DIET, ADIPOSITY AND METABOLISM IN PREGNANCY AND CHILDHOOD

Ellis Voerman

Diet, Adiposity and Metabolism in Pregnancy and Childhood

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CONTENTS

MANUSCRIPTS THAT FORM THE BASIS OF THIS THESIS

Chapter 2.1

Voerman E, Jaddoe VWV, Gisthi O, Hofman A, Franco OH, Gaillard R. Maternal caffeine intake during pregnancy, early growth, and body fat distribution at school age. *Obesity (Silver Spring). 2016;24(5):1170-7.*

Chapter 2.2

Voerman E, Jaddoe VWV, Hulst ME, Oei EHG, Gaillard R. Associations of maternal caffeine intake during pregnancy with abdominal and liver fat deposition in childhood. *Pediatr Obes. 2019;15(5):e12607*.

Chapter 2.3

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Chapter 3.1

Voerman E, Santos S, Patro Golab B, Amiano P, Ballester F, Barros H, Bergström A, Charles M, Chatzi L, Chevrier C, Chrousos GP, Corpeleijn E, Costet N, Crozier S, Devereux G, Eggesbø M, Ekström S, Fantini MP, Farchi S, Forastiere F, Georgiu V, Godfrey KM, Gori D, Grote V, Hanke W, Hertz-Picciotto I, Heude B, Hryhorczuk D, Huang R, Inskip H, Iszatt N, Karvonen AM, Kenny LC, Koletzko B, Küpers LK, Lagström H, Lehmann I, Magnus P, Majewska R, Mäkelä J, Manios Y, McAuliffe FM, McDonald SW, Mehegan J, Mommers M, Morgen CS, Mori TA, Moschonis G, Murray D, Chaoimh CN, Nohr EA, Nybo Andersen A, Oken E, Oostvogels AJJM, Pac A, Papadopoulou E, Pekkanen J, Pizzi C, Polanska K, Porta D, Richiardi L, Rifas-Shiman SL, Ronfani L, Santos AC, Standl M, Stoltenberg C, Thiering E, Thijs C, Torrent M, Tough SC, Trnovec T, Turner S, van Rossem L, von Berg A, Vrijheid M, Vrijkotte TGM, West J, Wijga A, Wright J, Zvinchuk O, Sørensen TIA, Lawlor DA, Gaillard R*, Jaddoe VWV*. Maternal body mass index, gestational weight gain and the risk of overweight and obesity across childhood: An individual participant data meta-analysis. *PLoS Med. 2019;16(2):e1002744.*

Chapter 3.2

Voerman E, Santos S, Inskip H, Amiano P, Barros H, Charles M, Chatzi L, Chrousos GP, Corpeleijn E, Crozier S, Doyon M, Eggesbø M, Fantini MP, Farchi S, Forastiere F, Georgiu V, Gori D, Hanke W, Hertz-Picciotto I, Heude B, Hivert M, Hryhorczuk D, Iñiguez C, Karvonen AM, Küpers LK, Lagström H, Lawlor DA, Lehmann I, Magnus P, Majewska R, Mäkelä J, Manios Y, Mommers M, Morgen CS, Moschonis G, Nohr EA, Nybo Andersen A, Oken E, Pac A, Papa-

dopoulou E, Pekkanen J, Pizzi C, Polanska K, Porta D, Richiardi L, Rifas-Shiman SL, Roeleveld N, Ronfani L, Santos AC, Standl M, Stigum H, Stoltenberg C, Thiering E, Thijs C, Torrent M, Trnovec T, van Gelder MMHJ, van Rossem L, von Berg A, Vrijheid M, Wijga A, Zvinchuk O, Sørensen TIA, Godfrey KM, Jaddoe VWV*, Gaillard R*. Association of gestational weight gain with adverse maternal and infant outcomes. *JAMA. 2019;321(17):1707-1715.*

Chapter 4.1

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Chapter 4.2

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Chapter 4.3

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Chapter 4.4

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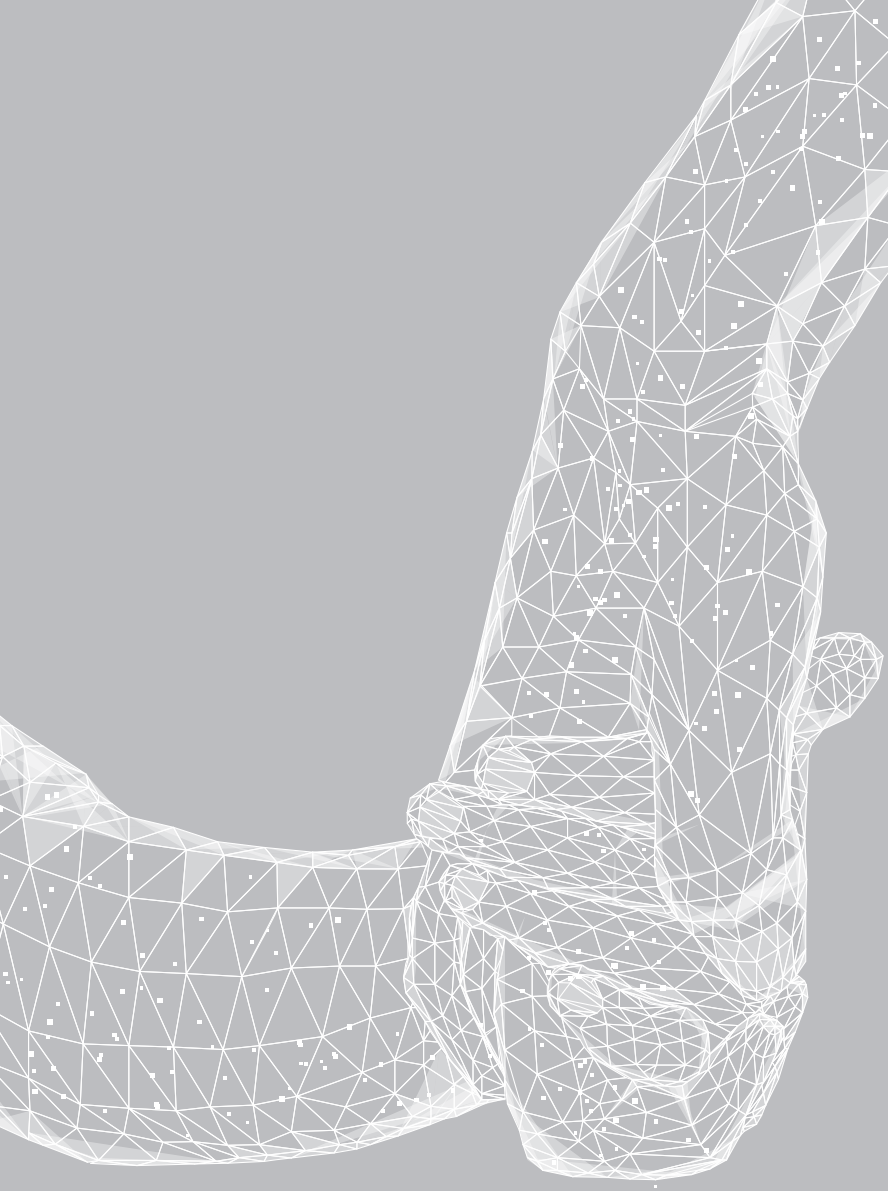
** Denotes shared last authors*



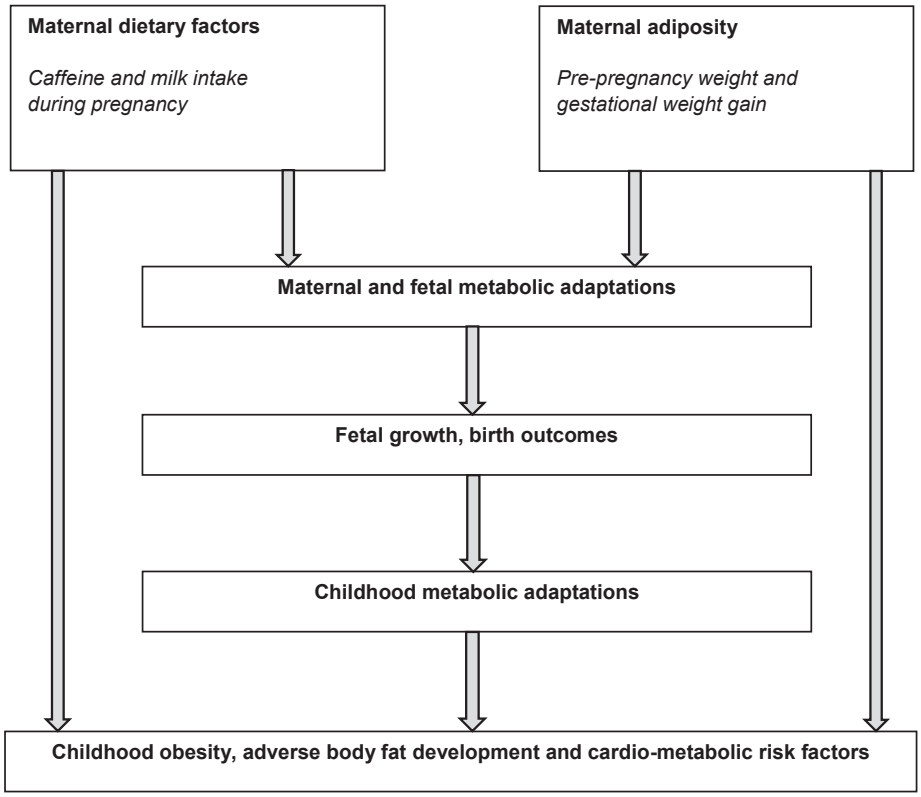
1 General introduction

Childhood overweight and obesity are of major public health concern. The global prevalence of obesity in boys increased from 0.7% to 5.6% between 1975 and 2016, whereas in girls the prevalence increased from 0.9% to 7.8% in the same period (1). Childhood overweight and obesity have serious consequences for health. Children with obesity are at increased risk of obesity in adulthood (2). In addition, obesity is a strong risk factor for a range of other health problems, including cardio-metabolic disease (type 2 diabetes, dyslipidemia, hypertension, coronary heart disease, stroke), asthma, osteoarthritis, mental health problems and premature death (3). Overweight and obesity are defined by the World Health Organization (WHO) as *"abnormal or excessive fat accumulation that may impair health"* (4). Overweight and obesity are defined using the body mass index, which is a measure of body weight relative to height. In adults, overweight and obesity are defined as a body mass index of 25.0-29.9 kg/m^2 and ≥ 30 kg/m^2, respectively (5). In children, the cut offs for overweight and obesity depend on the sex and age of the child and are defined according to reference charts for body mass index, such as those by Cole et al (6) and those by the WHO (7, 8). Body mass index is a simple and inexpensive measure of general adiposity. However, it is a suboptimal measure of fat mass, as it does not distinguish between fat mass and lean mass. Also, it does not provide any information on fat distribution. It has been suggested that an abdominal fat distribution, especially accumulation of visceral fat and liver fat, is strongly linked to metabolic disturbances and the risk of cardio-metabolic disease, independent of the total amount of body fat (9, 10).

The etiology of obesity and related cardio-metabolic diseases is complex and multifactorial. Risk factors include, but are not limited to, genetic predisposition, excess energy intake, sedentary behavior, lack of or excess of sleep, stress, and certain diseases. In addition, a large body of research suggests that obesity and other cardio-metabolic diseases might already originate in early life. The 'Developmental Origins of Health and Disease (DOHaD) Hypothesis' suggests that adverse exposures during fetal and early postnatal life may lead to developmental adaptations in organ structure or function, which may predispose these children to cardio-metabolic disease in later life (11). Early life adverse exposures suggested to influence offspring growth and development include for instance maternal pre-pregnancy obesity, unfavorable nutritional status, maternal smoking, gestational diabetes and gestational hypertensive disorders (11, 12). Studies assessing the early origins of adult disease often use birth weight, or its gestational age adjusted equivalent, as an indicator of suboptimal intra-uterine environment and fetal growth. These studies consistently showed that children born with a low birth weight are at increased risk of developing cardio-metabolic disease in later life (11, 13, 14). On the other side of the spectrum, children born with a high birth weight are also at risk of these diseases (11, 13, 14). However, birth weight is the result of different fetal growth patterns and is the starting point of infant growth. It has been observed that both children born with a low or a high birthweight that grow rapidly in early childhood are at the highest risk of later obesity and cardio-metabolic disease (15-18).

Thus, childhood obesity and more specifically an adverse body fat distribution are major health problems and important risk factors for other cardio-metabolic diseases. Susceptibility to these diseases might be partly established in early life, as reflected by different growth patterns from fetal life onwards. Identifying the factors related to adverse growth patterns and body fat development as well as the underlying mechanisms will broaden the understanding of the early origins of disease and is vital to effectively target interventions aiming to reduce the burden of these diseases. Therefore, the studies in this thesis were designed to assess the associations of common maternal dietary factors and maternal adiposity with growth, body fat development and cardio-metabolic risk factors in children, as well as the metabolic mechanisms that might underlie these associations (**Figure 1**).

Figure 1. Overview of hypotheses assessed in this thesis



MATERNAL COMMON DIETARY FACTORS

Adequate nutrition during pregnancy is important for the health status of both the pregnant woman and her child (19). The Dutch Famine Study, a birth cohort study of men and women born around the Dutch famine of 1944-1945 provided important first evidence that prenatal undernutrition increases the risk of a variety of diseases in adulthood, such as obesity, diabetes and cardiovascular disease (12). Up until now, numerous studies have shown associations of maternal dietary factors during pregnancy, such as diet quality, dietary patterns, total energy intake and macro- and micronutrient intake with both short- and long-term offspring health outcomes (19, 20). Less studied but common components of the human diet, such as caffeine-containing beverages and cow's milk, might also be important for offspring growth and development. Both caffeine-containing beverages and cow's milk are frequently consumed among pregnant women and are associated with fetal growth and the risk of adverse birth outcomes (21-30). The long-term offspring health effects of these common dietary factors remain unclear.

Caffeine is a component of several food products, including coffee and tea. It has the ability to cross the placental barrier and freely enters the fetal circulation (31). The activity of the principal enzyme in caffeine metabolism, cytochrome CYP1A2, decreases progressively during pregnancy and is absent in the placenta and fetus (32-34). Consequently, fetal exposure to caffeine is prolonged and might adversely affect fetal development. Previous research has suggested that caffeine intake by pregnant women is related to an increased risk of fetal death, impaired fetal growth and low birth weight (21-23). Based on the risks of adverse pregnancy and birth outcomes, the current recommendations for maximum caffeine intake range between 200-300 mg per day, equivalent to approximately 2-3 cups of coffee per day (35-37). In addition to these short-term outcomes, recent studies suggest that maternal caffeine intake might also affect offspring growth and body fat development, possibly by altering the development of the offspring hypothalamic-pituitary-adrenal (HPA) axis (38, 39). Also, studies in adult populations suggest that caffeine intake might affect body fat accumulation and the risks of several diseases (40-45).

Cow's milk has a high bioavailability of nutrients important for growth and development, including protein, vitamins, calcium and other minerals. It has been suggested that maternal cow's milk intake during pregnancy stimulates fetal growth (24-30). The current recommendation of the Dutch Nutrition Centre for pregnant and non-pregnant women is 375 mg of milk and milk products a day, equivalent to approximately 2-3 glasses (46). Translational research has suggested that milk intake during pregnancy may activate the nutrient-sensitive kinase *mechanistic target of rapamycin complex 1* (mTORC1) in the placenta, leading to an increased placental nutrient transfer and activated mTORC1 in the fetus (47, 48). mTORC1 is involved in the regulation of cell growth and adipogenesis, and overactivation of mTORC1 is related to a variety of diseases, including obesity, insulin resistance and cardiovascular

disease (47, 49, 50). This may suggest that maternal milk intake during pregnancy might also influence offspring cardio-metabolic development.

Thus, maternal caffeine intake during pregnancy and maternal milk intake during pregnancy might influence long-term body fat development and cardio-metabolic risk factors. Exploring these associations might provide insight in whether intake of these two commonly consumed beverages should be considered risks factors for offspring cardio-metabolic disease.

