy body mass index

A meta-analysis of observational studies showed that maternal pre-pregnancy overweight and obesity were associated with a 2-fold and a 3-fold higher risk of offspring overweight/obesity, respectively.[10] Similarly, maternal overweight and obesity have been strongly associated with increased risks of overweight and obesity in children aged 4 and 6 years within the Generation R Study cohort.[20,21] Thus far, not much is known about the associations of maternal obesity with offspring adiposity status throughout infancy, which is

a critical period for adiposity development in later life. In line with previous meta-analysis and single studies at older ages, we showed that higher maternal pre-pregnancy body mass index was associated with higher body mass index from 6 months onwards in infants from the Generation R Study. Body fat is mainly located subcutaneously in the first two years of life.[22,23] Thus, skinfold thickness, which is a valid measure to estimate subcutaneous fat mass,[24] might be particularly relevant in infancy. Also, information on total and regional subcutaneous fat mass can be obtained from these measurements, enabling the study of different locations of fat at younger ages. We showed that maternal pre-pregnancy body mass index was positively associated with total subcutaneous fat mass but not with central-to-total subcutaneous fat mass ratio at 24 months. No associations were observed with subcutaneous fat mass measures at 1.5 and 6 months. Thus, results from this thesis showed that maternal pre-pregnancy obesity seems to influence total body fat mass development already from early infancy onwards.

As compared to total or subcutaneous fat, excess visceral, heart and liver fat has been described as better indicators of cardiometabolic risk.[25-27] In 105 healthy mother-newborn pairs, higher maternal body mass index was associated with higher infant abdominal fat, independently of weight, and higher intrahepatocellular lipid content.[28] In another study among 25 newborns, infants born to obese mothers with gestational diabetes had higher intrahepatocellular fat compared with infants born to normal weight mothers.[29] Maternal pre-pregnancy obesity was also associated with higher visceral fat mass levels in 1,228 Greek children aged 9-13 years.[30] These studies have important limitations, such as their small sample size and the lack of properly control for confounding by general adiposity in these associations. The fat that is accumulated in a specific location is influenced by the general adiposity status. Thus, to assess the risk factors for accumulation in specific depots, it is important to control for the effect of general adiposity. A previous study in 6-year-old children from the Generation R Study reported that maternal pre-pregnancy body mass index was not associated with subcutaneous and preperitoneal abdominal fat measured by ultrasound, independently of child´s body mass index.[21] Contrarily, we showed that higher maternal body mass index was associated with higher subcutaneous, visceral, pericardial and liver fat assessed by magnetic resonance imaging in 10-year-old children from the Generation R Study. The associations of maternal body mass index with offspring subcutaneous and visceral fat seemed to be independent of offspring body mass index, suggesting that higher maternal body mass index is associated with a specific accumulation of fat in abdominal depots that is not a result of general adiposity. The different results reported in the studies

using data from 6- and 10-year-old children from the Generation R Study might be related to the use of different methods for measuring subcutaneous and visceral fat mass. Ultrasound has been shown to be a reliable method to differentiate between the abdominal preperitoneal and subcutaneous fat compartments but showed poorer accuracy and reproducibility as compared to magnetic resonance imaging that has been described as a gold standard technique.[31] Besides this, lower amounts of fat in these specific depots at younger ages might lead to lower variability, and might have, at least partly, contributed to the absence of effect in the sample of 6-year-old children. Results from this thesis showed that higher maternal body mass index is associated with organ fat accumulation, especially abdominal fat, in 10-year-old children.

Gestational weight gain

Excessive gestational weight gain has been associated with an increased risk of childhood overweight and obesity.[11-13] In a study among 948 mothers and their children from the Southampton Women's Survey, higher maternal total gestational weight gain was associated with higher neonatal total fat mass and higher total fat mass at 6 years but not at 4 years.[32] In a study among 1,044 mother-child pairs from Project Viva, higher weight gain during pregnancy was associated with higher sum of subscapular and triceps skinfold thickness.[33] In a retrospective cohort study in Germany of 6,837 mother-child pairs, excessive gestational weight gain was associated with higher risk of abdominal adiposity measured using waist circumference.[34] In 313 mother-child pairs, higher maternal body mass index was associated with higher childhood subcutaneous and visceral fat, particularly among mothers with excessive gestational weight gain.[35] It has been suggested that the associations of maternal weight gain with offspring fat mass outcomes may depend upon the timing of gestational weight gain. A prospective cohort study among 5,154 UK mothers and their children showed that maternal weight gain during early-pregnancy was positively associated with childhood total fat mass.[36] A study among 977 Greek mothers and their children aged 4 years showed that maternal weight gain during early-pregnancy was positively associated with childhood sum of skinfold thickness and waist circumference.[37] In 5,908 mothers and their 6-year-old children from the Generation R Study, maternal weight gain in early-, mid- and late-pregnancy was not associated with childhood subcutaneous and preperitoneal abdominal fat mass levels, independently of body mass index.[38] We showed with results from this thesis that maternal total and period-specific gestational weight gain were not consistently associated with body mass index, total subcutaneous fat mass and central-to-total

subcutaneous fat mass ratio in infants aged 1.5, 6 and 24 months from the Generation R Study. Higher maternal total gestational weight gain seems to be associated with higher body fat quantity assessed by using a body fat pattern combining body mass index, fat mass index and waist-to-height ratio in children aged 7 years from the Generation XXI. However, no associations were observed with body fat distribution assessed by using a body fat pattern combining waist-to-hip, waist-to-thigh and waist-to-weight ratios. Similarly, higher weight gain during pregnancy, especially in early-pregnancy, was associated with higher body mass index but no consistent associations were observed with subcutaneous, visceral, pericardial and liver fat, independently of body mass index in children aged 10 years from the Generation R Study. Results from this thesis showed that the effects of maternal weight gain during pregnancy on fat mass development seem to become more apparent at older offspring ages and seem to be restricted to the general adiposity and not to the accumulation in specific depots. The absence of effect, or less clear effect as compared to maternal pre-pregnancy body mass index, might be explained by the fact that gestational weight gain reflects maternal nutritional status but also other components such as the fetus, amniotic fluid, placenta, uterine and mammary tissue expansion, increased blood volume, and extracellular fluid.[39]

Main findings

- Higher maternal body mass index was associated with higher body mass index and total subcutaneous fat mass from 6 months onwards, but not with central-to-total subcutaneous fat mass ratio in infancy.
- Higher maternal body mass index was associated with higher abdominal, pericardial and liver fat measures at 10 years. The associations with abdominal subcutaneous and visceral fat were independent of body mass index.
- Maternal total and period-specific gestational weight gain were not associated with body mass index and subcutaneous fat mass measures in infancy.
- Maternal gestational weight gain was positively associated with body fat quantity but not with body fat distribution at 7 years.
- Maternal total and period-specific gestational weight gain were not consistently associated with subcutaneous, visceral, pericardial and liver fat measures, independently of body mass index at 10 years.