MATERNAL ADIPOSITY

Overweight and obesity in women of reproductive age are highly prevalent (51). Maternal pre-pregnancy obesity is an important and well-known risk factor for both short- and long-term adverse outcomes for both the mother and the child, including gestational hypertensive and diabetic disorders, fetal death, pre-term birth, large-size for gestational age at birth (LGA) and childhood obesity (13, 51-55). Studies have shown that children of mothers with obesity have an 54% increased risk to be born prematurely, a 2-fold increased risk to be born LGA and a 3-fold increased risk of childhood obesity, as compared to children of mothers with a normal pre-pregnancy weight (13, 53). Next to maternal weight before pregnancy, maternal weight gain during pregnancy is also a risk factor of these short- and long-term maternal and offspring outcomes (56-59). Gestational weight gain is usually categorized into inadequate, adequate and excessive according to pre-pregnancy body mass index using the criteria of the US Institute of Medicine (IOM, currently known as the National Academy of Medicine (NAM)) (60). For instance, studies have shown that excessive gestational weight gain according to these criteria was associated with an 85% increased risk of LGA, a 30% increased risk of caesarian delivery and an 40% increased risk of childhood obesity (57, 58).

Maternal pre-pregnancy obesity and gestational weight gain reflect different components. Maternal pre-pregnancy obesity reflects maternal genetic predisposition, nutritional status, fat accumulation and low-grade inflammation. Gestational weight gain additionally reflects maternal and amniotic fluid expansion, and growth of the fetus, placenta and uterus (61, 62). Intra-uterine programming mechanisms may, at least partly, underlie the associations of maternal pre-pregnancy obesity and gestational weight gain with offspring outcomes. Increased fetal exposure to nutrients in children from mothers with obesity has been suggested to lead to alterations in the structure and function of adipose tissue, appetite regulation, and energy metabolism (63, 64).

Thus, maternal pre-pregnancy BMI and gestational weight gain seem to be important modifiable risk factors of adverse maternal and offspring health outcomes. Despite the well-studied associations of maternal weight before and during pregnancy with offspring outcomes, refined and more in-depth understanding of the separate and combined relation-

ships of maternal pre-pregnancy body mass index and gestational weight gain with these outcomes is needed in order to effectively target future preventive strategies.

MATERNAL AND CHILDHOOD METABOLISM

The mechanisms linking adverse exposures in early life to later obesity and cardio-metabolic disease are incompletely understood, but might involve alterations in maternal or offspring metabolic pathways. Maternal metabolite profiles in pregnancy may influence fetal growth and development by direct influences on fetal nutrient availability, or indirectly by influences on fetal metabolic processes resulting from adaptions in endocrine function or placental metabolism (65, 66). Thus far, studies mainly focused on conventional metabolites to characterize maternal and offspring metabolic status. For instance, increased maternal glucose and lipid concentrations during pregnancy have been related to increased fetal and postnatal growth, and obesity, type 2 diabetes and the metabolic syndrome in the offspring (67-77). Detailed characterization of maternal and offspring metabolite profiles by metabolomics techniques may provide more in-depth insights in the metabolic mechanisms underlying the early origins of obesity and cardio-metabolic disease (78-80). Metabolomics is the study of a large number of small molecular weight metabolites in biological tissues and fluids. The metabolome is the most downstream component of the 'omics technologies and is therefore closely linked to the phenotype. It carries information about gene-expression, but also about lifestyle- and environmental factors (79, 80). Metabolomics studies have already been successfully performed in adults for characterization of disease status, development and progression as well as the underlying mechanisms (81-83). In studies addressing the early origins of health and disease metabolomics has been used less extensively, and existing studies are often small and assessed cross-sectional relationships only. Exploring the interrelationships of detailed maternal and offspring metabolite profiles as well as their determinants and outcomes over time, will help to disentangle the mechanisms underlying the early origins of cardio-metabolic disease.

GENERAL OBJECTIVE

The general objective of this thesis was to assess the associations of common maternal dietary factors and maternal adiposity with offspring growth, body fat development and cardio-metabolic risk factors and its potential underlying metabolic mechanisms.

GENERAL DESIGN

The studies presented in this thesis were embedded in the Generation R Study and in the Lifecycle - Maternal Obesity and Childhood Outcomes (MOCO) collaboration.

The Generation R Study

The Generation R Study is a population-based prospective cohort study from fetal life onwards in the city of Rotterdam, the Netherlands (84). The Generation R Study is designed to identify early environmental and genetic determinants of normal and abnormal growth, development and health from fetal life and young adulthood. All pregnant women living in the study area with a delivery date between April 2002 and January 2006 were eligible for enrollment in the 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 8879 (91%) were included during pregnancy. Assessments were planned in early pregnancy (<18 weeks of gestation), mid-pregnancy (18 - 25 weeks of gestation) and late pregnancy (≥ 25 weeks of gestation), and included parental physical examinations, maternal blood and urine collection, fetal ultrasound examinations and self-administered questionnaires. Assessments of the newborn at birth included a physical examination and cord blood collection. In the preschool period, from birth to 4 years of age, data collection was performed in all children by questionnaires and visits to the routine child health care centers. At the ages of 6 and 10 years, all children were invited to a dedicated research center in the Erasmus MC – Sophia Children's Hospital to participate in detailed body composition and cardiovascular follow-up measurements. Measurements during these visits included anthropometrics, body composition, cardiovascular development and collection of blood and urine. Currently, the 13-years old follow-up examination has been finished and the 17-years follow-up examination is ongoing.

The Lifecycle - Maternal Obesity and Childhood Outcomes collaboration

The Lifecycle - Maternal Obesity and Childhood Outcomes (MOCO) collaboration is an international collaboration of pregnancy and birth cohort studies, aiming to assess the associations of maternal pre-pregnancy body mass index and gestational weight gain with maternal and offspring outcomes. The collaboration consists of a total of 39 pregnancy and birth cohort studies from Europe, North-America and Australia, including a total of 277042 participants. Cohorts were selected based on existing collaborations on childhood health (the EarlyNutrition project, the CHICOS project and Birthcohorts.net assessed until July 2014). Inclusion criteria were the inclusion of mothers with singleton live-born children born from 1989 onwards, available information on maternal pre- or early pregnancy body mass index

and at least one offspring measurement (birth weight or childhood body mass index) and approval by the local ethical committee.

OUTLINE OF THIS THESIS

The general objective of this thesis is addressed in several studies presented in this thesis. **Chapter 2** describes studies on the influence of common maternal dietary factors on childhood growth, adiposity and cardio-metabolic risk factors. In **Chapter 2.1,** we assessed the associations of maternal caffeine intake during pregnancy with childhood growth and general adiposity, whereas in **Chapter 2.2** we assessed the association of maternal caffeine intake during pregnancy with detailed measures of abdominal and liver fat. In **Chapter 2.3**, we focused on the association of maternal milk intake during pregnancy with childhood general and organ fat mass and cardio-metabolic risk factors.

Chapter 3 describes studies on the influences of maternal adiposity before and during pregnancy on offspring adiposity at birth and in childhood. In **Chapter 3.1**, we examined the separate and combined associations of maternal body mass index and gestational weight gain with the risks of overweight and obesity across childhood. In **Chapter 3.2,** we estimated ranges of optimal gestational weight gain associated with adverse maternal and infant outcomes.

Chapter 4 describes studies on the potential metabolic mechanisms linking adverse exposures in early life to later obesity and cardio-metabolic disease. **Chapter 4.1** describes the associations of maternal glucose and insulin levels with childhood general and abdominal body fat and cardio-metabolic risk factors. In **Chapter 4.2**, we identified critical periods and longitudinal growth patterns from fetal life onwards associated with childhood insulin and c-peptide levels. In **Chapter 4.3**, we describe metabolite profiles in pregnant women, newborns and children as well as their interrelationships. In **Chapter 4.4** we describe the associations of metabolite profiles in pregnant women and newborns with detailed measures of fetal growth and the risks of adverse birth outcomes.

Finally, **Chapter 5** provides a general discussion in which the main findings and implications of studies described in this thesis are discussed. English and Dutch summaries are provided in **Chapter 6**.

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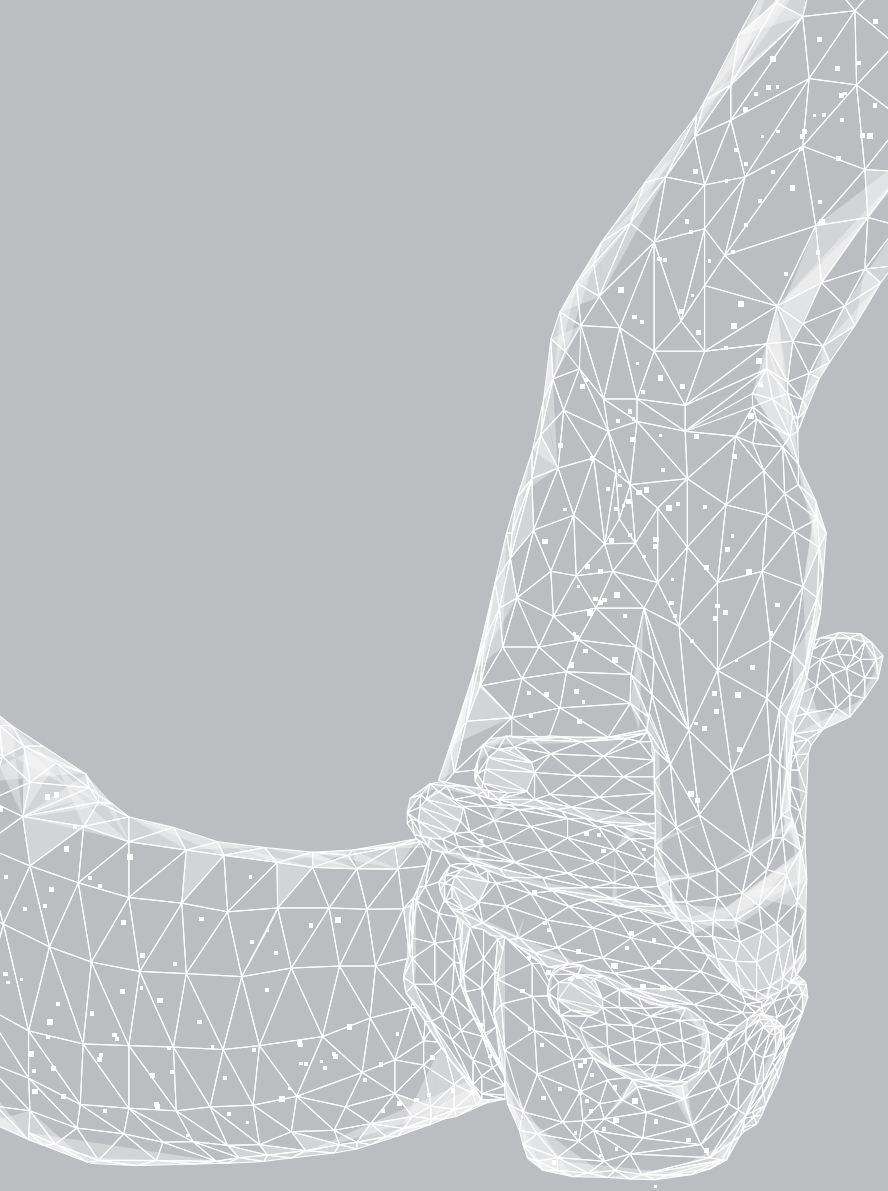
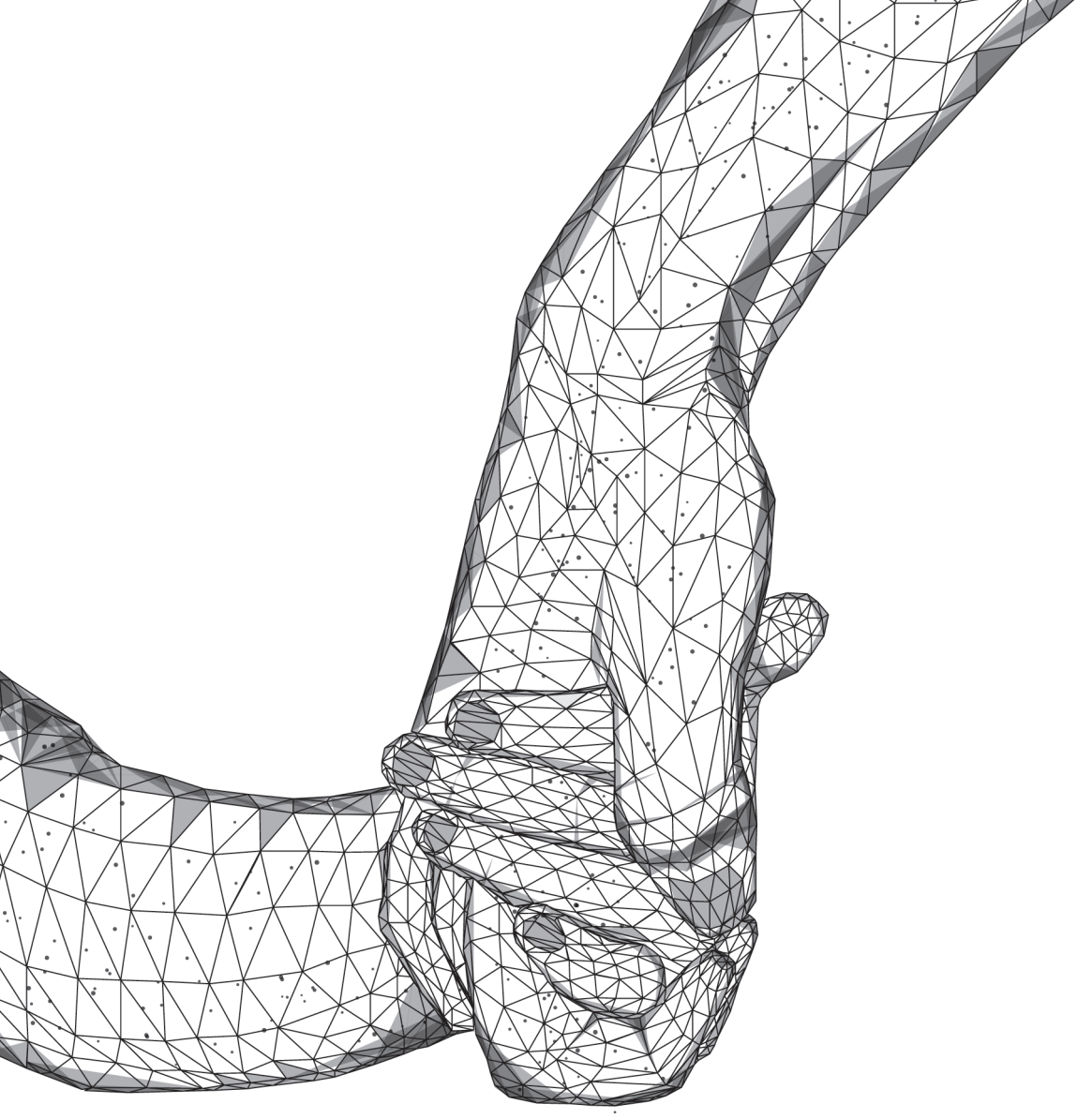


2 Maternal common dietary factors



2.1 Maternal caffeine intake during pregnancy, early growth, and childhood adiposity

Voerman E, Jaddoe VWV, Gisthi O, Hofman A, Franco OH, Gaillard R. Maternal caffeine intake during pregnancy, early growth, and body fat distribution at school age.

Adapted from: Obesity (Silver Spring). 2016;24(5):1170-7.

ABSTRACT

Objective: The associations of maternal caffeine intake during pregnancy with offspring growth patterns and body fat and insulin levels at school age were examined.

Methods: In a population-based birth cohort among 7,857 mothers and their children, maternal caffeine intake during pregnancy was assessed by questionnaires. Growth characteristics were measured from birth onward. At 6 years, body fat and insulin levels were measured.

Results: Compared to children whose mothers consumed <2 units of caffeine per day during pregnancy (1 unit of caffeine is equivalent to 1 cup of coffee (90 mg caffeine)), those whose mothers consumed ≥6 units of caffeine per day tended to have a lower weight at birth, higher weight gain from birth to 6 years, and higher body mass index from 6 months to 6 years. Both children whose mothers consumed 4-5.9 and ≥6 units of caffeine per day during pregnancy tended to have a higher childhood body mass index and total body fat mass. Only children whose mothers consumed ≥6 units of caffeine per day had a higher android/gynoid fat mass ratio.

Conclusions: These results suggest that high levels of maternal caffeine intake during pregnancy are associated with adverse offspring growth patterns and childhood body fat distribution.

INTRODUCTION

Caffeine is frequently consumed during pregnancy (1). Caffeine crosses the placenta and enters the fetal circulation freely (2). Fetal exposure to caffeine is prolonged as a result of a slow clearance of caffeine in pregnant women and slow fetal metabolism (3). Ours and other previous studies have reported associations of high levels of maternal caffeine intake during pregnancy with higher risks of low birth weight (4-7). High maternal caffeine intake during pregnancy also has been associated with impaired fetal length growth from the second trimester onward (7).