<u>Underlying mechanisms</u>

It remains unclear whether the associations of maternal adiposity during pregnancy with infant and childhood adiposity are explained by direct intrauterine mechanisms or confounded by environmental, lifestyle or genetic characteristics. To help disentangling the underlying mechanisms, previous studies have compared the strength of associations of maternal and paternal body mass index with infant and childhood adiposity measures. Although studies comparing associations of maternal and paternal body mass index with childhood body mass index have shown conflicting results,[40] other studies have shown that maternal pre-pregnancy body mass index tends to be more strongly associated with childhood total fat mass than paternal body mass index.[21,41] In this thesis, we showed stronger associations for paternal body mass index with infant body mass index at 1.5 months but stronger associations for maternal pre-pregnancy body mass index with infant body mass index and total subcutaneous fat mass from the age of 6 months onwards. These results suggest that intrauterine programming effects may become more apparent at later ages. We also showed a tendency for stronger associations of maternal pre-pregnancy body mass index with general and abdominal fat at 10 years. Even though there is a tendency in this thesis for stronger associations of maternal body mass index with infant and childhood adiposity measures, the confidence intervals of the maternal and paternal effect estimates were not statistically different and thus no clear conclusions can be drawn. In this thesis, we also showed, by using a path analysis, that the positive although small effect of gestational weight gain on child´s body fat quantity seemed to be mainly through intrauterine programming effects instead of indirect effects through birth weight.

The mechanisms by which maternal adiposity during pregnancy might affect offspring´s general, abdominal and organ fat accumulation are not fully known yet. A maternal obesogenic environment during pregnancy leads to higher maternal plasma concentrations of glucose, amino acids and free fatty acids that are transferred to the developing fetus. Fetal over-nutrition has been associated with a permanent increase in the capacity of adipocytes to store lipids.[42] Moreover, intrauterine exposure to a high-fat diet[43,44] and/or to hyperglycemia[45] is thought to induce excessive appetite postnatally due to a dysregulation in the expression of appetite-regulating genes and a reduced sensitivity of central hypothalamic neuropeptides to peripheral satiety signals (specifically leptin and insulin). Maternal over-nutrition might also lead to accumulation of fat in the liver and other developing organs of the fetus, especially during early- and mid-pregnancy due to the absence of adipose tissue.[46] The postnatal persistence of increased fat in these depots might be related to reduced fatty acid oxidation, changes in lipogenesis and lipoprotein export.[46]

Infant adiposity

Infant adiposity and childhood adiposity

Body mass index tends to track from infancy onwards.[47,48] Although total and central fat mass track moderately from childhood into adulthood,[49-55] the stability of these measures already from early infancy onwards is not known. We showed a moderate tracking of body mass index from 1.5 and 24 months to 6 years in children from the Generation R Study. Also, total subcutaneous fat mass at 24 months was positively associated with fat mass index at 6 years, suggesting tracking of total fat mass from infancy into school-age. Higher total subcutaneous fat mass and central-to-total subcutaneous fat mass ratio in infancy were associated with higher central-to-total and android-to-gynoid fat mass ratios measured by dual energy X-ray absorptiometry and preperitoneal fat mass area measured by abdominal ultrasound at 6 years old. We observed stronger effect estimates for infant total subcutaneous fat mass than for central-to-total subcutaneous fat mass ratio, in the associations with similar measures at school-age, although both showed weaker effect estimates compared with the tracking of body mass index from infancy to childhood. Stronger effect estimates were observed among girls than boys which might be explained by a more stable body fat development, driven by less hormonal fluctuations, during infancy in girls.[56] Also, the effect estimates were stronger for 24 months than for 1.5 months fat mass measures, which might be due to the shorter interval between 24 months and 6 years old. Also, 1.5 months might be more reflective of fetal growth patterns which seemed to be less predictive, as compared to postnatal growth, of later abdominal adiposity.[57] Thus, results from this thesis showed that body mass index and subcutaneous fat mass measures in infancy are positively associated with body mass index, total and abdominal fat mass at school-age. Compared with body mass index, subcutaneous fat mass measures in infancy add little value to estimate adiposity in childhood.

Infant adiposity and childhood cardiovascular risk factors

Rapid weight gain in the first 3 months of life has been associated with increased adiposity and an unfavorable cardiovascular profile in later life.[19] However, it remains unknown whether gain in body mass index or ponderal index (weight/height^3) in infancy predisposes individuals to cardiovascular risk. A study among 4,601 UK subjects has shown that changes in ponderal index from 0 to 2 years were not associated with cardiovascular risk factors in

adolescence.[58] Similarly, we observed no associations of body mass index at the ages of 1.5 and 24 months or its change in this period with cardiovascular risk factors at 6 years. Previous studies have also shown that high total and central subcutaneous fat mass measures, estimated from skinfold thicknesses, are associated with an adverse cardiovascular risk profile in childhood.[59-65] Currently, it is not known whether total and central subcutaneous fat mass measures in infancy are associated with cardiovascular risk factors in later life. We observed no associations of infant subcutaneous fat mass measures with childhood blood pressure and triglycerides or insulin levels in children from the Generation R Study. At 1.5 months, total subcutaneous fat mass was inversely associated with low-density lipoprotein (LDL)-cholesterol at 6 years old. Residual confounding by children´s dietary intake and physical activity might have contributed to this finding, since we had only available information about breastfeeding, age at introduction of solid foods and TV watching time at 6 years. At 24 months, total subcutaneous fat mass, but not central-to-total subcutaneous fat mass ratio, was positively but weakly associated with childhood total- and LDL-cholesterol, independently of body mass index at 6 years old. Results from this thesis suggest that body mass index and subcutaneous fat mass measures in infancy seem to be poor indicators of cardiovascular risk profile in later childhood. These findings may be partially explained by the fact that fat mass measures in infancy represent the accumulation of fat mass over a short period of time, which may not be long enough to influence cardiovascular risk profile.

Main findings

- Infant body mass index and subcutaneous fat mass measures were positively associated with body mass index, total and abdominal fat mass at school-age.
- Infant body mass index and subcutaneous fat mass measures were not associated with childhood blood pressure and triglycerides or insulin levels. Total subcutaneous fat mass was weakly associated with cholesterol levels at 6 years, independently of body mass index.

Methodological considerations

Strengths and limitations for each study are described in **Chapter 2** and **Chapter 3** of this thesis. In the following paragraphs, general methodological considerations regarding selection bias, information bias, confounding and causality are discussed.

Selection bias

Selection bias is a bias in the estimated association or effect of an exposure on an outcome that arises from the procedures used to select individuals into the study or the analysis.[66] It is present when the association between exposure and outcome in study participants is different as compared to that in eligible non-participants. Selection bias may occur due to selective non-response at baseline or selective loss to follow-up.

Generation XXI

Of the invited mothers, 91% accepted to participate. When compared to the mothers from the catchment area, delivering in 2005 and 2006, cohort participants were slightly younger but similar in marital status. The prevalence of caesarean section was slightly higher than the prevalence in Portugal in 2004. The cohort had a similar gender proportion but higher proportion of multiple fetuses as compared to the national percentages. When compared to the Portuguese deliveries in 2005 and 2006, the cohort presented a higher proportion of preterm and low birth weight newborns, probably because all the maternities enrolled had highly differentiated perinatal support.

In this thesis, we used data from the 7-year-old re-evaluation, in which 80% of the entire cohort participated. Mothers participating in follow-up studies were slightly older and higher educated, although the magnitude of the differences was small.