Although previous studies have consistently suggested that children born with a low birth weight are at higher risk of an adverse body fat distribution and insulin resistance in later life (8-12), not much is known about the direct long-term offspring consequences of maternal caffeine intake during pregnancy. A recent prospective cohort study in the United States among 615 mothers and children reported a higher overall risk of obesity before the age of 15 years in children exposed to any caffeine during pregnancy (13). Another recent study among 1,986 mothers and children in the United States did not observe consistent associations between maternal serum paraxanthine concentrations, the primary metabolite of caffeine, during pregnancy and childhood body mass index at the ages of 4 and 7 years (14). In addition, animal studies have shown a decreased expression of insulin-like growth factor-1 (IGF-1), IGF-1 receptors, and insulin receptors in the offspring of rats exposed to caffeine during pregnancy, suggesting that fetal exposure to caffeine may disturb early growth and glucose metabolism (15, 16). To the best of our knowledge, no previous studies have assessed the associations of maternal caffeine intake during pregnancy with early growth, detailed body fat outcomes, or insulin levels in childhood.

Therefore, in a population-based prospective cohort study from early pregnancy onward among 7,857 mothers and their children, we examined the associations of maternal caffeine intake from coffee and tea during pregnancy with repeatedly measured growth characteristics from birth until the age of 6 years and detailed body fat measures and insulin and c-peptide levels at the age of 6 years.

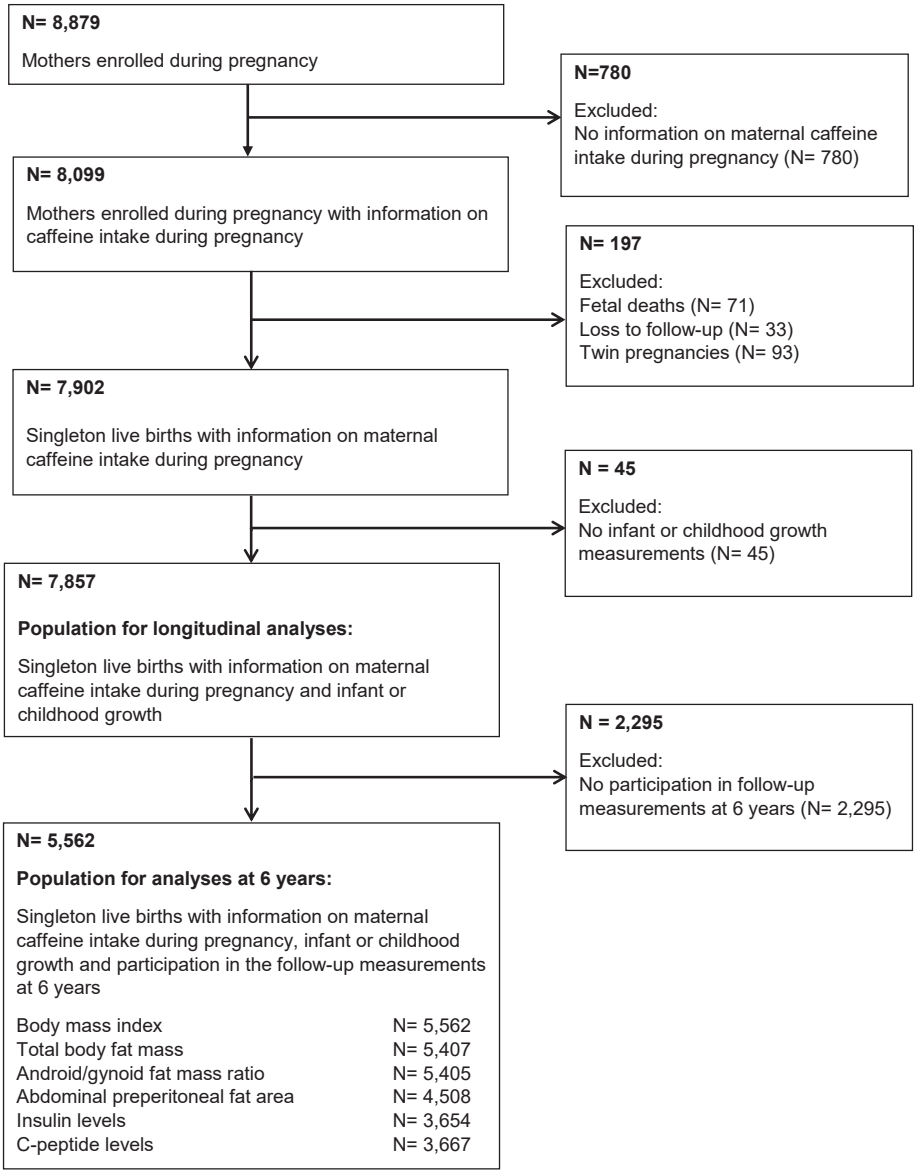
METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life until young adulthood performed in Rotterdam, the Netherlands (17, 18). Pregnant women were enrolled between 2001 and 2005. Of all eligible children, 61% participated in the study at birth. The study was approved by the local Medical Ethical Committee (MEC 198.782/2001/31). Written informed consent was obtained from all mothers.

In total, 8,879 mothers were enrolled during pregnancy, of whom 8,099 had information available on maternal caffeine intake during pregnancy. Of their children, 7,902 were singleton and live-born, 7,857 had data available on infant or childhood growth, and 5,562 participated in the follow-up measurements at 6 years and had data available on body mass index, body fat, insulin, or c-peptide levels (flow-chart is given in **Figure 1**).

Figure 1. Flow-chart of study participants



Maternal caffeine intake during pregnancy

Information on maternal caffeine intake during pregnancy was obtained by postal questionnaires in the first, second, and third trimester of pregnancy (7). Response rates for these questionnaires were 91%, 80%, and 77%, respectively (7). Mothers who reported to drink any coffee or tea were asked how many cups of coffee or tea on average they consumed per day and what type of coffee or tea they consumed (caffeinated, decaffeinated, or a combination of both). According to standard values for caffeine content, a regular coffee serving (125 mL) in the Netherlands contains ~90 mg caffeine, decaffeinated coffee contains ~3 mg, and tea contains ~45 mg (19). To calculate the total caffeine intake in each trimester, the type of coffee or tea was weighted according to its caffeine content (caffeinated coffee = 1, caffeinated and decaffeinated coffee = 0.5, decaffeinated coffee = 0, caffeinated tea = 0.5, caffeinated and decaffeinated tea = 0.25, decaffeinated tea = 0; herbal tea = 0, and green tea = 0.5) (7). Thus, in our analyses, each unit of caffeine intake reflects caffeine exposure based on 1 cup of caffeinated coffee (90 mg caffeine). Total maternal caffeine intake was subsequently categorized (<2, 2-3.9, 4-5.9, ≥6 units per day, equivalent to <180, 180-359, 360-539, and ≥540 mg per day, respectively). The average maternal caffeine intake of the trimesters of pregnancy was used for further analyses. When we used maternal caffeine intake in each trimester separately, results were similar (results not shown).

Infant and childhood growth

Information about length and weight at birth was obtained from medical records. Infant and childhood height and weight were measured using standardized methods at the ages of 6, 12, 24, 36, 48, and 72 months. We calculated body mass index (kg/m^2) from the age of 6 months onward. We created age- and sex-adjusted standard deviation scores (SDS) within our study population using North-European reference growth charts for birth measurements (20) and Dutch reference growth charts for infant and childhood measurements (Growth Analyzer 3.5, Dutch Growth Research Foundation) (21). We defined childhood overweight or obesity at the age of 72 months using the International Obesity Task Force cut offs (boys: body mass index ≥17.55 and ≥19.78, girls: body mass index ≥17.37 and ≥19.65 for overweight and obesity, respectively) (22).

Childhood body fat distribution

At the age of 6 years, we measured total and regional body fat mass using Dual-Energy X-ray absorptiometry (DXA) (iDXA, General Electrics-Lunar, 2008, Madison, WI) (23). Total body fat mass was calculated as a percentage of total body weight measured by DXA. Android/gynoid fat mass ratio was calculated (23). Pre-peritoneal fat mass was used as a proxy for visceral fat and was measured using abdominal ultrasound examinations with ultrasound LOGIQ E9 (GE Medical System, Wauwatosa, WI) and ATL-Philips Model HDI 5000 (Seattle, WA), as described in detail previously (24). Briefly, a linear (L12-5 MHz) transducer was placed

perpendicular to the skin surface on the median upper abdomen (25). We scanned longitudinally from the xiphoid process to the navel along the midline (linea alba). Pre-peritoneal fat mass was measured as areas of 2 cm length along the midline starting from the reference point in direction of the navel.

Childhood insulin and c-peptide levels

Childhood insulin (pmol/L) and c-peptide levels (nmol/L) were obtained enzymatically from 30 min fasting venous blood samples at the age of 6 years using a Cobas 8000 analyzer (Roche, Almere, the Netherlands). Quality control samples demonstrated intra- and inter-assay coefficients of variation of 1.39% and 2.40%, respectively.

Covariates

We assessed maternal age, pre-pregnancy body mass index, parity, ethnicity, educational level, and folic acid supplementation use by questionnaire at enrolment in the study. Smoking and alcohol consumption during pregnancy were repeatedly assessed by questionnaire. We obtained information on gestational hypertensive disorders (gestational hypertension and pre-eclampsia) and gestational diabetes, date of birth, and the child's sex from midwife and hospital registries. We obtained information on breastfeeding and the timing of introduction to solid foods by questionnaire during infancy. Average television-watching time was assessed by questionnaire at the age of 6 years.

Statistical analysis

First, we used unbalanced repeated measurement regression models to examine the associations of maternal caffeine intake during pregnancy with longitudinally measured growth characteristics. These models take the correlation between repeated measurements of the same subject into account and allow for incomplete outcome data. The models are described in more detail in **Supplemental Methods 1**.These models were adjusted for child's sex, and maternal and childhood socio-demographic and lifestyle-related characteristics.

Second, we used multiple linear regression models to examine the associations of maternal caffeine intake during pregnancy with childhood body fat distribution and insulin and c-peptide levels. These models were first adjusted for child's sex, age at follow-up measurement, and height at follow-up measurement (for fat mass outcomes only) and subsequently additionally adjusted for maternal and childhood socio-demographic and lifestyle-related characteristics. We included covariates in the models based on their associations with the outcomes of interest in previous studies, a significant association with the determinants and outcomes, or a change in effect estimates of >10%. To examine whether a dose–response relationship is present, we performed tests for trends by entering the categorized variable as a continuous term to the models. Finally, we used logistic regression models to examine the

associations of maternal caffeine intake during pregnancy with childhood overweight at the age of 6 years using similar adjustments.

In order to obtain normal distributions, we log transformed fat mass outcomes and square root transformed insulin and c-peptide levels. We constructed standard deviation scores (SDS) for all outcomes. Since no significant interaction between maternal caffeine intake during pregnancy and child's sex was present (all P-values >0.05), we performed no sex-stratified analyses. We used Multiple Imputation for missing values of covariates, by generating five independent datasets using the Markov Chain Monte Carlo (MCMC) method (26). We included all covariates in the imputation model. In addition, childhood body mass index and insulin levels at the age of 6 years, maternal pre-pregnancy weight, maternal height, income, maternal caffeine intake, and paternal body mass index were used as predictors only, and were not imputed themselves. Percentages missing values in the population for analysis were all lower than 22%, except for timing of introduction of solid foods (37.6%). Pooled effect estimates were presented. The repeated measurement analysis was performed using the Statistical Analysis System version 9.3 (SAS, Institute Inc., Cary, NC). All other analyses were performed using the Statistical Package of Social Sciences version 22.0 for Windows (IBM Corp., Armonk, NY).

RESULTS

Study population

Table 1 shows that, as compared to mothers who consumed <2 units of caffeine per day during their pregnancy, those who consumed ≥6 units per day were more likely to be higher educated, nulliparous, and from European descent. Their children had a lower birth weight and a higher body mass index at the age of 6 years (P-values< 0.05). **Supplemental Table 1** shows infant and childhood growth characteristics. **Supplemental Table 2** shows limited to moderate correlations between the outcome measures at 6 years. Non-response analyses at baseline (**Supplemental Table 3**) and at follow-up measurement (**Supplemental Table 4**) showed that both mothers excluded because of missing data on caffeine intake during pregnancy and mothers lost to follow-up were lower educated and less often of European descent, compared to those included in the analysis. Their children had a lower birth weight. However, no large differences were observed between the caffeine intake during pregnancy of mothers of children not included in the analyses at 6 years and mothers of children included in the analyses (median (95% range) caffeine intake: 1.3 units (0, 5.0) vs. 1.5 units (0, 5.0)).

Table 1. Characteristics of the mothers and their children (N=7,857)[1]

	Maternal caffeine intake during pregnancy[2]					
	Total group	**<2 units**	**2-3.9 units**	**4-5.9 units**	**≥6 units**	**P-value[3]**
	N=7,857	**N=4,804**	**N=2,447**	**N=496**	**N=110**	
Maternal characteristics						
Age, median (95% range), years	30.4 (19.3, 39.2)	29.3 (18.7, 38.8)	31.5 (20.5, 39.9)	32.6 (21.2, 40.3)	32.8 (22.2, 40.8)	<0.001
Height, mean (SD), cm	167.4 (7.4)	166.6 (7.4)	168.4 (7.2)	169.4 (7.3)	167.9 (7.1)	<0.001
Pre-pregnancy weight, median (95% range), kg	64.0 (48.0, 99.0)	63.0 (48.0, 100.0)	65.0 (49.0, 95.0)	65.0 (49.0, 100.1)	67.5 (48.1, 100.3)	0.076
Pre-pregnancy BMI, median (95% range), kg/m^2	22.6 (17.9, 35.0)	22.6 (17.8, 35.6)	22.6 (18.1, 34.1)	22.6 (18.0, 35,5)	22.8 (18.4, 33.0)	0.108
Education, No. (%)						
Primary	421 (5.9)	295 (6.8)	106 (4.8)	18 (4.0)	2 (1.9)	<0.001
Secondary	3,070 (43.3)	2,021 (46.8)	814 (36.6)	176 (39.5)	59 (56.7)	
Higher	3,600 (50.8)	2,003 (46.4)	1,302 (58.6)	252 (56.5)	43 (41.3)	
Parity, No. nulliparous (%)	4,405 (56.4)	2,838 (59.5)	1,278 (52.4)	243 (49.2)	46 (42.2)	<0.001
Ethnicity, No. European (%)	4,485 (58.5)	2,387 (51.1)	1,636 (67.9)	378 (77.6)	84 (77.1)	<0.001
Folic acid supplementation use, No. Yes (%)	4,375 (71.3)	2,558 (69.1)	1,466 (75.1)	302 (75.7)	49 (61.3)	<0.001
Smoking during pregnancy, No. Yes (%)	1,336 (18.6)	645 (14.7)	493 (22.0)	144 (31.7)	54 (51.4)	<0.001
Alcohol consumption during pregnancy, No. Yes (%)	2,672 (37.6)	1,339 (30.7)	1,062 (48.0)	215 (48.8)	56 (54.4)	<0.001
Gestational diabetes, No. Yes (%)	80 (1.1)	48 (1.0)	25 (1.1)	7 (1.5)	0 (0.0)	N/A[4]
Pre-eclampsia, No. Yes (%)	153 (2.1)	99 (2.2)	37 (1.6)	16 (3.4)	1(1.0)	0.072
Gestational hypertension, No Yes (%)	293 (3.9)	187 (4.1)	87 (3.7)	15 (3.2)	4 (3.8)	0.775

Table 1. Characteristics of the mothers and their children (N=7,857)[1] (continued)

	Maternal caffeine intake during pregnancy[2]					
	Total group	**<2 units**	**2-3.9 units**	**4-5.9 units**	**≥6 units**	**P-value[3]**
	N=7,857	**N=4,804**	**N=2,447**	**N=496**	**N=110**	
Child characteristics						
Males, No. (%)	3,957 (50.4)	2,452 (51.1)	1,176 (48.1)	267 (53.8)	62 (56.4)	0.018
Gestational age at birth, median (95% range), weeks	40.1 (35.7, 42.3)	40.1 (35.6, 42.3)	40.1 (36.0, 42.3)	40.3 (33.3, 42.4)	40.2 (33.3, 42.5)	0.001
Birth weight, median (95% range), g	3435 (2242, 4490)	3420 (2250, 4450)	3460 (2260, 4520)	3490 (2144, 4567)	3380 (1980, 4300)	<0.001
Ever breastfeeding, No. Yes (%)	5,282 (91.9)	3,131 (92.1)	1,733 (92.0)	353 (90.7)	65 (87.8)	0.467
Introduction of solid foods, No. before 6 months (%)	3,859 (89.5)	2,208 (88.9)	1,321 (90.0)	282 (91.6)	48 (92.3)	0.349
Age at 6 year follow-up measurement, median (95% range), years	6.0 (5.6, 7.9)	6.0 (5.6, 8.0)	6.0 (5.6, 7.8)	6.0 (5.5, 7.4)	6.1 (5.6, 7.5)	0.042
Television watching, No. More than 2 hours/day (%)	934 (19.2)	588 (21.0)	284 (17.3)	47 (13.4)	15 (23.4)	0.001
Body mass index at 6 years, median (95% range), kg/m2	15.9 (13.6, 21.3)	15.9 (13.6, 21.3)	15.7 (13.7, 20.9)	16.0 (13.8, 21.4)	16.2 (13.9, 21.0)	0.001
Overweight at 6 years, No. Yes (%)	991 (17.8)	633 (19.2)	280 (15.4)	63 (17.1)	15 (18.8)	0.008
Total body fat mass, median (95% range), %	24.0 (16.3, 38.8)	24.1 (16.2, 39.2)	23.9 (16.6, 38.2)	24.0 (16.3, 38.2)	23.2 (15.8, 41.6)	0.198
Android/gynoid fat mass ratio, median (95% range)	0.24 (0.16, 0.42)	0.24 (0.16, 0.42)	0.24 (0.16, 0.42)	0.24 (0.16, 0.43)	0.25 (0.17, 0.43)	0.197
Abdominal preperitoneal fat area, median (95% range), cm^2	0.39 (0.16, 1.21)	0.40 (0.16, 1.27)	0.39 (0.16, 1.15)	0.39 (0.15, 1.03)	0.33 (0.14, 1.19)	0.002
Insulin, median (95% range), (pmol/L)	115 (17, 402)	115 (16, 395)	114 (18, 408)	132 (16, 467)	91 (14, 369)	0.015
C-peptide median (95% range), (nmol/L)	0.96 (0.30, 2.14)	0.97 (0.30, 2.15)	0.95 (0.32, 2.09)	1.06 (0.26, 2,48)	0.88 (0.36, 2,16)	0.039

[1]Values represent means (SD), median (95% range) or number of subjects (valid %).
[2]1 unit of caffeine intake represents the equivalent of 1 cup of coffee (90 mg caffeine).
[3] Differences in subject characteristics between the groups were tested using One-Way ANOVA for continuous variables and Chi-square tests for proportions.
[4]Not available as a result of small numbers.

Infant and childhood growth patterns

Figure 2 A-C shows the associations of maternal caffeine intake during pregnancy with repeatedly measured growth characteristics from birth to 72 months, obtained from repeated measurement regression models. As compared to children whose mothers consumed <2 units of caffeine per day during pregnancy, children whose mothers consumed ≥6 units of caffeine per day were shorter, but these differences decreased over time. At the age of 6 years, children whose mothers consumed ≥6 units of caffeine per day still tended to be shorter. These children also had lower birth weights and had higher weight gain from birth to 72 months. Body mass index tended to be higher from 6 months to 72 months in children whose mothers consumed ≥6 units of caffeine per day during pregnancy, as compared to children whose mothers consumed <2 units of caffeine per day.

Childhood body fat distribution

Compared to children whose mothers consumed <2 units of caffeine per day during their pregnancy, both those whose mothers consumed 4-5.9 units and ≥6 units of caffeine per day tended to have a higher childhood body mass index (differences: 0.09 Standard deviation score (SDS) (95% confidence interval (CI): -0.01, 0.19) and 0.16 SDS (95% CI: -0.03, 0.36), respectively) and a higher childhood total body fat mass (differences: 0.10 SDS (95% CI: 0.01, 0.20) and 0.18 SDS (95% CI: -0.01, 0.37), respectively) (**Table 2**). Only children whose mothers consumed ≥6 units of caffeine per day during their pregnancy had a higher childhood android/gynoid fat mass ratio (difference: 0.27 SDS (95% CI: 0.05, 0.49)). Similar tendencies were present when we combined the upper two maternal caffeine intake categories into one category (results not shown). **Supplemental Table 5** shows similar results from the basic models. **Supplemental Table 6** shows that as compared to children whose mothers consumed <2 units of caffeine per day during pregnancy, those whose mothers consumed ≥6 units of caffeine per day tended to have higher risks of childhood overweight (odds ratio (OR): 1.25 (95% CI: 0.68, 2.30)) in the fully adjusted model.

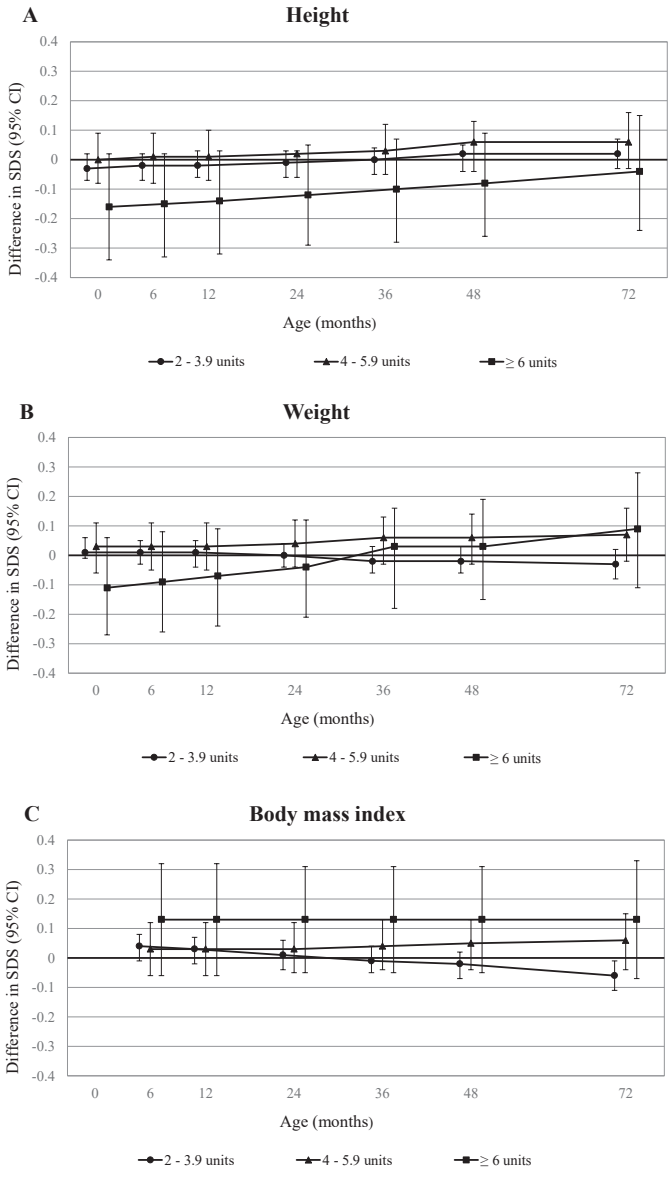
Childhood insulin and c-peptide levels

Table 3 shows no consistent associations of maternal caffeine intake during pregnancy with childhood insulin and c-peptide levels in the fully adjusted models. Similar results were present in the basic models (**Supplemental Table 7**).

DISCUSSION

We observed that, as compared to children whose mothers consumed no or less than 2 units of caffeine per day during their pregnancy, children whose mothers consumed 6 units of caffeine or more per day tended to have a lower weight at birth, higher weight gain from

Figure 2. Associations of maternal caffeine intake during pregnancy with longitudinally measured growth characteristics (N=7,857)



Results are based on repeated linear regression models and reflect the differences in SDS of (A) height (based on 51,691 measurements), (B) weight (based on 58,124 measurements) and (C) body mass index (based on 36,953 measurements) growth in children whose mothers consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day during pregnancy, respectively, as compared to those whose mothers consumed <2 units of caffeine per day. 1 unit of caffeine intake represents the equivalent of 1 cup of coffee (90 mg caffeine). The reference value is an SDS of 0. The models were adjusted for child's sex, maternal age, pre-pregnancy body mass index, parity, ethnicity, educational level, folic acid supplementation use, smoking and alcohol consumption during pregnancy, pregnancy complications, breastfeeding and timing of introduction of solid foods. All p-values for interaction <0.05.

Table 2. Maternal caffeine intake during pregnancy and childhood body fat distribution at 6 years (fully adjusted models) (N=5,562)

	Body mass index (SDS)	Total body fat mass (SDS)	Android/gynoid fat mass ratio (SDS)	Abdominal preperitoneal fat area (SDS)
	N=5,562	**N=5,407**	**N=5,405**	**N=4,508**
Maternal caffeine intake categories				
< 2 units	Reference	Reference	Reference	Reference
	N=3,295	*N= 3,199*	*N=3,197*	*N=2,658*
2 – 3.9 units	-0.03 (-0.08, 0.03)	0.02 (-0.03, 0.07)	0.04 (-0.02, 0.09)	-0.05 (-0.11, 0.01)
	N= 1,819	*N=1,771*	*N=1,771*	*N= 1,475*
4 – 5.9 units	0.09 (-0.01, 0.19)	0.10 (0.01, 0.20)*	0.02 (-0.09, 0.13)	-0.04 (-0.15, 0.07)
	N=368	*N=357*	*N=357*	*N=306*
≥ 6 units	0.16 (-0.03, 0.36)	0.18 (-0.01, 0.37)	0.27 (0.05, 0.49)*	-0.15 (-0.37, 0.07)
	N=80	*N=80*	*N=80*	*N=69*
P-value for trend	0.220	0.015	0.068	0.075

Values are regression coefficients (95% confidence interval) that reflect the difference in childhood outcomes in children whose mothers consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day during pregnancy, respectively, as compared to those whose mothers consumed <2 units of caffeine per day. 1 unit of caffeine intake represents the equivalent of 1 cup of coffee (90 mg caffeine). The models were adjusted for child's sex, age at follow-up measurement, height at follow-up measurement, maternal age, pre-pregnancy body mass index, parity, ethnicity, educational level, folic acid supplementation use, smoking and alcohol consumption during pregnancy, gestational diabetes, gestational hypertensive disorders, birth weight, gestational age at birth, breastfeeding, introduction of solid foods and television-watching time. P-values for trend were obtained from models in which the categorized caffeine intake variable was entered as continuous variable. * P-value <0.05.

birth to 6 years and a higher body mass index from 6 months to 6 years. Also, at the age of 6 years, children of mothers with higher levels of caffeine intake during pregnancy tended to have a higher childhood total body fat mass and android/gynoid fat mass ratio. We did not observe differences for childhood insulin or c-peptide levels.

Strengths and limitations

We used a large population-based cohort followed from early pregnancy onward. In total, 30% of the eligible participants with information on maternal caffeine intake during pregnancy were not participating in follow-up measurements at 6 years. This loss to follow-up could have reduced the statistical power of our study and could have led to biased effect estimates if associations of interest differ between children included and not included in the analysis. This seems unlikely, since only minor differences were observed between the caffeine intake during pregnancy of mothers of children not included in the analysis and the caffeine intake of mothers of children included in the analysis. Since maternal caffeine intake during pregnancy was self-reported, misclassification by underreporting may be present. In addition, in accordance with the Netherlands Nutrition Centre (27), we assumed that coffee

Table 3. Maternal caffeine intake during pregnancy and childhood insulin and c-peptide levels at 6 years (fully adjusted models) (N=3,667)

	Insulin (SDS)	C-peptide (SDS)
	N=3,654	**N=3,667**
Maternal caffeine intake categories		
< 2 units	Reference	Reference
	N= 2,116	*N=2,128*
2 -3.9 units	-0.03 (-0.11, 0.04)	-0.05 (-0.12, 0.03)
	N= 1,239	*N=1,237*
4 – 5.9 units	0.14 (0.01, 0.28)*	0.10 (-0.03, 0.24)
	N= 241	*N=243*
≥ 6 units	-0.18 (-0.45, 0.08)	-0.13 (-0.39, 0.14)
	N= 58	*N=59*
P-value for trend	0.900	0.869

Values are regression coefficients (95% confidence interval) that reflect the difference in childhood outcomes in children whose mothers consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day during pregnancy, respectively, as compared to those whose mothers consumed <2 units of caffeine per day. 1 unit of caffeine intake represents the equivalent of 1 cup of coffee (90 mg caffeine). The models were adjusted for child's sex, age at follow-up measurement, maternal age, pre-pregnancy body mass index, parity, ethnicity, educational level, folic acid supplementation use, smoking and alcohol consumption during pregnancy, gestational diabetes, gestational hypertensive disorders, birth weight, gestational age at birth, breastfeeding, introduction of solid foods and television-watching time. P-values for trend were obtained from models in which the categorized caffeine intake variable was entered as continuous variable. *P-value <0.05.

was consumed in cups of 125 mL. However, this might have differed between participants, which may have led to some misclassification of the categories of maternal caffeine intake. We also assessed only caffeine intake from coffee and tea and not intake from other sources, such as soft drinks, chocolate, and medications. However, at the time of data collection (2002-2006), coffee and tea accounted for 70 and 26%, respectively, of all caffeine ingested (1). We categorized maternal caffeine intake during pregnancy in units of caffeine instead of calculating the exact milligrams of caffeine consumed per day. The highest category of maternal caffeine intake in our study (≥6 units) should be considered equivalent to a caffeine intake of ≥540 mg per day. However, since caffeine contents per unit of coffee might differ between countries, our results should be interpreted carefully with regard to other populations. We were able to adjust our analyses for many possible confounders. However, as in any observational study, residual confounding might still be an issue. For example, in our study we were unable to adjust the analyses for detailed maternal and childhood dietary habits.

Interpretation of main findings

Maternal caffeine intake during pregnancy may affect fetal growth and development. Among the same population as the present study and in line with other large observational studies

(4-6), we previously reported that high levels of maternal caffeine intake during pregnancy are associated with impaired fetal growth and higher risks of low birth weight (7).

Not much is known about the long-term offspring consequences of maternal caffeine intake during pregnancy. A recent study in the United States among 615 mothers and their children suggested that any caffeine intake during pregnancy was associated with an 87% higher overall risk of childhood obesity before the age of 15 years. Also, a dose–response relation for maternal caffeine intake was observed (13). In contrast, another recent study among 1,986 mothers and their children in the United States did not observe consistent associations of maternal serum paraxanthine concentrations, caffeine's primary metabolite, during pregnancy with childhood body mass index (14). We observed that children of mothers with the highest caffeine intake during pregnancy were shorter and had lower weights at birth, as compared to children of mothers with low caffeine intake, but gained more weight from birth to 6 years. Also, they tended to have higher body mass indexes from 6 months to 6 years. We observed a tendency to a higher risk of overweight at the age of 6 years in children of mothers with the highest caffeine intake during pregnancy, although not statistically significant. This may be due to smaller numbers, since only 80 mothers in our study consumed 6 units of caffeine per day or more. Thus, these findings suggest that maternal caffeine intake during pregnancy might not only affect fetal development, but may have persistent consequences for childhood growth.

Although body mass index is a widely accepted measure of adiposity, previous studies have shown that more specific body fat measures, such as total fat mass, waist circumference, and waist to hip ratio, are predictors of cardiovascular risk factors and disease in children and adults, independent of body mass index (28-30). Thus, detailed body composition measures provide useful additional information in assessing adiposity and its consequences. To the best of our knowledge, no previous studies have been performed focused on the associations of maternal caffeine intake during pregnancy with detailed measures of childhood body fat distribution. We observed that high maternal caffeine intake during pregnancy tended to be associated with a higher childhood total body fat mass and an adverse childhood body fat distribution, as reflected by a higher android/gynoid fat mass ratio. We observed no effect of maternal caffeine intake during pregnancy on pre-peritoneal fat mass. This discrepancy could be attributed to a larger measurement error for preperitoneal fat mass measurements in childhood (31, 32). Thus, our results suggest that maternal caffeine intake during pregnancy may affect childhood total fat and body fat distribution, next to body mass index.

Studies in adults showed that coffee consumption is consistently associated with lower risks of insulin resistance and type 2 diabetes (33-35). However, associations with both caffeinated and decaffeinated coffee were observed, suggesting that next to caffeine, other coffee components may also play a role in the underlying mechanisms. In contrast to these findings in non-pregnant adults, animal studies suggest that maternal caffeine intake during pregnancy may increase insulin resistance and disturb glucose metabolism in the offspring

(15, 16). We did not observe consistent associations of maternal caffeine intake during pregnancy with childhood insulin and c-peptide levels. Our results should be interpreted carefully, since the fasting period before blood draw for the childhood insulin and c-peptide measurements was limited. This may have led to some non-differential misclassification and an underestimation of the observed effect estimates. This may especially affect childhood insulin levels, which are less stable and have a shorter half-life as compared to c-peptide levels. Further studies are needed to assess the detailed associations of maternal caffeine intake during pregnancy with offspring glucose and insulin metabolism.