Generation R Study

The participation rate at birth was 61%. As compared to the general population in Rotterdam, women from the Generation R Study were less likely to be from ethnic minority groups and of lower socioeconomic status. Also, participating women were less likely to develop gestational hypertensive disorders and have children born preterm or with low birth weight.

In this thesis, we used data from the subgroup of Dutch mothers and their children participating in additional detailed assessments of growth and development from late pregnancy onwards, in which the loss to follow-up at 1.5, 6 and 24 months and 6 years old was low. We also used data from the follow-up at the age of 10 years, in which 76% of the original cohort participated in the study. A lower percentage of children participated in the magnetic resonance imaging measurements of abdominal and organ fat at 10 years, which was mainly due to non-consent. Compared to the baseline characteristics, mothers who did visit the research center in the follow-up evaluations were older, more frequently of Dutch nationality and higher educated.[67]

Generation XXI might have included mothers that were more likely to have children born preterm or with low birth weight. It is difficult to speculate whether this could have biased our effect estimates. On the other hand, the selective non-response at baseline in the Generation R Study suggests a selection towards a more affluent and healthy population, which may have led to lower prevalence rates of maternal, infant and childhood adiposity and cardiovascular risk factors, and subsequently reduced statistical power. Also, it may affect the generalizability of our findings to less healthy and affluent populations. However, several studies have shown that in cohort studies associations are not strongly influenced by selective non-participation at baseline, and therefore it seems unlikely that the results of this thesis are biased due to the selection procedures.[68,69]

Similarly, the selective loss to follow-up in both cohorts suggests a selection towards a healthier population. Although difficult to speculate if this might have biased our effect estimates, this seems unlikely since participants did not differ from non-participants regarding the exposures of interest, such as maternal pre-pregnancy body mass index.

Information bias

Information bias is a bias in an estimate arising from measurement errors or misclassification.[66] In this thesis, we relied on anthropometric measurements, such as body mass index, indices based on waist circumference and skinfold thicknesses, which might have greater measurement error and be less accurate, but on the other hand be easier and cheaper to obtain in large epidemiological studies as compared to imaging techniques of body composition.[24,70,71] High accuracy and reproducibility have been reported for dual energy X-ray absorptiometry, abdominal ultrasound and magnetic resonance imaging that were also used in this thesis.[24,31,72-74]

Misclassification can be non-differential or differential. Non-differential misclassification occurs when the determinant status is not related to the outcome status, and vice-versa and generally leads to an underestimation of the effect estimates. Differential misclassification occurs when the determinant status is related to the outcome status, and vice-versa and generally leads to biased results, which can be either overestimated or underestimated. In this thesis, differential misclassification is unlikely. Exposure data used in our studies were collected longitudinally and before assessment of the outcomes, the data collectors were blinded to the exposure status when assessing the outcomes and parents as well as data collectors were unaware of the specific research questions under study. However,

non-differential misclassification might have occurred. In the studies included in this thesis, information on maternal pre-pregnancy weight and maximum gestational weight was self-reported. Women tend to underestimate their weight on self-report,[75] which might have led to an underestimation of observed effects for maternal pre-pregnancy body mass index. Since pre-pregnancy weight and maximum gestational weight are both self-reported and probably underestimated, the influence on maximum weight gain is likely to be minimal. The attendance of prenatal visits could also have made pregnant women more aware of their weight status, minimizing the error of self-reported final pre-delivery weight. The bias in the results for maternal pre-pregnancy body mass index and maximum gestational weight gain seems also unlikely since similar results were observed when using maternal weight measured at enrolment and weight gain measured until late-pregnancy. The use of thirty-minutes fasting blood samples may have resulted in misclassification and thus may have led to underestimation of the observed associations. However, it has been shown in adults that non-fasting blood lipid levels can accurately predict increased risks of cardiovascular events in later life.[76]

Confounding

Confounding occurs when all or part of the effect between an exposure and an outcome is in fact explained by other variables that affect the outcome but are not themselves affected by the exposure.[66] To take account for confounding, we adjusted all analyses for multiple potential confounders. We selected covariates based on their associations with the exposures and outcomes of interest in our or previous studies or a change in effect estimate of more than 10%. In this thesis, adjustment for potential confounders only moderately affected the effect estimates, which suggests that the observed associations are possibly true associations between the exposures and the outcomes. Although information about many potential confounders was available in the studies included in this thesis, residual confounding might still be present. Also, information about several confounders was self-reported and misclassification might have occurred, contributing to residual confounding and an overestimation of the observed effect estimates. To help understanding whether the associations of maternal obesity with infant and childhood adiposity are explained by direct intrauterine mechanisms or confounded by environmental, lifestyle or genetic characteristics, we compared the associations of both maternal and paternal body mass index. A similar effect size for the maternal and paternal association would suggest that the association of maternal obesity with infant and childhood adiposity is explained by unmeasured

environmental factors, rather than direct intrauterine mechanisms. A limitation of this approach is the assumption that both parents contribute equally to the shared lifestyle-related characteristics between parents and their offspring. We also performed a comprehensive path analysis to help understanding whether the effects of prenatal exposures (gestational weight gain, diabetes and smoking) on child´s body fat are mainly explained by a direct (through intrauterine programming) or indirect pathway (through birth weight). Although we included the most important paths described to date, and tested several covariates as confounders, we cannot exclude the possibility of existing other paths not considered in this thesis and also residual confounding by lifestyles or genetics that might have biased our estimates for the intrauterine programming effects.

Causality

The causality of the associations observed in this thesis remains to be established. The Bradford Hill criteria, that can be useful for providing evidence of a causal relationship between an exposure and an outcome, include strength, consistency, specificity, temporality, dose-response relationship, biological plausibility, coherence, experiment and analogy.[66] Overall, our associations may be relatively small. Although stronger associations are more likely to be causal, weak relationships may also be causal. The main findings of this thesis were consistent with previous studies, our studies were longitudinal supporting the temporality between exposures and outcomes, several potential biological mechanisms for these associations have been described, and our findings were coherent with animal studies. Although not explored in all studies presented in this thesis, we did observe some dose-response effects between maternal body mass index and general and organ fat at 10 years. The specificity, experiment and analogy criteria were not addressed in this thesis. Although this thesis was not designed to fully clarify the causality of these associations, our observational studies seem to provide some evidence for causal relationships based on the Bradford Hill criteria.

Future research

Although findings from this thesis suggest that maternal adiposity during pregnancy and infant adiposity adversely affect infant and childhood adiposity and cardiovascular health, the following major issues remain to be addressed in future studies.

Causality

We performed an extensive adjustment for potential confounding factors, used parental body mass index comparison analyses and path analyses and found some evidence for causality in our studies based on the Bradford Hill criteria. However, the causality of the associations for maternal adiposity during pregnancy observed in this thesis remains unclear. Randomized controlled trials, sibling comparison studies, and Mendelian randomization studies can be used to obtain further insight into the causality of the associations.

Randomized controlled trials are the gold standard study design to establish causality. Previous randomized controlled trials focused on controlling obesity in pregnant women or reducing the risk of excessive gestational weight gain by improving diet and physical activity showed small reductions in maternal pre-pregnancy weight and gestational weight gain.[77-79] The effect of these interventions on infant and childhood outcomes remains to be explored. A small randomized controlled trial that provided dietary advice and exercise to obese pregnant women showed no differences in their infant body mass index or metabolic risk factors.[80] Long-term follow-up of participants in these randomized controlled trials might provide a unique opportunity to examine whether maternal obesity and excessive gestational weight gain are causally related to childhood adiposity status and cardiovascular risk. It will also provide insight into the effectiveness of these interventions for improving health outcomes in the offspring. Many randomized controlled trials have started their interventions from the second trimester onwards.[79,81] Considering the findings of this thesis that suggest that promoting a healthy maternal pre-pregnancy body mass index might be of greater importance than influencing gestational weight gain, randomized controlled trials focused on the preconception or early pregnancy period are needed.

Sibling comparison studies allow control for environmental characteristics as well as maternal genotype that are shared among siblings. However, besides major exposures of interest, other lifestyle-related characteristics may also differ between siblings, limiting the potential of this study design to assess causality.[82] Mendelian randomization studies use genetic variants, known to be robustly associated with the exposure of interest and not affected by confounding, to examine whether the exposure is causally related to the outcome.[83] Mendelian randomization studies with large sample sizes to have adequate statistical power, using multiple maternal genetic variants as instruments and focused on childhood outcomes are needed.

Assessment of exposures and outcomes

In this thesis, information on self-reported maternal pre-pregnancy body mass index, measured early-, mid-, and late-pregnancy weight and self-reported maximum gestational weight was available. Studies with measured pre-pregnancy weight and maximum gestational weight might reduce the risk for misclassification and bias. Studies with serial repeated maternal weight measurements during pregnancy are also needed to allow a more thorough identification of critical time points for adiposity and cardiovascular development. Gestational weight gain reflects multiple components, which might complicate the interpretation of the associations. Studies disentangling the different components of gestational weight gain might provide further insight into the underlying mechanisms. Infant and childhood outcomes studied in this thesis included total, abdominal and organ fat, blood pressure, lipids and insulin levels. We used magnetic resonance imaging that is a gold standard technique for measuring intra-abdominal and ectopic fat deposition.[31,72-74] However, we only have magnetic resonance imaging measurements available at 10 years. Future studies should measure these body fat compartments, which have important adverse metabolic consequences, at younger ages to obtain further insight into its development and related risk factors and health consequences. Additional measurements of childhood cardiovascular development might also provide further insight into the underlying mechanisms linking early life exposures to cardiovascular events in later life. Measures of endothelial function, intima media thickness, insulin/glucose metabolism and coronary circulation imaging in children might be of interest to gain more knowledge about the cardiovascular development. To obtain further insight into the underlying mechanisms for the associations addressed in this thesis, large population-based studies focused on epigenetics and metabolomics are needed. Further research needs to explore whether and which critical periods are involved in these associations.

Implications for clinical practice and policy

Maternal adiposity during pregnancy and infant adiposity might influence the adiposity status and cardiovascular profile in later life. The observed effect estimates were small to moderate, but are of interest from a developmental perspective since obesity and cardiovascular risk factors tend to track into adulthood. Also, even subclinical differences in cardiovascular risk factors during childhood are related to the development of cardiovascular disease in later life. Thus, prevention of maternal adiposity during pregnancy and infant adiposity is important to reduce the burden of obesity and cardiovascular disease throughout the life course and in future generations.

Preventive strategies or interventions focused on the preconception period or during pregnancy should be implemented. Women might be more motivated to make lifestyle changes in preconception period and pregnancy and might also keep these new lifestyle habits after pregnancy, highlighting the potential for health gains. In particular, based on our findings, promoting a healthy body mass index in women when entering pregnancy might be of greater importance than influencing gestational weight gain. So, it is essential to promote knowledge about the importance of a healthy weight in women of reproductive age. General practitioners, gynecologists, and other health professionals should inform and advise young women. Also, specific health care programs aiming to encourage healthy eating and physical activity for overweight or obese mothers-to-be should be conducted.

Information about the benefits of an adequate body composition during infancy should also be provided to families together with proper recommendations for breastfeeding, introduction of solid foods and other environmental and behavioral factors that might impact the health of the child later in life. Since the education sector plays a critical role in providing nutrition and health education, increasing the opportunities for physical activity and promoting healthy school environments is of utmost importance in preventing obesity in childhood.[5]

Conclusion

Findings from this thesis suggest that maternal adiposity during pregnancy and infant adiposity are associated with infant and childhood adiposity and cardiovascular health outcomes. The observed associations are relatively small, but may be important for the burden of obesity and cardiovascular disease on a population level.

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CHAPTER 5

Summary

Resumo

Samenvatting

Summary

Chapter 1 provides the background, hypothesis, aims and design for this thesis. Obesity is increasing in prevalence worldwide and has large clinical implications in childhood and adulthood. An accumulating body of evidence suggests that obesity and cardiometabolic diseases in childhood and adulthood at least partly originate in fetal or early postnatal life. Adverse exposures, acting during fetal and early postnatal development, lead to permanent adaptations in the structure, physiology and function of various organ systems. In particular, maternal adiposity during pregnancy might lead to a higher risk of obesity in the offspring through intrauterine programming mechanisms. A maternal obesogenic environment during pregnancy leads to fetal over-nutrition and might cause permanent changes in the development of adipocytes, and in the appetite control system. Infant growth patterns, such as rapid postnatal weight gain, seem also to be critical for the development of obesity and cardiovascular diseases in later life. Assessing the influence of maternal adiposity during pregnancy and infant growth and adiposity on detailed offspring fat measures and cardiovascular risk factors is important to obtain a better understanding of the influence of mother´s and infant´s health on health outcomes in later life. Therefore, the general aim of this thesis was to assess the adiposity and cardiovascular health related consequences of maternal and infant adiposity. The studies presented in this thesis used data from the Generation XXI, a population-based birth cohort in Porto, Portugal and the Generation R Study, a population-based cohort from fetal life onwards in Rotterdam, the Netherlands.

In **Chapter 2**, studies on the influence of maternal adiposity during pregnancy on infant and childhood outcomes are described. In **Chapter 2.1,** we reviewed the childhood health consequences of maternal obesity during pregnancy. Evidence from observational studies strongly suggests that maternal pre-pregnancy obesity and excessive gestational weight gain are associated with increased risks of fetal pregnancy complications and adverse childhood cardiometabolic, respiratory and cognitive-related health outcomes. It remains unclear whether these associations are due to intrauterine mechanisms or explained by confounding. The underlying epigenetic mechanisms have been assessed in animal studies and small human studies, but have not been tested yet in large human studies. In **Chapter 2.2**, we studied the influence of maternal body mass index, and gestational weight gain on body mass index and subcutaneous fat mass measures at the ages of 1.5, 6 and 24 months. We observed that higher maternal pre-pregnancy body mass index was associated with higher body mass index and total subcutaneous fat mass from 6 months onwards. No associations

were observed for central-to-total subcutaneous fat mass ratio in infancy. Maternal total and period-specific gestational weight gain were not associated with body mass index and subcutaneous fat mass measures at 1.5, 6 and 24 months. Next, we examined the associations of maternal gestational weight gain, diabetes and smoking on total and regional adiposity at the age of 7 years and explored whether these associations were through direct intrauterine mechanisms or through birth weight (**Chapter 2.3**). We observed a positive total effect of maternal gestational weight gain, diabetes and smoking during pregnancy on child´s body fat quantity. Maternal smoking was also associated with a central fat distribution in children. These effects seem to be mainly through intrauterine programming rather than indirect effects through birth weight. In **Chapter 2.4**, we assessed the associations of maternal body mass index, and gestational weight gain with general, abdominal, pericardial and liver fat at the age of 10 years. We observed that higher maternal body mass index was associated with higher body mass index, fat mass index, and subcutaneous, visceral, pericardial and liver fat. The associations with abdominal fat were independent of child´s body mass index. Higher weight gain during pregnancy, especially in early-pregnancy, was associated with higher body mass index but no consistent associations were observed with subcutaneous, visceral, pericardial and liver fat, independently of body mass index. In summary, findings from **Chapter 2** suggest that maternal obesity and excessive gestational weight gain are important modifiable factors during pregnancy that are associated with adverse health outcomes in the offspring.