The mechanisms by which maternal caffeine intake during pregnancy might influence childhood body fat distribution are not clear. It has been suggested that caffeine induces an increase in circulating maternal and fetal glucocorticoid concentrations (36, 37). Studies in rats suggest that fetal overexposure to glucocorticoids leads to an altered development of the hypothalamic–pituitary–adrenal axis (HPA-axis), impaired fetal growth, altered structure of the endocrine pancreas, insulin target-tissues and adipose depots, and increased HPA-axis activity (37-39). Whether this mechanism partly underlies the observed associations needs to be further studied.

Although the observed effect estimates are small and without direct individual clinical consequence, our results suggest that maternal caffeine intake during pregnancy is associated with infant and childhood growth and body fat distribution. As caffeine is frequently consumed during pregnancy and the prevalence of obesity is still rising (40), our results underline the need to study the long-term health consequences of maternal caffeine intake during pregnancy.

Conclusion

Our results suggest that high levels of maternal caffeine intake during pregnancy are associated with adverse offspring growth patterns and childhood body fat distribution, but not with childhood insulin and c-peptide levels. Further studies are needed to assess whether maternal caffeine intake during pregnancy affects long-term offspring health outcomes, as well as the causality and underlying mechanisms.

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SUPPLEMENTAL MATERIAL

Supplemental Methods 1. Unbalanced repeated measurement regression models

We used unbalanced repeated measurement regression models to analyze the infant and childhood growth patterns among children whose mothers consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day during their pregnancy, as compared to children whose mothers consumed <2 units of caffeine per day. These models allow for incomplete outcome data and take the correlation between repeated measurements of the same subject into account by modelling the correlated errors of these measurements (1, 2). To model the correlated errors, a compound symmetry covariance structure was assumed. The models can be written as:

Height (SDS) = ß0 + ß1 × caffeine intake category + ß2 × age + ß3 × caffeine intake category × age

Weight (SDS) = ß0 + ß1 × caffeine intake category + ß2 × age + ß3 × caffeine intake category × age

Body mass index (SDS) = ß0 + ß1 × caffeine intake category + ß2 × age + ß3 × caffeine intake category × age

In these models, 'ß0 + ß1 × caffeine intake category' reflects the intercept. The intercept reflects the mean growth characteristic value in SDS for each caffeine intake category. The term 'ß2 × age' reflects the change in growth characteristics per month. The term 'ß3 × caffeine intake category × age', reflects the difference in change in growth characteristics per month between the different caffeine intake categories.

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Supplemental Table 1. Childhood growth characteristics (N=7,857)[1]

	Maternal caffeine intake during pregnancy[2]					
	Total group	< 2 units	2 -3.9 units	4-5.9 units	≥6 units	P-value[3]
	N=7,857	N=4,804	N=2,447	N=496	N=110	
Birth						
Gestational age at birth, median (95% range), weeks	40.1 (35.7, 42.3)	40.1 (35.6, 42.3)	40.1 (36.0, 42.3)	40.3 (33.3, 42.4)	40.2 (33.3, 42.5)	0.001
Birth length, mean (SD), cm	50.2 (2.4)	50.2 (2.4)	50.2 (2.4)	50.3 (2.2)	50.6 (2.5)	0.216
Birth weight, median (95% range), g	3435 (2242, 4490)	3420 (2250, 4450)	3460 (2260, 4520)	3490 (2144, 4567)	3380 (1980, 4300)	<0.001
6 months						
Age at follow-up, median (95% range), months	6.2 (5.2, 8.3)	6.2 (5.2, 8.3)	6.2 (5.2, 8.3)	6.2 (5.3, 8.1)	6.2 (5.3, 9.4)	0.530
Length, mean (SD), cm	67.6 (2.7)	67.7 (2.7)	67.6 (2.7)	67.8 (2.7)	67.2 (2.4)	0.375
Weight, median (95% range), kg	7.9 (6.2, 9.8)	7.9 (6.2, 9.8)	7.8 (6.2, 9.8)	7.8 (6.3, 10.0)	7.8 (6.4, 9.8)	0.110
Body mass index, median (95% range), kg/m^2	17.2 (14.7, 20.3)	17.2 (14.7, 20.3)	17.1 (14.7, 20.3)	17.2 (14.4, 20.4)	17.6 (15.1, 20.0)	0.012
12 months						
Age at follow-up, median (95% range), months	11.1 (10.1, 12.5	11.1 (10.1, 12.6)	11.1 (10.1, 12.5)	11.1 (10.2, 12.5)	11.1 (10.0, 12.7)	0.705
Length, mean (SD), cm	74.4 (2.7)	74.4 (2.7)	74.3 (2.6)	74.5 (2.9)	74.1 (2.7)	0.329
Weight, median (95% range), kg	9.6 (7.7, 12.0)	9.7 (7.6, 11.9)	9.6 (7.6, 11.9)	9.6 (7.7, 12.4)	9.5 (7.9, 12.1)	0.672
Body mass index, median (95% range), kg/m^2	17.4 (14.9, 20.3)	17.4 (14.9, 20.2)	17.4 (14.9, 20.5)	17.4 (14.6, 20.6)	17.4 (15.3, 20.7)	0.959
24 months						
Age at follow-up, median (95% range), months	25.0 (23.4, 31.4)	25.0 (23.4, 31.2)	24.9 (23.4, 31.4)	25.1 (23.5, 31.7)	24.6 (23.4, 30.4)	0.374
Height, mean (SD), cm	88.7 (3.8)	88.8 (3.8)	88.6 (3.7)	88.9 (4.0)	88.2 (3.2)	0.216
Weight, median (95% range), kg	12.9 (10.3, 16.5)	12.9 (10.3, 16.5)	12.9 (10.3, 16.6)	13.0 (10.4, 16.3)	13.2 (9.7, 16.1)	0.924
Body mass index, median (95% range), kg/m^2	16.5 (14.1, 19.6)	16.5 (14.0, 19.5)	16.5 (14.1, 20.0)	16.6 (14.1, 18.9)	16.8 (13.9, 19.0)	0.296

Supplemental Table 1. Childhood growth characteristics (N=7,857)[1] (continued)

	Maternal caffeine intake during pregnancy[2]					
	Total group	< 2 units	2 -3.9 units	4-5.9 units	≥6 units	P-value[3]
	N=7,857	N=4,804	N=2,447	N=496	N=110	
36 months						
Age at follow-up, median (95% range), months	36.7 (35.4, 40.8)	36.7 (35.3, 408)	36.7 (35.4, 40.8)	36.7 (35.4, 40.8)	36.7 (35.2, 41.5)	0.997
Height, mean (SD), cm	97.4 (3.8)	97.4 (3.8)	97.4 (3.8)	97.5 (3.8)	97.1 (3.4)	0.833
Weight, median (95% range), kg	15.1 (12.0, 19.5)	15.0 (12.0, 19.5)	15.1 (12.1, 19.5)	15.3 (12.3, 19.0)	15.5 (12.2, 21.7)	0.549
Body mass index, median (95% range), kg/m^2	15.9 (13.7, 19.0)	15.9 (13.7, 19.0)	15.9 (13.8, 19.1)	16.0 (14.0, 18.8)	16.6 (14.2, 20.8)	0.149
48 months						
Age at follow-up, median (95% range), months	45.8 (44.4, 48.6)	45.8 (44.5, 48.7)	45.8 (44.4, 48.4)	45.9 (44.4, 48.7)	46.0 (44.3, 49.1)	0.807
Height, mean (SD), cm	103.2 (4.2)	103.2 (4.2)	103.2 (4.3)	103.3 (4.2)	103.1 (3.9)	0.986
Weight, median (95% range), kg	16.7 (13.3, 22.1)	16.7 (13.2, 22.2)	16.7 (13.3, 22.1)	17.0 (13.3, 21.4)	17.3 (12.8, 25.7)	0.269
Body mass index, median (95% range), kg/m^2	15.7 (13.6, 19.2)	15.7 (13.5, 19.3)	15.7 (13.5, 19.9)	15.9 (13.8, 19.0)	16.1 (14.0, 20.8)	0.068
72 months						
Age at follow-up, median (95% range), months	72.6 (67.8, 95.6)	72.7 (67.9, 96.9)	72.5 (67.6, 93.3)	72.6 (66.7, 88.8)	73.0 (67.7, 90.7)	0.042
Height, mean (SD), cm	119.5 (6.0)	119.5 (6.2)	119.5 (5.9)	119.5 (5.9)	119.1 (5.1)	0.960
Weight, median (95% range), kg	22.6 (17.4, 34.2)	22.6 (14.4, 34.4)	22.4 (17.6, 33.8)	22.8 (17.8, 33.8)	22.8 (16.8, 34.2)	0.071
Body mass index at 6 years, median (95% range), kg/m^2	15.9 (13.6, 21.3)	15.9 (13.6, 21.3)	15.7 (13.7, 20.9)	16.0 (13.8, 21.4)	16.2 (13.9, 21.0)	0.001

[1]Values represent means (SD), median (95% range) or number of subjects (valid %). [2]1 unit of caffeine intake represents the equivalent of 1 cup of coffee (90 mg caffeine).
[3]Differences in subject characteristics between the groups were tested using One-Way ANOVA for continuous variables and Chi-square tests for proportions.

Supplemental Table 2. Correlation coefficients between childhood body fat measures and insulin and c-peptide levels (N=5,562) [1]

Spearman r_s	Body mass index	Total body fat mass	Android/ gynoid fat mass ratio	Preperitoneal fat area	Insulin	C-peptide
Body mass index	1	0.57 P<0.001	0.47 P<0.001	0.42 P<0.001	0.13 P<0.001	0.09 P<0.001
Total body fat mass	0.57 P<0.001	1	0.55 P<0.001	0.55 P<0.001	0.09 P<0.001	0.06 P=0.001
Android/gynoid fat mass ratio	0.47 P<0.001	0.55 P<0.001	1	0.40 P<0.001	0.09 P<0.001	0.08 P<0.001
Preperitoneal fat area	0.42 P<0.001	0.55 P<0.001	0.40 P<0.001	1	0.09 P<0.001	0.06 P=0.001
Insulin	0.13 P<0.001	0.09 P<0.001	0.09 P<0.001	0.09 P<0.001	1	0.88 P<0.001
C-peptide	0.09 P<0.001	0.06 P=0.001	0.08 P<0.001	0.06 P=0.001	0.88 P<0.001	1

[1]Values are correlation coefficients using Spearman's rho tests for skewed variables.

Supplemental Table 3. Non-response analysis at baseline (N=8,879)[1]

	Maternal caffeine intake during pregnancy available	Excluded because of missing information on maternal caffeine intake during pregnancy	P-value[2]
	N= 8,099	N= 780	
Maternal characteristics			
Age, median (95% range), years	30.4 (19.3, 39.3)	28.4 (18.7, 39.3)	<0.001
Height, mean (SD), cm	167.4 (7.4)	164.3 (7.3)	<0.001
Pre-pregnancy weight, median (95% range), kg	64.0 (48.0, 99.0)	63.0 (46.6, 100.0)	0.418
Pre-pregnancy body mass index, median (95% range), kg/m^2	22.6 (17.9, 35.0)	23.3 (17.6, 35.6)	0.048
Education, No. (%)			
Primary	436 (6.0)	47 (14.1)	<0.001
Secondary	3160 (43.2)	179 (53.6)	
Higher	3712(50.8)	108 (32.3)	
Parity, No. nulliparous (%)	4536 (56.4)	386 (54.3)	0.001
Ethnicity, No. European (%)	4618 (58.4)	150 (31.5)	<0.001
Folic acid supplementation use, No. Yes (%)	4504 (71.2)	128 (53.8)	<0.001
Smoking during pregnancy, No. Yes (%)	1381 (18.6)	59 (18.6)	0.997
Alcohol consumption during pregnancy, No. Yes (%)	2737 (37.3)	49 (16.1)	<0.001
Gestational diabetes, No. Yes (%)	82 (1.1)	9 (1.3)	0.600
Pre-eclampsia, No. Yes (%)	169 (2.3)	18 (2.5)	0.633
Gestational hypertension, No. Yes (%)	301 (4.0)	17 (2.4)	0.039
Child characteristics			
Males, No. (%)	4024 (50.4)	377 (51.3)	0.627
Birth weight, median (95% range), g	3425 (2200, 4482)	3340 (1948, 4460)	<0.001
Gestational age at birth, median (95% range), weeks	40.1 (35.4, 42.3)	40.0 (32.3, 42.4)	<0.001
Ever breastfeeding, No. Yes (%)	5336 (91.9)	275 (95.5)	0.028
Introduction of solid foods, No. before 6 months (%)	3899 (89.4)	123 (89.8)	0.900

[1]Values represent means (SD), median (95% range) or number of subjects (valid %). [2]Differences in subject characteristics between the groups were tested using Independent Samples T-tests for continuous variables and Chi-square tests for proportions.

Supplemental Table 4. Non-response analysis at follow-up measurement (N=7,902)[1]

	Follow-up at 6 years	Lost to follow-up at 6 years	P-value[2]
	N= 5,562	N= 2,340	
Maternal characteristics			
Age, median (95% range), years	30.9 (19.8, 39.4)	28.8 (18.5, 38.5)	<0.001
Height, mean (SD), cm	167.7 (7.4)	166.6 (7.3)	<0.001
Pre-pregnancy weight, median (95% range), kg	64.0 (49.0, 98.0)	63.0 (47.0, 100.0)	0.145
Pre-pregnancy body mass index, median (95% range), kg/m^2	22.6 (18.1, 34.6)	22.6 (17.7, 35.6)	0.300
Caffeine intake during pregnancy, median (95% range), units	1.5 (0, 5.0)	1.3 (0, 5.0)	<0.001
Caffeine intake during pregnancy, No. (%)			
<2 units	3,295 (59.2)	1,545 (66.0)	<0.001
2 – 3.9 units	1,819 (32.7)	635 (27.1)	
4 – 5.9 units	368 (6.6)	130 (5.6)	
≥ 6 units	80 (1.4)	30 (1.3)	
Education, No. (%)			
Primary	272 (5.4)	152 (7.2)	<0.001
Secondary	1,976 (39.3)	1,116 (53.1)	
Higher	2,780(55.3)	833 (39.6)	
Parity, No. nulliparous (%)	3,195 (57.7)	1,238 (53.5)	0.001
Ethnicity, No. European (%)	3,412 (62.1)	1,091 (49.2)	<0.001
Folic acid supplementation use, No. Yes (%)	3,287(75.2)	1,105 (61.6)	<0.001
Smoking during pregnancy, No. Yes (%)	871 (17.1)	478 (22.2)	<0.001
Alcohol consumption during pregnancy, No. Yes (%)	2,056 (40.9)	630 (29.6)	<0.001
Gestational diabetes, No. Yes (%)	55 (1.0)	25 (1.1)	0.679
Pre-eclampsia, No. Yes (%)	99 (1.9)	55 (2.6)	0.082
Gestational hypertension, No. Yes (%)	228 (4.3)	66 (3.1)	0.012
Child characteristics			
Males, No. (%)	2,772 (49.8)	1,207 (51.6)	0.147
Birth weight, median (95% range), g	3,450 (2260, 4460)	3,400 (2205, 4528)	0.002
Gestational age at birth, median (95% range), weeks	40.1 (35.9, 42.3)	40.0 (35.0, 42.4)	<0.001
Ever breastfeeding, No. Yes (%)	4,114 (92.5)	1,168 (90.1)	0.005
Introduction of solid foods, No. before 6 months (%)	3,105 (89.4)	755 (89.8)	0.754

[1]Values represent means (SD), median (95% range) or number of subjects (valid %). [2]Differences in subject characteristics between the groups were tested using Independent Samples T-tests for continuous variables and Chi-square tests for proportions.