In **Chapter 3**, studies on the influence of infant adiposity on childhood outcomes are described. In **Chapter 3.1**, we compared the accuracy of body fat patterns and single measures for assessing body fat and clarified the use of indices based on waist circumference as measures of adiposity in children. Pattern 1 (body mass index, fat mass index from tetra-polar bioelectric impedance and waist-to-height ratio) showed stronger correlations with dual energy X-ray absorptiometry (DXA)-fat mass index and pattern 2 (waist-to-hip, waist-to-thigh and waist-to-weight ratios) showed stronger correlations with DXA-central fat. We observed that as compared to single measures, body fat patterns showed similar correlations with DXA-derived measures. Single anthropometric indices seem to be enough to accurately estimate body fat. Waist-to-height ratio might be a proxy for total fat, while waist-to-hip, waist-to-thigh and waist-to-weight ratios might be proxies for central fat. The influence of infant body mass index and subcutaneous fat mass measures on childhood adiposity and cardiovascular risk factors is studied in **Chapter 3.2** and **Chapter 3.3**, respectively. We observed that infant body mass index and subcutaneous fat mass measures are positively associated with body mass index, total and abdominal fat mass at school-age. The effect

estimates were stronger for body mass index and total subcutaneous fat mass than for central-to-total subcutaneous fat mass ratio, and for girls compared with boys. Also, the effect estimates were stronger for 24 months than for 1.5 months fat mass measures (**Chapter 3.2**). Infant body mass index and subcutaneous fat mass measures were not associated with childhood blood pressure, triglycerides or insulin levels. Higher total subcutaneous fat mass at 1.5 months was associated with lower low-density lipoprotein (LDL)-cholesterol levels while higher total subcutaneous fat mass at 24 months was associated with higher total-cholesterol and LDL-cholesterol levels at 6 years, independently of body mass index. There were no associations of body mass index and central-to-total subcutaneous fat mass ratio with childhood cholesterol levels (**Chapter 3.3**). In summary, findings from **Chapter 3** suggest that infant adiposity is related to childhood adiposity and weakly related to childhood cholesterol levels. Finally, in **Chapter 4,** a general discussion of all studies included in this thesis, suggestions for future research and implications for clinical practice and policy are presented.

In conclusion, findings from this thesis suggest that maternal adiposity during pregnancy and infant adiposity are associated with infant and childhood adiposity and cardiovascular health outcomes. The observed associations are relatively small, but may be important for the burden of obesity and cardiovascular disease on a population level.

Resumo

O **Capítulo 1** inclui a introdução, os objetivos e a população em estudo desta tese. A prevalência de obesidade está a aumentar em todo o mundo e tem importantes implicações clínicas nas crianças e nos adultos. Evidência científica sugere que a obesidade e as doenças cardiometabólicas nas crianças e nos adultos têm origem, pelo menos parcialmente, no período pré- e pós-natal. Exposições adversas, durante o período pré- e pós-natal, podem resultar em adaptações permanentes na estrutura, fisiologia e função de vários sistemas de órgãos. Em particular, a adiposidade das mães durante a gravidez pode aumentar o risco de obesidade nos seus filhos através de mecanismos de programação intrauterina. Um ambiente materno obesogénico durante a gravidez está associado a sobrenutrição fetal e pode causar alterações permanentes no desenvolvimento dos adipócitos e no sistema de controlo do apetite. Os padrões de crescimento na infância, tais como um rápido ganho de peso pós-natal, parecem ser críticos para o desenvolvimento de obesidade e doenças cardiovasculares na vida adulta. Estudar a influência da adiposidade materna durante a gravidez e do crescimento e adiposidade infantil em medidas de adiposidade e em fatores de risco cardiovascular ao longo da vida é importante para melhorar o conhecimento sobre a influência da saúde materna e infantil na saúde futura. Por isso, o objetivo geral desta tese foi estudar as consequências relacionadas com a adiposidade e com a saúde cardiovascular da adiposidade materna e infantil. Os estudos apresentados nesta tese usaram dados da Geração XXI, uma coorte de nascimento de base populacional no Porto, Portugal e da Generation R, uma coorte de base populacional desde a vida fetal em Roterdão, Holanda.

No **Capítulo 2,** os estudos sobre a influência da adiposidade materna durante a gravidez na saúde das crianças foram descritos. No **Capítulo 2.1,** as consequências na saúde das crianças relacionadas com a obesidade materna durante a gravidez foram sumariadas numa revisão. Evidência de estudos observacionais sugere que a obesidade materna e o ganho de peso excessivo durante a gravidez estão associados com um aumento do risco de complicações da gravidez e de problemas de saúde cardiometabólicos, respiratórios e cognitivos nas crianças. Permanece por clarificar se as associações são resultado de mecanismos intrauterinos ou explicadas por confundimento. Os mecanismos epigenéticos subjacentes têm sido explorados em estudos com animais e com amostras pequenas de seres humanos, mas ainda não foram testados em estudos com grande tamanho amostral. No **Capítulo 2.2**, nós estudamos a influência do índice de massa corporal materno e ganho de peso gestational no índice de massa corporal e medidas de massa gorda subcutânea aos 1,5, 6

e 24 meses. Nós observamos que um aumento no índice de massa corporal materno estava associado com um aumento no índice de massa corporal e massa gorda subcutânea total a partir dos 6 meses. Não foram observadas associações com a razão de massa gorda subcutânea central-total na infância. O ganho de peso gestacional total ou por trimestre não estava associado com o índice de massa corporal e medidas de massa gorda subcutânea aos 1,5, 6 e 24 meses. De seguida, nós avaliamos as associações do ganho de peso gestacional, diabetes e hábitos tabágicos na adiposidade total e localizada aos 7 anos e exploramos se essas associações ocorriam através de mecanismos intrauterinos diretos ou através do peso ao nascimento (**Capítulo 2.3**). Nós observamos um efeito total positivo do ganho de peso, diabetes e hábitos tabágicos durante a gravidez na quantidade de gordura corporal da criança. Os hábitos tabágicos da mãe estavam também associados com uma distribuição de gordura central nas crianças. Estes efeitos parecem ocorrer maioritariamente através de programação intrauterina em vez de serem efeitos indiretos através do peso ao nascimento. No **Capítulo 2.4**, nós avaliamos as associações do índice de massa corporal materno e ganho de peso gestacional com a gordura corporal total, abdominal, do pericárdio e do fígado aos 10 anos. Nós observamos que um aumento do índice de massa corporal materno estava associado com um aumento do índice de massa corporal, índice de massa gorda e gordura subcutânea, visceral, do pericárdio e do fígado. As associações com a gordura abdominal eram independentes do índice de massa corporal da criança. Um aumento do ganho de peso durante a gravidez, especialmente no início da gravidez, estava associado com um aumento do índice de massa corporal mas não foram observadas associações consistentes com a gordura subcutânea, visceral, do pericárdio e do fígado, independentemente do índice de massa corporal. Em síntese, os resultados do **Capítulo 2** sugerem que a obesidade materna e o ganho de peso gestacional excessivo são importantes fatores de risco modificáveis que estão associados com problemas de saúde nas crianças.