Supplemental Table 5. Maternal caffeine intake during pregnancy and childhood body fat distribution at 6 years (basic models) (N=5,562)

	Body mass index (SDS)	Total body fat mass (SDS)	Android/gynoid fat mass ratio (SDS)	Abdominal preperitoneal fat area (SDS)
	N=5,562	**N=5,407**	**N=5,405**	**N=4,508**
Maternal caffeine intake categories				
< 2 units	Reference	Reference	Reference	Reference
	N=3,295	*N=3,199*	*N=3,197*	*N=2,658*
2 -3.9 units	-0.10 (-0.15, -0.04)*	-0.08 (-0.13, -0.03)*	-0.02 (-0.08, 0.04)	-0.12 (-0.18, -0.06)*
	N=1,819	*N= 1,771*	*N=1,771*	*N=1,475*
4- 5.9 units	0.03 (-0.08, 0.13)	-0.01 (-0.11, 0.09)	-0.04 (-0.15, 0.07)	-0.13 (-0.24, -0.02)*
	N= 368	*N=357*	*N=357*	*N=306*
≥ 6 units	0.08 (-0.13, 0.29)	0.09 (-0.11, 0.29)	0.24 (0.02, 0.46)*	-0.23 (-0.45, 0)*
	N=80	*N=80*	*N=80*	*N=69*
P-value for trend	0.245	0.202	0.852	<0.001

Values are regression coefficients (95% confidence interval) that reflect the difference in childhood outcomes in children whose mothers consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day during pregnancy, respectively, as compared to those whose mothers consumed <2 units of caffeine per day. 1 unit of caffeine intake represents the equivalent of 1 cup of coffee (90 mg caffeine).The models were adjusted for child's sex, age at follow-up measurement and height at follow-up measurement (fat mass outcomes only). P-values for trend were obtained from models in which the categorized caffeine intake variable was entered as continuous variable. *P-value <0.05.

Supplemental Table 6. Maternal caffeine intake during pregnancy and childhood overweight at 6 years (N=5,562)

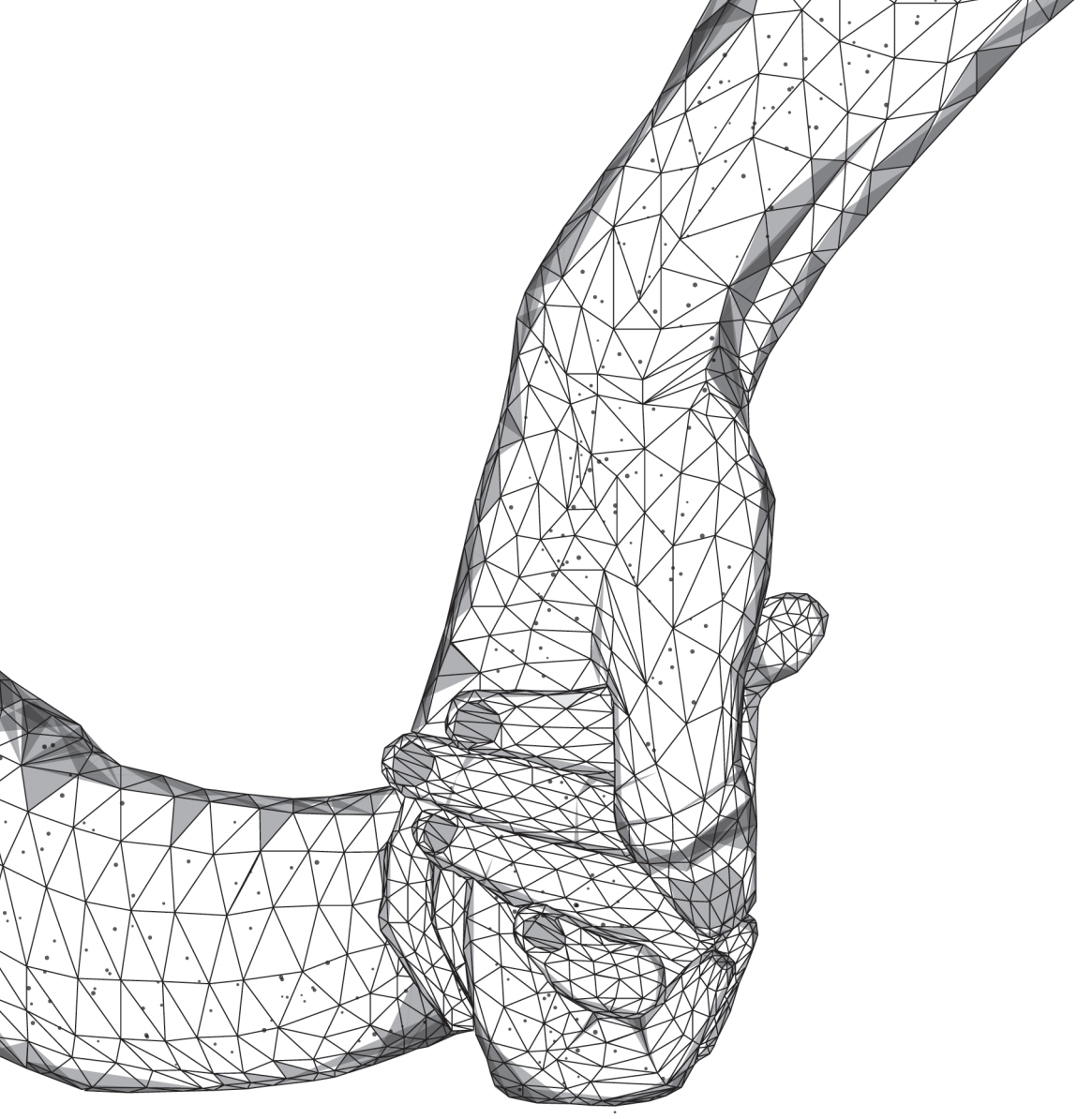
	Odds Ratio (95% Confidence Interval)
Maternal caffeine intake categories	
< 2 units	Reference
	N=3,295
2 -3.9 units	0.91 (0.77, 1.08)
	N=1,819
4 – 5.9 units	1.09 (0.80, 1.49)
	N=368
≥ 6 units	1.25 (0.68, 2.30)
	N=80
P-value for trend	0.937

Values are Odds Ratio's (95% confidence interval) that reflect the risk of overweight/obesity in children whose mothers consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day during pregnancy, respectively, as compared to those whose mothers consumed <2 units of caffeine per day. 1 unit of caffeine intake represents the equivalent of 1 cup of coffee (90 mg caffeine).The model was adjusted for the child's sex, age at follow-up measurement, maternal age, pre-pregnancy body mass index, parity, ethnicity, educational level, folic acid supplementation use, smoking and alcohol consumption during pregnancy, gestational diabetes, gestational hypertensive disorders, birth weight, gestational age at birth, breastfeeding, timing of introduction of solid foods and television-watching time. P-values for trend were obtained from models in which the categorized caffeine intake variable was entered as continuous variable.

Supplemental Table 7. Maternal caffeine intake during pregnancy and childhood insulin and c-peptide levels at 6 years (basic models) (N=3,667)

	Insulin (SDS)	C-peptide (SDS)
	N=3,654	**N=3,667**
Maternal caffeine intake categories		
< 2 units	Reference	Reference
	N=2,116	*N=2,128*
2 – 3.9 units	-0.02 (-0.09, 0.05)	0.01 (-0.09, 0.06)
	N= 1,239	*N=1,237*
4 – 5.9 units	0.16 (0.02, 0.29)*	0.16 (0.03, 0.29)*
	N=241	*N=244*
≥ 6 units	-0.19 (-0.45, 0.07)	-0.10 (-0.36, 0.16)
	N=58	*N=59*
P-value for trend	0.738	0.415

Values are regression coefficients (95% confidence interval) that reflect the difference in childhood outcomes in children whose mothers consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day during pregnancy, respectively, as compared to those whose mothers consumed <2 units of caffeine per day. 1 unit of caffeine intake represents the equivalent of 1 cup of coffee (90 mg caffeine).The models were adjusted for child's sex and age at follow-up measurement. P-values for trend were obtained from models in which the categorized caffeine intake variable was entered as continuous variable. *P-value <0.05.



2.2 Maternal caffeine intake during pregnancy and childhood abdominal and liver fat deposition

Voerman E, Jaddoe VWV, Hulst ME, Oei EHG, Gaillard R. Associations of maternal caffeine intake during pregnancy with abdominal and liver fat deposition in childhood.

Adapted from: Pediatr Obes. 2020; 15(5):e12607.

ABSTRACT

Background: Maternal caffeine intake during pregnancy is associated with an increased risk of childhood obesity. Studies in adults suggest that caffeine intake might also directly affect visceral and liver fat deposition, which are strong risk factors for cardio-metabolic disease.
Objective: To assess the associations of maternal caffeine intake during pregnancy with childhood general, abdominal, and liver fat mass at 10 years of age.
Methods: In a population-based cohort from early pregnancy onwards among 4770 mothers and children, we assessed maternal caffeine intake during pregnancy and childhood fat mass at age 10 years.
Results: Compared with children whose mothers consumed <2 units of caffeine per day during pregnancy, those whose mothers consumed 4-5.9 and ≥6 units of caffeine per day had a higher body mass index, total body fat mass index, android/gynoid fat mass ratio, and abdominal subcutaneous and visceral fat mass indices. Children whose mothers consumed 4-5.9 units of caffeine per day had a higher liver fat fraction. The associations with abdominal visceral fat and liver fat persisted after taking childhood total body fat mass into account.
Conclusions: High maternal caffeine intake during pregnancy was associated with higher childhood body mass index, total body fat, abdominal visceral fat, and liver fat. The associations with childhood abdominal visceral fat and liver fat fraction were independent of childhood total body fat. This suggests differential fat accumulation in these depots, which may increase susceptibility to cardio-metabolic disease in later life.

INTRODUCTION

Caffeine is a methylxantine that occurs naturally in several food products. Caffeine-containing beverages, including coffee and tea, are widely consumed by pregnant women. Caffeine crosses the placenta and enters the fetal circulation freely (1). The activity of the principal enzyme in caffeine metabolism, cytochrome CYP1A2, decreases progressively during pregnancy and is absent in placenta and fetus (2-4). As a consequence, fetal exposure to caffeine is prolonged and might adversely influence the development of organ systems. Consumption of caffeine-containing beverages during pregnancy has been related to an increased risk of fetal death, impaired fetal growth, and low birth weight (5-9). In addition to these short-term outcomes, maternal caffeine intake during pregnancy may also influence long-term offspring body fat development. We previously observed among 7857 mothers and their children from the Netherlands that high maternal caffeine intake during pregnancy was associated with a higher childhood body mass index and total body fat mass at the age of 6 years (10). Similarly, studies among 615, 50 943, and 558 mothers and children from the United States, Norway, and Ireland, respectively, observed that any maternal caffeine intake during pregnancy was associated with an increased risk of obesity in childhood (11-13).

In contrast, consumption of caffeine-containing beverages by non-pregnant adults seems to have beneficial effects on body fat accumulation and the risks of several diseases (14-19). Previous studies suggest that consumption of caffeine-containing beverages is associated with lower visceral fat accumulation and lower risks of non-alcoholic fatty liver disease (NAFLD), possibly by influencing blood concentrations of adiponectin and pro-inflammatory cytokines (15-19). Previous research suggests that blood concentrations of adipokines and cytokines in pregnant women are related to childhood body fat development (20-22). However, it is not known whether maternal caffeine intake during pregnancy is also related to offspring abdominal and liver fat accumulation. Thus far, only animal studies have shown that in utero exposure to caffeine increases intra-hepatic fat content and the susceptibility to NAFLD (23, 24). As visceral and liver fat accumulation are related to the development of hypertension, type 2 diabetes, NAFLD, and the metabolic syndrome independent of excess body fat per se (25, 26), it is important to obtain further insight into whether maternal caffeine intake during pregnancy differentially affects offspring visceral and liver fat deposition.

Therefore, in a population-based cohort among 4770 mothers and children from early pregnancy onwards, we assessed the associations of maternal caffeine intake during pregnancy with childhood general, abdominal, and liver fat at the age of 10 years, with the main focus on abdominal and liver fat.

METHODS

Study design

This study was embedded in the Generation R Study, a prospective population-based cohort study from early pregnancy onwards performed in Rotterdam, the Netherlands (27). The study was approved by the Medical Ethical Committee of the Erasmus Medical Center, University Medical Center, Rotterdam (MEC 198.782/2001/31). Written informed consent was obtained from all mothers at enrolment in the study. The response rate at birth was 61%. Of the 8879 mothers that were prenatally included in the study, 8097 had information available on caffeine intake during pregnancy. Of their children, 7900 were singleton and live born. Of these children, 4770 participated in body composition follow-up measurements at 10 years of age and were included in the analyses (**Supplemental Figure 1**).

Maternal caffeine intake during pregnancy

As described previously, information on maternal caffeine intake from coffee and tea during pregnancy was obtained by postal questionnaires in the first, second, and third trimesters of pregnancy (7, 10). Response rates for these questionnaires were 91%, 80%, and 77%, respectively (7, 10). Mothers who reported to drink any coffee or tea were asked how many cups of coffee or tea they consumed on average per day and what type of coffee or tea they consumed (caffeinated, decaffeinated, or a combination of both). According to standard values for caffeine content, a regular coffee serving (125 mL) in the Netherlands contains ~90 mg caffeine, decaffeinated coffee contains ~3 mg, and black tea contains ~45 mg (28). To calculate the total caffeine intake in each trimester, the type of coffee or tea was weighted according to its caffeine content (caffeinated coffee = 1, caffeinated and decaffeinated coffee = 0.5, decaffeinated coffee = 0, caffeinated tea = 0.5, caffeinated and decaffeinated tea = 0.25, decaffeinated tea = 0, herbal tea = 0, and green tea = 0.5) (7). Thus, in our analyses, each unit of caffeine intake reflects caffeine exposure based on one cup of caffeinated coffee (90 mg caffeine) (10). Based on data availability, total caffeine intake was categorized into categories of <2, 2-3.9, 4-5.9, and ≥6 units per day (equivalent to <180, 180-359, 360-539, and ≥540 mg per day, respectively). For the main analyses using caffeine intake during the full pregnancy, caffeine intake of the trimesters was averaged.

Childhood body fat mass

At the age of 10 years, we measured height and weight without shoes and heavy clothing and calculated body mass index (kg/m^2). We created age- and sex-adjusted standard deviation scores (SDS) of body mass index using a Dutch reference chart (29). In addition, we defined childhood overweight/obesity according to the International Obesity Task Force cut-offs (30). We measured total and regional body fat mass using dual-energy X-ray absorptiometry (DXA) (iDXA, General Electrics–Lunar, 2008, Madison, Wisconsin) (31). Android/gynoid fat

mass ratio was calculated and used as a measure of body fat distribution comparable with waist/hip ratio (31). Abdominal and organ fat were measured in a subgroup by magnetic resonance imaging (MRI), as described previously (27). Briefly, all children were scanned using a 3.0 Tesla MRI (Discovery MR750w, General Electric Healthcare, Milwaukee, Wisconsin). The MRI protocol included an axial 3-point Dixon sequence for fat and water separation (IDEAL IQ) for liver fat measurements. This technique also enables the generation of liver fat fraction images (32). An axial abdominal scan from lower liver to pelvis and a coronal scan centred at the head of the femurs were also performed with a 2-point Dixon acquisition (LavaFlex). The obtained fat scans were analysed by the Precision Image Analysis company (PIA, Kirkland, Washington), using the sliceOmatic (TomoVision, Magog, Canada) software package. All extraneous structures and any image artefacts were removed manually (33). Total subcutaneous and visceral fat volumes ranged from the dome of the liver to the superior part of the femoral head. Fat masses were obtained by multiplying the total volumes by the specific gravity of adipose tissue, 0.9 g/mL. Liver fat fraction was determined by defining four regions of interest of at least 4 cm^2 in 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 fat measures independent of child's height, we estimated the optimal adjustment by log-log regression analyses and subsequently divided total and subcutaneous fat mass by $height^4$ and visceral fat mass by $height^3$ (**Supplemental Methods 1**) (34, 35).

Covariates

Information on maternal age, pre-pregnancy body mass index, parity, ethnicity, educational level, and folic acid supplementation use was obtained by questionnaire at enrolment in the study. Smoking and alcohol intake during pregnancy were repeatedly assessed by questionnaire. Information on gestational diabetes, gestational hypertensive disorders (gestational hypertension and pre-eclampsia), date of birth, child's sex, and birth weight was obtained from midwife and hospital registries. Average television watching time was assessed by questionnaire at the age of 10 years.

Statistical analysis

First, we performed a non-response analysis comparing participants included the analysis with those lost to follow up at the age of 10 years. Second, we assessed the associations of maternal caffeine intake during pregnancy with childhood general fat measures and the risk of overweight/obesity at age 10, using linear and logistic regression models. Third, we assessed the associations of maternal caffeine intake during pregnancy with childhood abdominal subcutaneous and visceral fat mass indices and liver fat fraction, using linear regression models. Non-normally distributed outcome variables were log-transformed. To enable comparison of effect estimates across the different outcomes, we calculated SDS for each of the outcomes. The models were first adjusted for child's age and sex only (basic models). Next, we addition-

ally adjusted the models for maternal ethnicity, education, smoking during pregnancy, alcohol consumption during pregnancy, folic acid supplementation use, and childhood television watching time (confounder models). These confounders were selected based on existing literature, associations with the exposure and outcome in the study sample, and a change in effect estimates of >10%. Maternal age, pre-pregnancy body mass index, parity, and gestational hypertensive disorders were also considered, but were not associated with either the exposure or the outcome or did not change the effect estimates with >10% and were therefore not included in the models. To explore whether any observed associations of caffeine intake during pregnancy with the outcomes were mediated by gestational age at birth and birth weight, we added these variables to the confounder models (mediator models). We performed tests for trend by adding the categorized caffeine intake variable to the models as continuous variable. Fourth, to further explore whether maternal caffeine intake during pregnancy was specifically associated with childhood abdominal fat mass and liver fat fraction, independent from total body fat mass, we used conditional regression analyses. We created measures of childhood abdominal subcutaneous fat mass, abdominal visceral fat mass, and liver fat fraction that are independent of total body fat mass by regressing these detailed childhood fat measures on childhood total body fat mass index. The standardized residuals from these models were used as an outcome for the regression models focused on the associations of maternal caffeine intake during pregnancy with conditional childhood abdominal and liver fat measures (36). Fifth, to identify potential critical periods, we assessed the associations of trimester-specific maternal caffeine intake with childhood general, abdominal, and liver fat using linear regression models. As sex differences in childhood body fat development have been reported (37, 38), we tested for interactions between maternal caffeine intake during pregnancy and child's sex, but these interaction terms were not statistically significant (*P* values > .05). Missing values of covariates (maximum percentage of missing values: 20.8%) were imputed using Multiple Imputation, and pooled results from five imputed datasets were reported. All statistical tests were two-sided, with a significance threshold of 0.05. The analyses were performed using the Statistical Package for the Social Sciences version 24.0 (IBM Corp, Armonk, New York, USA) and R version 3.3.4 (R Foundation for Statistical Computing).

RESULTS

Participants' characteristics

Table 1 shows that, of the 4770 women included, 2780 (58.3%), 1583 (33.2%), 329 (6.9%), and 78 (1.6%) consumed <2 units, 2-3.9 units, 4-5.9 units, and ≥6 units of caffeine per day, respectively, during pregnancy. Women who had higher caffeine intakes were older and were more likely to be higher educated, multiparous, and from European descent. They used less often folic acid supplementation and smoked and consumed alcohol more

Table 1. Characteristics of the mothers and their children

	Total group	<2 units	2-3.9 units	4-5.9 units	≥6 units	P-value
	N= 4,770	N=2,780 (58.3%)	N= 1,583 (33.2%)	N=329 (6.9%)	N=78 (1.6%)	
Maternal characteristics						
Caffeine intake during pregnancy, median (95% range), units	1.6 (0.0, 5.2)	1.0 (0.0, 1.8)	2.6 (2.0, 3.8)	4.5 (4.0, 5.7)	6.7 (6.0, 10.3)	<0.001
Age, median (95% range), years	31.2 (20.4, 39.6)	30.4 (19.6, 39.1)	32.0 (21.9, 40.1)	32.9 (23.5, 39.8)	33.7 (23.2, 40.8)	<0.001
Pre-pregnancy BMI, median (95% range), kg/m^2	22.6 (18.1, 34.5)	22.6 (18, 34.6)	22.4 (18.3, 34.1)	22.8 (18.5, 34.5)	22.7 (18.4, 31.5)	0.242
Education, N (%)						
Primary	340 (7.4)	233 (8.7)	87 (5.6)	13 (4.0)	7 (9.2)	<0.001
Secondary	1,944 (42.2)	1,247 (46.7)	543 (35.3)	116 (36.1)	38 (50.0)	
Higher	2,324 (50.4)	1,191 (44.6)	910 (59.1)	192 (59.8)	31 (40.8)	
Parity, nulliparous (%)	2,793 (58.8)	1,715 (62.0)	873 (55.4)	168 (51.1)	37 (47.4)	<0.001
Ethnicity, European (%)	3,113 (65.8)	1,624 (59.1)	1,163 (73.8)	264 (80.7)	62 (80.5)	<0.001
Folic acid supplementation use, Yes (%)	2,955 (78.2)	1,663 (75.9)	1,039 (81.7)	216 (82.4)	37 (67.3)	<0.001
Smoking during pregnancy, Yes (%)	1,065 (24.3)	493 (19.3)	412 (28.3)	116 (38.3)	44 (58.7)	<0.001
Alcohol consumption during pregnancy, Yes (%)	2,495 (57.5)	1,271 (50.2)	969 (67.3)	203 (69.0)	52 (70.3)	<0.001
Gestational hypertensive disorders, Yes (%)	272 (5.9)	166 (6.2)	80 (5.2)	22 (6.8)	4 (5.3)	NA
Gestational diabetes, Yes (%)	46 (1.0)	26 (1.0)	15 (1.0)	5 (1.6)	0 (0.0)	NA
Child characteristics						
Males, No. (%)	2361 (49.5)	1410 (50.7)	732 (46.2)	176 (53.5)	43 (55.1)	0.009
Gestational age at birth, median (95% range), weeks	40.1 (35.9, 42.3)	40.1 (35.5, 42.3)	40.3 (36.4, 42.3)	40.3 (36.0, 42.4)	40.3 (34.5, 42.4)	0.023
Birth weight, median (95% range), g	3,460 (2,262, 4,480)	3,450 (2,250, 4,430)	3,490 (2,368, 4,520)	3,530 (2,392, 4,534)	3,390 (1,999, 4,263)	0.007
Gestational age adjusted birth weight, mean (SD)	-0.1 (1.0)	-0.1 (1.0)	0.0 (1.0)	0.0 (1.1)	-0.3 (1.1)	0.027
Ever breastfeeding, Yes (%)	3,627 (92.6)	2,063 (92.3)	1,244 (93.3)	266 (92.7)	54 (91.5)	NA
Introduction of solid foods, before 6 months (%)	2,800 (89.1)	1569 (88.7)	979 (89.4)	214 (90.7)	38 (90.5)	NA

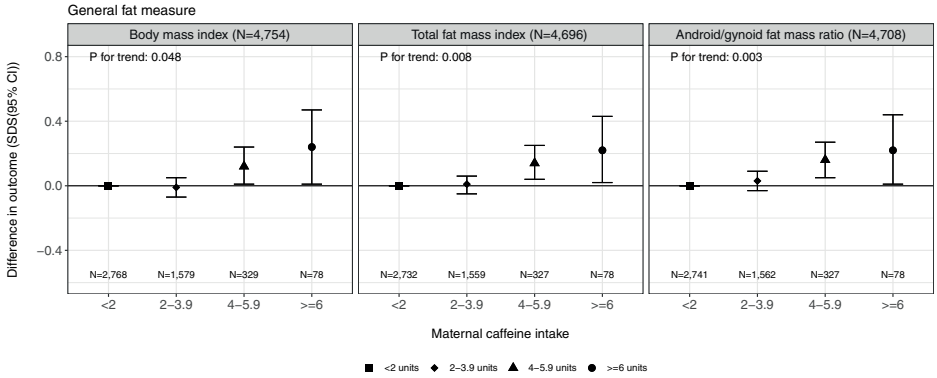
Table 1. Characteristics of the mothers and their children (continued)

	Total group	<2 units	2-3.9 units	4-5.9 units	≥6 units	P-value
	N= 4,770	N=2,780 (58.3%)	N= 1,583 (33.2%)	N=329 (6.9%)	N=78 (1.6%)	
Age at 10 year follow-up measurement, median (95% range), years	9.7 (9.4, 10.7)	9.7 (9.3, 10.8)	9.7 (9.4, 10.7)	9.7 (9.4, 10.4)	9.7 (9.3, 10.3)	0.650
Television watching, More than 2 hours/day (%)	1168 (30.9)	717 (33.3)	346 (26.8)	80 (28.7)	25 (40.3)	<0.001
Body mass index at 10 years, median (95% range), kg/m^2	17.0 (14.0, 24.8)	17.0 (14.0, 25.1)	16.8 (14.1, 24.2)	17.2 (14, 24.1)	17.8 (14.3, 24.0)	<0.001
Overweight, N (%)	862 (3.7)	556 (16.2)	231 (11.8)	54 (13.8)	21 (22.0)	NA
Total body fat mass, median (95% range), kg	8.5 (4.5, 22.0)	8.6 (4.5, 22.2)	8.4 (4.5, 21.7)	8.5 (4.8, 21.9)	9.7 (4.6, 20.9)	0.044
Android/gynoid fat mass ratio, median (95% range)	0.2 (0.2, 0.5)	0.2 (0.2, 0.5)	0.2 (0.2, 0.5)	0.2 (0.2, 0.5)	0.3 (0.2, 0.5)	0.023
Abdominal subcutaneous fat, median (95% range), kg	1.3 (0.6, 5.4)	1.3 (0.6, 5.4)	1.3 (0.6, 4.9)	1.4 (0.7, 4.7)	1.5 (0.8, 6.2)	0.017
Abdominal visceral fat, median (95% range),kg	0.4 (0.2, 1.0)	0.4 (0.2, 1.0)	0.4 (0.2, 0.9)	0.4 (0.2, 0.9)	0.5 (0.2, 1.2)	0.002
Liver fat fraction, median (95% range), %	2.0 (1.2, 5.2)	2.0 (1.2, 5.3)	2.0 (1.3, 4.6)	2.1 (1.4, 5.4)	2.1 (1.3, 8.4)	0.444

Values represent mean (SD), median (95% range) or number of participants (valid %).
1 unit of caffeine represents the equivalent of 1 cup of coffee (90 mg of caffeine).
NA: Chi-square test not available as a result of low expected cell counts.

often during pregnancy**. Supplemental Table 1** shows that, as compared with women included in the analyses, those lost to follow up had slightly lower caffeine intakes and a lower pre-pregnancy BMI, were younger, were more often multiparous, and were less often from European descent. These women used folic supplementation less often, smoked more often, and consumed alcohol less often during pregnancy.

Figure 1. Associations of maternal caffeine intake during pregnancy with childhood general body fat mass



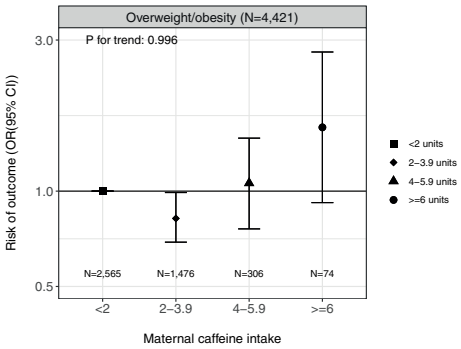
Values are regression coefficients (95% confidence intervals) from the confounder models that reflect the difference in childhood body mass index, total body fat mass index, android/gynoid fat mass ratio in children of mothers who consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day, as compared to those whose mothers consumed <2 units of caffeine per day. 1 unit of caffeine represents the equivalent of 1 cup of coffee (90 mg). The models are adjusted for child's sex, child's age at follow-up measurement, maternal ethnicity, maternal education, maternal smoking, maternal alcohol use, folic acid supplementation and television watching time. P-values for trend were obtained from models in which the categorized caffeine intake variable (<2, 2-3.9, 4-5.9 and ≥6 units) was entered as continuous variable.

Maternal caffeine intake during pregnancy and childhood general body fat mass

Figure 1 shows that in the confounder model, as compared with children whose mothers consumed <2 units of caffeine per day during pregnancy, those whose mothers consumed 4-5.9 and ≥6 units of caffeine per day during pregnancy had a higher body mass index (differences: 0.12 standard deviation [SD] [95% confidence interval (CI), 0.01-0.24] and 0.24 [95% CI, 0.01-0.47], respectively), total body fat mass index (differences: 0.14 SD [95% CI, 0.04-0.25] and 0.22 [95% CI, 0.02-0.43], respectively), and android/gynoid fat mass ratio (differences: 0.16 SD [95% CI, 0.05-0.27] and 0.22 [95% CI, 0.01-0.44], respectively) at the age of 10 years (exact differences are given in **Supplemental Table 2**). A dose-response relationship was present for each of these outcomes (*P*-values for trend < .05). Children whose mothers consumed ≥6 units of caffeine per day also tended to have a higher risk of overweight/obesity (odds ratio: 1.59 [95% CI, 0.92-2.75], **Figure 2**). Results from the basic model were similar (**Supplemental Table 3**). Additional adjustment for gestational age at birth and birth weight did not change the results (**Supplemental Table 4**). **Supplemental**

Table 5 shows that no trimester-specific associations were present, but rather that associations were similar across pregnancy.

Figure 2. Associations of maternal caffeine intake during pregnancy with the risk of childhood overweight/obesity



Values are odds ratios (95% confidence intervals) from the confounder models that reflect the risk of overweight/obesity in children of mothers who consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day, as compared to those whose mothers consumed <2 units of caffeine per day. 1 unit of caffeine represents the equivalent of 1 cup of coffee (90 mg). The models are adjusted for child's sex, child's age at follow-up measurement, maternal ethnicity, maternal education, maternal smoking, maternal alcohol use, folic acid supplementation and television watching time. P-values for trend were obtained from models in which the categorized caffeine intake variable (<2, 2-3.9, 4-5.9 and ≥6 units) was entered as continuous variable.

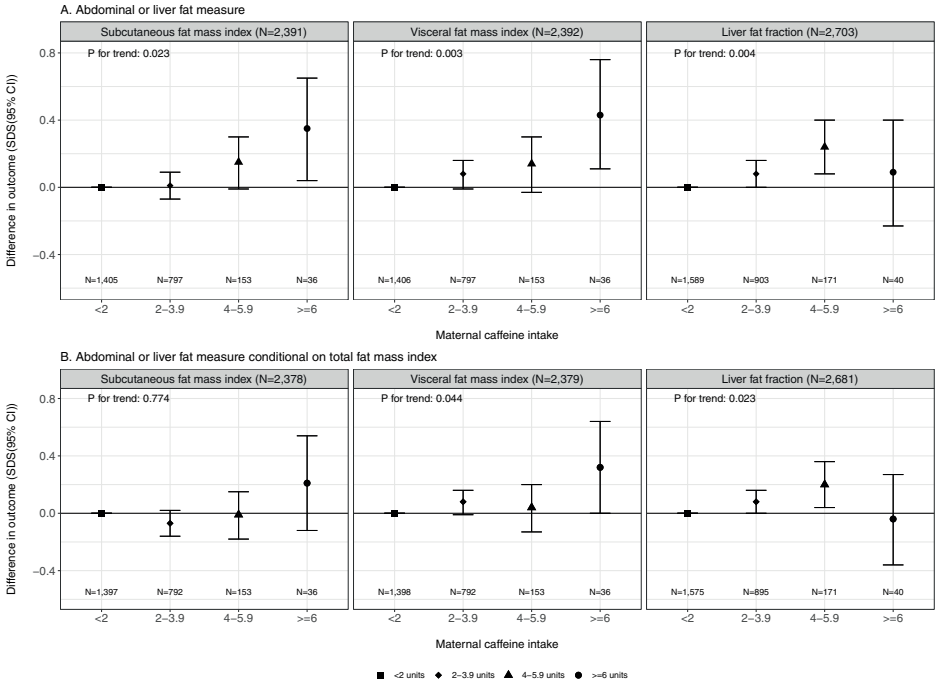
Maternal caffeine intake during pregnancy and childhood abdominal fat mass and liver fat fraction

Figure 3A shows that in the confounder model, as compared with children whose mothers consumed <2 units of caffeine per day during pregnancy, those whose mothers consumed 4-5.9 and ≥6 units of caffeine per day during pregnancy had a higher abdominal subcutaneous fat mass index (differences: 0.15 SD [95% CI, –0.01 to 0.30] and 0.35 SD [95% CI, 0.04-0.65], respectively) and a higher abdominal visceral fat mass index (differences: 0.14 SD [95% CI, –0.03 to 0.30] and 0.43 SD [95% CI, 0.11-0.76], respectively). Children whose mothers consumed 4-5.9 units of caffeine per day also had a higher liver fat fraction, as compared with those whose mothers consumed <2 units per day during pregnancy (difference: 0.20 SD [95% CI, 0.04-0.36]); exact differences are given in **Supplemental Table 6**). A dose-response relationship was present for each of the outcomes (*P* values for trend < .05). Results from the basic model were similar (**Supplemental Table 7**). Additional adjustment for gestational age at birth and birth weight did not influence the observed estimates (**Supplemental Table 8**). **Supplemental Table 9** shows that the associations for each trimester separately were comparable with those for the full pregnancy.