No **Capítulo 3**, os estudos sobre a influência da adiposidade infantil na saúde das crianças foram descritos. No **Capítulo 3.1,** nós comparamos padrões de gordura corporal e medidas individuais para avaliar gordura corporal e clarificamos o uso de índices baseados no perímetro da cintura como medidas de adiposidade nas crianças. O padrão 1 (índice de massa corporal, índice de massa gorda obtido a partir da bioimpedância tetrapolar e razão cintura-altura) apresentou correlações mais fortes com o índice de massa gorda obtido a partir da absorciometria de raios X de dupla energia (DXA) e o padrão 2 (razão cintura-anca, cintura-coxa e cintura-peso) apresentou correlações mais fortes com a gordura central obtida a partir do DXA. Nós observamos que quando comparado com as medidas individuais, os padrões de

gordura corporal apresentaram correlações semelhantes com as medidas obtidas a partir do DXA. Os índices antropométricos individuais parecem ser suficientes para estimar a gordura corporal. A razão cintura-altura pode ser usada para estimar gordura total, enquanto as razões cintura-anca, cintura-coxa e cintura-peso podem ser usadas para estimar gordura central. A influência do índice de massa corporal e medidas de massa gorda subcutânea durante os primeiros 2 anos de vida na adiposidade e fatores de risco cardiovascular nas crianças foi estudada no **Capítulo 3.2** e **Capítulo 3.3**, respetivamente. Nós observamos que o índice de massa corporal e as medidas de massa gorda subcutânea nos primeiros 2 anos de vida estão positivamente associadas com o índice de massa corporal, e com a massa gorda total e abdominal em idade escolar. As estimativas de efeito eram mais fortes para o índice de massa corporal e massa gorda subcutânea total do que para a razão de massa gorda subcutânea central-total, e para as raparigas do que para os rapazes. Além disso, as estimativas de efeito eram mais fortes para as medidas de massa gorda aos 24 meses do que aos 1,5 meses (**Capítulo 3.2**). O índice de massa corporal e as medidas de massa gorda subcutânea não estavam associados com a pressão arterial, triglicerídeos e insulina nas crianças. Um aumento da massa gorda subcutânea total aos 1,5 meses estava associado com uma diminuição dos níveis de colesterol da lipoproteína de baixa densidade (LDL) enquanto um aumento da massa gorda subcutânea total aos 24 meses estava associado com um aumento dos níveis de colesterol total e LDL aos 6 anos, independentemente do índice de massa corporal. Não se observaram associações do índice de massa corporal e da razão de massa gorda subcutânea central-total com os níveis de colesterol nas crianças (**Capítulo 3.3**). Em síntese, os resultados do **Capítulo 3** sugerem que a adiposidade durante os primeiros 2 anos de vida está associada com a adiposidade e fracamente associada com os níveis de colesterol nas crianças. Finalmente, no **Capítulo 4,** apresentamos uma discussão geral de todos os estudos incluídos nesta tese, sugestões para investigação futura e implicações para a prática clínica e políticas de saúde.

Em conclusão, os resultados desta tese sugerem que a adiposidade materna durante a gravidez e a adiposidade durante os primeiros 2 anos de vida estão associadas com a adiposidade e saúde cardiovascular nas crianças. As associações observadas são relativamente pequenas, mas podem ser importantes para o impacto da obesidade e da doença cardiovascular ao nível populacional.

Samenvatting

Hoofdstuk 1 beschrijft de achtergrond, hypothese, de doelstelling en de opzet van het onderzoek beschreven in dit proefschrift. De wereldwijde prevalentie van obesitas neemt toe. Obesitas heeft belangrijke gevolgen voor de gezondheid van zowel kinderen als volwassenen. Eerder onderzoek suggereert dat obesitas en cardiometabole ziekten in de kindertijd en op volwassen leeftijd hun grondslag deels al vinden in de foetale en vroege postnatale ontwikkeling. Blootstelling aan ongunstige factoren tijdens deze foetale of vroeg postnatale periode leidt tot blijvende aanpassingen in de structuur, fysiologie en functie van verschillende orgaansystemen. In het bijzonder zou maternale adipositas tijdens de zwangerschap via intra-uteriene programmering kunnen leiden tot een hoger risico op obesitas bij het kind. Een obesogene omgeving tijdens de zwangerschap leidt tot foetale overvoeding en zou kunnen leiden tot blijvende verandering in de ontwikkeling van adipocyten en het verzadigingssysteem. Groeipatronen tijdens de peutertijd, zoals een snelle postnatale gewichtstoename, lijken ook belangrijk te zijn voor de ontwikkeling van obesitas en cardiovasculaire ziekten later in het leven. Het is van belang om meer inzicht te verkrijgen in de invloed van maternale adipositas tijdens de zwangerschap en de groei en adipositas van het kind tijdens de peutertijd op gedetailleerde maten van lichaamssamenstelling en cardiovasculaire risicofactoren op latere leeftijd. Het doel van dit proefschrift was daarom om de gevolgen te onderzoeken van adipositas bij de moeder en in de peutertijd met betrekking tot adipositas en cardiovasculaire gezondheid later in het leven. Voor de studies beschreven in dit proefschrift zijn gegevens gebruikt van de Generation XXI, een populatie-gebaseerd geboortecohort in Porto, Portugal, en van de Generation R Studie, een populatie-gebaseerd cohort dat gevolgd wordt vanaf het vroege foetale leven in Rotterdam, Nederland.

In **Hoofdstuk 2** worden studies beschreven betreffende de invloed van maternale adipositas tijdens de zwangerschap op gezondheidsuitkomsten tijdens de peuter- en kindertijd. In **Hoofdstuk 2.1** hebben we het bestaande onderzoek naar de gevolgen van maternale obesitas tijdens de zwangerschap op de gezondheid van het kind samengevat. Observationele studies stellen dat maternale obesitas vooraf aan de zwangerschap en een verhoogde gewichtstoename tijdens de zwangerschap geassocieerd zijn met een verhoogd risico op complicaties bij de foetus tijdens de zwangerschap en ongunstige cardiometabole, respiratoire en cognitieve gezondheidsuitkomsten op de kinderleeftijd. Het is niet duidelijk of deze associaties het gevolg

zijn van intra-uteriene mechanismen of verklaard kunnen worden door confounding. De epigenetische mechanismen onderliggend aan deze associaties zijn bestudeerd in dierstudies en kleine studies bij mensen, maar zijn nog niet onderzocht in grotere populatie gebaseerde studies. In **Hoofdstuk 2.2** hebben we de invloed van maternale body mass index en gewichtstoename tijdens de zwangerschap op de body mass index en maten voor subcutaan vet bij het kind op de leeftijden van 1.5, 6 en 24 maanden onderzocht. We lieten zien dat hogere maternale body mass index was geassocieerd met een hogere body mass index en totale subcutane vetmassa vanaf de leeftijd van 6 maanden. Maternale body mass index was niet geassocieerd met de centraal/totaal subcutaan vet ratio tijdens de peutertijd. Maternale totale en periode-specifieke gewichtstoename tijdens de zwangerschap waren niet geassocieerd met body mass index en maten van subcutaan vet op de leeftijden van 1.5, 6 en 24 maanden. Daarnaast hebben we gekeken naar de associaties van gewichtstoename tijdens de zwangerschap, diabetes, en roken met totale en regionale adipositas op de leeftijd van 7 jaar, en hebben we onderzocht of deze associaties verklaard werden door directe intra-uteriene mechanismen of door het geboortegewicht (**Hoofdstuk 2.3**). We zagen een positief totaal effect van gewichtstoename, diabetes en roken tijdens de zwangerschap op de hoeveelheid lichaamsvet bij het kind. Roken tijdens de zwangerschap was ook geassocieerd met een centrale vetverdeling bij kinderen. Deze effecten lijken vooral verklaard te worden door intra-uteriene programmering en niet door indirecte effecten via geboortegewicht. In **Hoofdstuk 2.4** onderzochten we de associaties van maternale body mass index en gewichtstoename tijdens de zwangerschap met totaal-, abdominaal-, pericardiaal- en levervet op de leeftijd van 10 jaar. We observeerden dat een hogere body mass index van de moeder was geassocieerd met een hogere body mass index en een hogere massa van het subcutane-,viscerale-, pericardiale- en levervet.De associaties met abdominaalvet waren onafhankelijk van de body mass index van het kind. Meer gewichtstoename tijdens de zwangerschap, vooral tijdens de vroege zwangerschap, was geassocieerd met een hogere body mass index, maar er waren geen consistente associaties met subcutaan-, visceraal-, pericardiaal- en levervet, onafhankelijk van de body mass index. Samengevat, de bevindingen beschreven in **Hoofdstuk 2** suggereren dat maternale obesitas en meer gewichtstoename tijdens de zwangerschap belangrijke factoren zijn die geassocieerd zijn met ongunstige gezondheidsuitkomsten bij kinderen.

In **Hoofdstuk 3** worden studies naar de invloed van adipositas tijdens de peutertijd op gezondheidsuitkomsten op de kinderleeftijd beschreven. In **Hoofdstuk 3.1** vergeleken we de patronen van lichaamsvet met enkelvoudige maten van lichaamsvet en beschreven we het gebruik van indexen gebaseerd op middelomtrek als maten van adipositas bij kinderen. Patroon 1 (body mass index, vetmassa index verkregen met tetra-polar bioelectric impedance, en middelomtrek/lengte ratio) was sterker gecorreleerd aan vetmassa index verkregen met dual energy X-ray absorptiometry (DXA) scans en patroon 2 (middelomtrek/heupomtrek, middelomtrek/dijomtrek en middelomtrek/gewicht ratio's) was sterker gecorreleerd met centraal vet, verkregen met DXA scans. De correlaties van lichaamsvetpatronen met maten verkregen met DXA scans waren gelijk aan die van de enkelvoudige maten van lichaamsvet. Enkelvoudige antropometrische indexen lijken voldoende te zijn om de hoeveelheid lichaamsvet te schatten. Middelomtrek/lengte ratio zou een proxy kunnen zijn voor totaal vet, terwijl middelomtrek/heupomtrek, middelomtrek/dijomtrek en middelomtrek/gewicht ratio's proxies zouden kunnen zijn voor centraal vet. De invloed van body mass index en maten voor subcutaan vet tijdens de peutertijd op adipositas en cardiovasculaire risicofactoren tijdens de kindertijd zijn onderzocht in respectievelijk **Hoofdstuk 3.2** en **Hoofdstuk 3.3**. We hebben laten zien dat body mass index en maten voor subcutaan vet tijdens de peutertijd positief geassocieerd zijn met body mass index en totale en abdominale vetmassa op schoolgaande leeftijd. De associaties waren sterker voor body mass index en totaal subcutaan vet dan voor de centraal/totaal subcutaan vet ratio en waren ook sterker voor meisjes dan voor jongens. Daarnaast waren de effecten sterker op de leeftijd van 24 maanden dan op de leeftijd van 1.5 maand (**Hoofdstuk 3.2**). Body mass index en maten voor subcutaan vet waren niet geassocieerd met bloeddruk, triglyceriden of insuline waarden op de kinderleeftijd. Een hogere totale subcutane vetmassa op de leeftijd van 1.5 maand was geassocieerd met lagere LDL-cholesterol waarden, terwijl een hogere totale subcutane vetmassa op de leeftijd van 24 maanden geassocieerd was met hogere totaal-cholesterol waarden en LDL-cholesterol waarden op de leeftijd van 6 jaar, onafhankelijk van body mass index. Body mass index en centraal/totaal vetmassa ratio waren niet geassocieerd met cholesterol waarden op de kinderleeftijd (**Hoofdstuk 3.3**). Samengevat, de bevindingen beschreven in **Hoofdstuk 3** suggereren dat adipositas op de peuterleeftijd gerelateerd is aan adipositas op de kinderleeftijd en zwak gerelateerd is aan cholesterol waarden op de kinderleeftijd. In **Hoofdstuk 4** is een algemene discussie gegeven van alle studies beschreven in dit proefschrift. Daarnaast worden er

suggesties gedaan voor verder onderzoek en worden de implicaties voor de klinische praktijk en beleid beschreven.

Concluderend, de bevindingen van dit proefschrift suggereren dat maternale adipositas tijdens de zwangerschap en adipositas tijdens de peutertijd geassocieerd zijn met adipositas tijdens de peuter- en kindertijd en cardiovasculaire gezondheidsuitkomsten tijdens de kindertijd. Hoewel de gevonden effecten relatief klein zijn, zijn ze van belang voor de impact van obesitas en cardiovasculaire zieken op populatieniveau.

CHAPTER 6

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Publication list

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1. **Santos S**, Severo M, Gaillard R, Santos AC, Barros H, Oliveira A. The role of prenatal exposures on body fat patterns at 7 years: intrauterine programming or birthweight effects? *Nutr Metab Cardiovasc Dis 2016;26(11):1004:1010.*

2. **Santos S**, Gaillard R, Oliveira A, Barros H, Abrahamse-Berkeveld M, van der Beek EM, Hofman A, Jaddoe VW. Associations of infant subcutaneous fat mass with total and abdominal fat mass at school-age: The Generation R Study. *Paediatr Perinat Epidemiol 2016;30(5):511-520.*

3. **Santos S**, Gaillard R, Oliveira A, Barros H, Hofman A, Franco OH, Jaddoe VW. Subcutaneous fat mass in infancy and cardiovascular risk factors at school-age: The Generation R Study. *Obesity (Silver Spring) 2016;24(2):424-429.*

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8. **Santos S**, Monnereau C, Felix JF, Duijts L, Gaillard R, Jaddoe VW. Maternal body mass index, gestational weight gain and childhood abdominal, pericardial and liver fat assessed by magnetic resonance imaging. *Submitted.*

9. **Santos S**, Severo M, Lopes C, Oliveira A. Single or combined anthropometric indices for assessing body fat in children: are different anthropometric indices based on waist circumference measuring the same? *Submitted.*

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10. Jharap VV, **Santos S**, Steegers EA, Jaddoe VW, Gaillard R. Associations of maternal obesity and excessive weight gain during pregnancy with subcutaneous fat mass in infancy. *Early Hum Dev 2017;108:23-28.*

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14. Afonso L, Lopes C, Severo M, **Santos S**, Real H, Durão C, Moreira P, Oliveira A. Bidirectional association between parental child-feeding practices and body mass index at 4 and 7 years of age. *Am J Clin Nutr 2016;103(3):861-867.*

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17. Monnereau C, **Santos S**, van der Lugt A, Jaddoe VW, Felix JF. Influence of known genetic variants associated with adiposity on childhood abdominal, liver and pericardial fat assessed by Magnetic Resonance Imaging. *Submitted.*

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About the author

Susana Santos was born October 14th 1986 in Porto, Portugal. From 2005 to 2009, she was enrolled in the Bachelor of Nutrition Science at the Faculty of Nutrition and Food Sciences of the University of Porto, Porto, Portugal. During the last year, she spent 6 months in the Centre for Food Policy, City University London, London, United Kingdom as an intern researcher. From 2010 to 2012, she worked as a researcher with a scientific initiation grant at the Institute of Public Health, University of Porto, Porto, Portugal. She obtained her Master in Epidemiology degree at the University of Porto Medical School, Porto, Portugal in 2012. In the same year, she started her PhD in Public Health at the University of Porto Medical School and Institute of Public Health, University of Porto under supervision of Dr. Andreia Oliveira. In 2014, she started developing her PhD project at the Generation R Study Group, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands under supervision of Prof dr. V.W.V. Jaddoe and Dr. Romy Gaillard. In 2016, she started a joint doctorate between both universities and expanded her research project in this current thesis entitled ´Maternal and infant adiposity: metabolic health related consequences´. She will continue working at the Generation R Study Group until June 2018. She would like to pursue a research career.

PhD portfolio

Name PhD student:	Susana Santos
Faculties/Universities:	Faculty of Medicine/University of Porto (Porto, Portugal)
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	The Generation R Study Group, Departments of Epidemiology and Pediatrics, Erasmus Medical Center (Rotterdam, the Netherlands)
PhD period:	2012 until 2017
Supervisors:	Dr. Andreia Oliveira (Porto, Portugal)
	Prof. dr. V.W.V. Jaddoe, Dr. Romy Gaillard (Rotterdam, the Netherlands)

	Year	Workload (ECTS)
1. PhD Training		
Courses		
Scientific Integrity	2017	0.3
Analysis of longitudinal data in health	2013	0.6
Introduction to Epidemiology	2012/13	4.0
Health Research Methods	2012/13	2.0
Advanced Epidemiologic Methods	2012/13	4.0
Systematic Review and Meta-analysis	2012/13	4.0
Journal Club	2012/13	3.0
Topical seminars	2012/13	3.0
Health Statistics Methods I	2012/13	4.0
Health Statistics Methods II	2012/13	4.0
Occupational Health	2012/13	4.0
Measuring Health	2012/13	4.0
Nutrition and Health	2012/13	4.0
Clinical Epidemiology	2012/13	4.0
Epidemiology of Cardiovascular Diseases	2012/13	4.0
Genetic Epidemiology	2012/13	4.0
Epidemiology of cancer	2012/13	4.0

	Year	Workload (ECTS)
Mental Health	2012/13	2.0
Ethics, Solidarity and Public Health	2012	0.3
Meetings		
Generation R Research Meetings	2014-17	1.0
Maternal and Child Health meetings	2014-17	1.0
ISPUP internal scientific meetings	2012-14	1.0
Nutrition and Obesity Research group meetings	2012-14	1.0
ISPUP Scientific Committee meetings	2012-14	1.0
National public health PhD students meeting	2013	0.2
Communications in scientific meetings		
Oral communications		
Porto Birth Cohorts Meeting, Porto, Portugal	2016	0.7
The Power of Programming, Munich, Germany	2016	1.4
III World Congress of Public Health Nutrition, Las Palmas de Gran Canaria, Spain	2014	1.4
10th International Symposium on Body Composition, Cascais, Portugal	2014	1.4
International Society for Behavioral Nutrition and Physical Activity Annual Meeting, San Diego, California, USA	2014	1.4
54th Annual Meeting European Society for Paediatric Research, Porto, Portugal	2013	1.4
XII Congress of Food and Nutrition, Lisbon, Portugal	2013	0.7
XI Congress of Food and Nutrition, Porto, Portugal	2012	0.7
Poster/Poster-oral communications		
9th World Congress on Developmental Origins of Health and Disease, Cape Town, South Africa	2015	1.0
European Congress of Epidemiology, Aarhus, Denmark	2013	1.0
European Congress of Epidemiology, Porto, Portugal	2012	1.0
IX Congress of Portuguese Society of Nutrition and Food Sciences, Porto, Portugal	2012	0.7
Communications by invitation		
European Birth Cohorts Network meeting, Barcelona, Spain	2017	1.4
XIV Congress of Food and Nutrition, Lisbon, Portugal	2015	0.7
Prizes and awards		
New investigator award. The Power of Programming 2016, Munich, Germany	2016	

	Year	Workload (ECTS)
1st prize for best poster-oral communication. 9th World Congress on Developmental Origins of Health and Disease, Cape Town, South Africa	2015	
1st prize for best oral communication. XII Congress of Food and Nutrition, Lisbon, Portugal	2013	
1st prize for best oral communication. XI Congress of Food and Nutrition, Porto, Portugal	2012	
Grants		
Individual doctoral grant (SFRH/BD/81123/2011) by Foundation for Science and Technology, Portugal	2012-16	
Vereniging Trustfonds Erasmus Universiteit Rotterdam, several travel grants	2015-16	
Other activities		
Co-chair session:		
European Birth Cohorts Network meeting, Barcelona, Spain	2017	
The Power of Programming, Munich, Germany	2016	
Peer review of articles for scientific journals	2012-17	
Participation in an international collaboration of pregnancy and birth cohorts focused on maternal obesity and childhood outcomes (MOCO)	2015-17	
Collaboration in the ultrasound measurements and analyses of abdominal fat in a randomized controlled trial (Venus study, Danone)	2016-17	
2. Teaching activities		
Varsha Jharap, Master in Clinical Epidemiology, NIHES, the Netherlands, Project title: Associations of maternal obesity and excessive weight gain during pregnancy with subcutaneous fat mass in infancy	2015-16	4.0
Teaching assistant in Biostatistical Methods I, NIHES, the Netherlands	2016	0.5

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The ultimate measure of a man is not where he stands in moments of comfort and convenience, but where he stands at times of challenge and controversy.

Martin Luther King, Jr.