Figure 3B shows that after conditioning on total body fat mass index to assess the effects of maternal caffeine intake during pregnancy on childhood abdominal fat and liver fat fraction independent of childhood total body fat, maternal caffeine intake during pregnancy

of ≥6 units and 4-5.9 units per day remained associated with abdominal visceral fat mass index and liver fat fraction, respectively (differences: 0.32 SD [95% CI, 0.00-0.64] and 0.20 SD [95% CI, 0.04-0.36]). A significant dose-response relationship remained also present for these outcomes (*P* values for trend < .05). No associations were present with childhood abdominal subcutaneous fat mass index conditioned on childhood total body fat mass index.

Figure 3. Associations of maternal caffeine intake during pregnancy with childhood abdominal fat mass and liver fat fraction



Values are regression coefficients (95% confidence intervals) from the confounder models that reflect the difference in (A) childhood outcomes in SDS and (B) childhood outcomes in standardized residuals in children of mothers who consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day, as compared to those whose mothers consumed <2 units of caffeine per day. 1 unit of caffeine represents the equivalent of 1 cup of coffee (90 mg). The models are adjusted for child's sex, child's age at follow-up measurement, maternal ethnicity, maternal education, maternal smoking, maternal alcohol use, folic acid supplementation and television watching time. P-values for trend were obtained from models in which the categorized caffeine intake variable (<2, 2-3.9, 4-5.9 and ≥6 units) was entered as continuous variable.

DISCUSSION

In this population-based prospective cohort study, high maternal caffeine intake during pregnancy was associated with higher childhood general body fat mass, abdominal fat mass, and liver fat fraction at the age of 10 years. The associations of high maternal caffeine intake with childhood abdominal visceral fat mass and liver fat fraction seemed to be independent from childhood total body fat mass.

Interpretation of main findings

Caffeine-containing beverages are frequently consumed during pregnancy. Increasing evidence suggests that maternal caffeine intake during pregnancy might be related to long-term offspring body fat development (10-12, 39). We previously showed among 7857 6-year-old children from the same cohort as the current study that maternal caffeine intake during pregnancy of ≥4 units per day was associated with a higher body mass index and total body fat mass. Maternal caffeine intake during pregnancy of ≥6 units per day was also associated with a higher android/gynoid fat mass ratio, reflecting a central body fat accumulation (10). A study among 272 mother-child pairs from Brazil observed that any caffeine intake by women with an uncomplicated pregnancy was associated with a higher offspring sum of skinfold thicknesses at age 3 months (39). A study among 50 943 participants from Norway showed that any caffeine intake during pregnancy was associated with an increased risk of childhood overweight at ages 3 and 5, whereas at 8 years, this association was only present for high caffeine intakes (12). In an Irish study among 558 mother-child pairs, higher maternal caffeine intake during pregnancy was associated with higher risks of overall and central obesity at the ages of 5 and 9 years (13). In line with these previous studies, we observed in the current study that higher maternal caffeine intake during pregnancy was associated with higher body mass index, total body fat mass, and android/gynoid fat mass ratio at the age of 10 years, as indicated by significant tests for trend. The strongest effects were present for maternal caffeine intake during pregnancy 4 or more units per day. For instance, as compared with children whose mothers consumed <2 units of caffeine per day, children whose mothers consumed ≥6 units of caffeine per day during their pregnancy had a 0.24 SD higher body mass index, corresponding to a difference of approximately 0.7 kg/m^2. These effect sizes are comparable with those observed for well-known determinants of childhood body mass index, such as maternal pre-pregnancy overweight and smoking during pregnancy (40-42). These associations were similar across the trimesters of pregnancy. Mothers with high caffeine intakes during pregnancy also had higher alcohol intakes and smoked more often during their pregnancies. However, associations were present across the full range of maternal caffeine intake, and adjusting the models for these lifestyle-related factors did not influence the results. We therefore do not consider it likely that the observed associations can be fully explained by differences in these factors. Thus, these findings suggest that maternal caffeine intake throughout pregnancy has long-term consequences for offspring body fat development, as reflected by higher total body fat mass and a central body fat distribution.

Studies in adults suggest that consumption of caffeine-containing beverages might also be associated with abdominal and ectopic fat deposition, although the direction of these associations might be different from the direction of the associations of maternal caffeine intake during pregnancy with offspring body fat development (15-17). A study among 364 Japanese men showed inverse associations of coffee consumption with visceral fat mass and visceral to subcutaneous fat mass ratio (15). A meta-analysis of five studies showed that

the risk of NAFLD was 30% lower in participants who consumed coffee as compared with those who did not (18). It is not known whether caffeine intake by pregnant women is also related to offspring fat deposition in these specific fat depots. Only one study, among 7857 participants from our cohort from the Netherlands, showed that maternal caffeine intake during pregnancy was not associated with pre-peritoneal fat mass measured by abdominal ultrasound at age 6, which was used as proxy of visceral fat (10). In the current study, we observed that higher maternal caffeine intake during pregnancy was associated with higher childhood abdominal subcutaneous fat mass, abdominal visceral fat mass, and liver fat fraction measured by MRI at age 10. This inconsistency might be explained by differences in measures of abdominal visceral fat mass. Pre-peritoneal fat mass provides an estimation of abdominal visceral fat mass, while MRI provides more precise measures and is the gold standard for the measurement of intra-abdominal and organ fat deposition (33). Also, the associations of maternal caffeine intake during pregnancy might only become apparent at older childhood ages. The results for each of the trimesters separately were comparable with those for the full pregnancy. The associations with abdominal visceral fat mass and liver fat fraction persisted after taking total body fat mass into account. This suggests that maternal caffeine intake throughout pregnancy might differentially affect fat deposition in these depots in the offspring, independent of their total body fat development. As visceral and liver fat accumulation are related to the development of cardio-metabolic disease independently of total body fat, these children might be at risk of later cardio-metabolic disease (25, 26). The associations with abdominal subcutaneous fat mass were not independent from total body fat mass. This might be explained by subcutaneous fat being the main compartment of fat storage across the full body. Thus, maternal caffeine intake throughout pregnancy might affect offspring visceral and liver fat deposition, independent from their total amount of body fat.

The mechanisms underlying the observed associations are not well known. Studies in adults have suggested that consumption of caffeine-containing beverages might increase adiponectin concentrations and decrease concentrations of pro-inflammatory cytokines, subsequently influencing visceral and liver fat masses (15, 19). Although high maternal adiponectin concentrations during pregnancy have been related to a higher risk of childhood obesity (20), the role of adipokines and cytokines in the association of maternal caffeine intake during pregnancy with offspring fat deposition is unknown. We speculate that caffeine intake by pregnant women may affect adiponectin concentrations and the pro-inflammatory state, which may affect fetal nutrient supply and subsequently lead to developmental adaptations in adipose tissue. Alternatively, animal studies have suggested that in utero exposure to caffeine may overexpose the developing fetus to glucocorticoids, leading to an altered development of the HPA-axis (43, 44). High glucocorticoid concentrations have been related to increased central obesity. In addition, the concentration of glucocorticoid receptors is higher in visceral adipose tissue as compared with other fat depots, possibly resulting in dif-

ferential fat deposition in these depots (45). Rats exposed to caffeine in utero had increased intra-hepatic fat concentrations and increased susceptibility to NAFLD (23, 33), possibly by similar mechanisms. Finally, the associations might be explained by confounding by unhealthy lifestyle factors that are shared within families. However, a negative control analysis among 50 943 participants showed stronger associations for maternal caffeine intake during pregnancy with the risk of childhood overweight at the age of 3 years, as compared with those for paternal caffeine intake at the time of their partners pregnancy (12). Similarly, in another recent negative control analysis among 558 participants, maternal caffeine intake, but not paternal caffeine intake, was associated with childhood body mass index and waist circumference at ages 5 and 10 years (13). These results suggest that an intra-uterine programming mechanism might at least partly underlie these associations. Further studies are needed to disentangle the mechanisms underlying the associations of maternal caffeine intake and childhood abdominal and liver fat deposition.

Our results are consistent with those of previous studies and further highlight the importance of limiting maternal caffeine intake during pregnancy with respect to its potential adverse effects on long-term body fat development in the offspring. The current recommendations for maternal caffeine intake during pregnancy range between 200 and 300 mg per day and are based on the risks of adverse pregnancy and birth outcomes (46-48). The most pronounced effects observed in our study were for caffeine intakes above these guidelines. However, the dose-response relationship in our and previous studies (5, 6, 8-13) suggest that the adverse effects of maternal caffeine intake with respect to both pregnancy outcomes and long-term body fat development are not restricted to high caffeine intakes, but increase across the range of maternal caffeine intake. A review of randomized controlled trials had insufficient evidence to confirm that avoiding caffeine consumption during pregnancy is beneficial with respect to adverse pregnancy outcomes (49). Based on our findings and findings from other observational studies, further adequately powered randomized controlled trials are needed to assess whether avoiding caffeine consumption during pregnancy improves both pregnancy and long-term offspring health outcomes, as compared with current recommendations. Our findings and findings from other observational studies need to be incorporated in future guidelines regarding maternal caffeine consumption during pregnancy, and these guidelines need to further emphasize potential beneficial effects on offspring health outcomes by further reducing caffeine intake during pregnancy below the current recommendations.

Strengths and limitations

This study was embedded in a large population-based cohort from early pregnancy onwards, enabling us to prospectively study the associations of interest. Of all participants with information on maternal caffeine intake during pregnancy, 39.6% did not participate in the follow-up measurements at age 10. This non-response might have led to biased estimates if the associations of interest differ between participants included and lost to follow up. This

seems unlikely as only a minor difference in maternal caffeine intake was observed between these groups. However, the selection towards a higher educated, healthier population might have affected the generalizability of our results. Maternal caffeine intake might have been underreported, possibly leading to misclassification of the caffeine intake categories and underestimation of the effect estimates. In accordance with the Dutch Nutrition Centre, we assumed that coffee and tea were consumed in cups of 125 mL (46). This might have differed between participants, which may have led to some misclassification of maternal caffeine intake. We only had data available about caffeine intake from coffee and tea and not from other sources, such as soft drinks, energy drinks, chocolate, and medications. However, at the time of data collection (2002-2006), coffee and tea accounted for 70% and 26%, respectively, of all caffeine consumed (50). We had data available on many possible confounders. However, residual confounding might still be present, for example, by maternal and child's physical activity and dietary habits.

Conclusions

Our results suggest that high maternal caffeine intake during pregnancy is associated with higher general body fat, abdominal subcutaneous and visceral fat mass and liver fat fraction in childhood. The associations of maternal caffeine intake during pregnancy with childhood visceral fat mass and liver fat seem to be largely independent from childhood total body fat mass. This suggests differential fat accumulation in these depots, which may increase susceptibility to cardio-metabolic disease.

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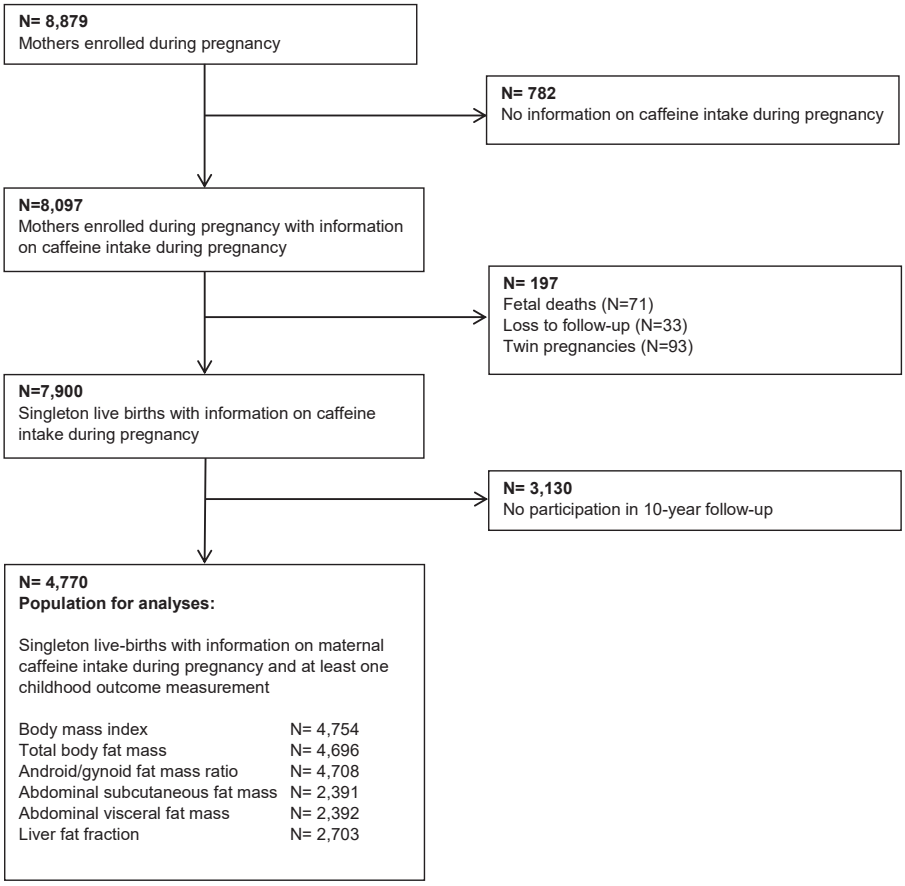
SUPPLEMENTAL MATERIAL

Supplemental Figure 1. Flow-chart of the participants



Supplemental Methods 1. Log-log regression analyses

As adiposity is highly dependent on the current height of the child, we created measures of adiposity that are independent of height using log-log regression analyses. We log-transformed total fat mass, abdominal subcutaneous fat mass, abdominal visceral fat mass, and height, using natural logs. We subsequently regressed the log-adiposity measures on log-height. The regression slope corresponds to the power by which height should be raised in order to calculate an index of the adiposity measure that is uncorrelated with height. Thus, we divided total fat mass by height^4, abdominal subcutaneous fat mass by height^4, and abdominal visceral fat mass by height^3.

References

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Supplemental Table 1. Non-response analysis

	Follow-up at 10 years	Lost to follow-up at 10 years	P-value
	N= 4,770	**N= 3,130**	
Maternal characteristics			
Caffeine intake during pregnancy, median (95% range), units	1.6 (0.0, 5.2)	1.3 (0.0, 5.0)	<0.001
Caffeine intake during pregnancy, N (%)			
<2 units	2,780 (58.3)	2,059 (65.8)	<0.001
2-3.9 units	1,583 (33.2)	870 (27.8)	
4-5.9 units	329 (6.9)	169 (5.4)	
≥6 units	78 (1.6)	32 (1.0)	
Age, median (95% range), years	31.2 (20.4, 39.6)	28.6 (18.5, 38.5)	<0.001
Pre-pregnancy BMI, median (95% range), kg/m^2	22.6 (18.1, 34.5)	22.7 (17.7, 35.6)	<0.001
Education, N (%)			<0.001
Primary	340 (7.4)	481 (16.7)	
Secondary	1,944 (42.2)	1,520 (52.8)	
Higher	2,324 (50.4)	876 (30.4)	
Parity, nulliparous (%)	2,793 (58.8)	1,640 (52.9)	<0.001
Ethnicity, European (%)	3,113 (65.8)	1,389 (46.6)	<0.001
Folic acid supplementation use, Yes (%)	2,955 (78.2)	1,437 (60.2)	<0.001
Smoking during pregnancy, Yes (%)	1,065 (24.3)	908 (31.8)	<0.001
Alcohol consumption during pregnancy, Yes (%)	2,495 (57.5)	1,152 (41.0)	<0.001
Gestational hypertensive disorders, Yes (%)	272 (5.9)	176 (5.9)	0.999
Gestational diabetes, Yes (%)	46 (1.0)	34 (1.1)	0.614
Child characteristics			
Males, No. (%)	2,361 (49.5)	1,618 (51.7)	0.057
Gestational age at birth, median (95% range), weeks	40.1 (35.9, 42.3)	40.0 (35.3, 42.3)	<0.001
Birth weight, median (95% range), g	3,460 (2,261, 4,479)	3,390 (2,200, 4,490)	<0.001
Gestational age adjusted birth weight, mean (SD)	-0.1 (1.0)	-0.1 (1.0)	<0.001
Ever breastfeeding, Yes (%)	3,627 (92.6)	1,655 (90.4)	0.004
Introduction of solid foods, before 6 months (%)	2,800 (89.1)	1,059 (90.4)	0.271

Values represent mean (SD), median (95% range) or number of participants (valid %).
1 unit of caffeine represents the equivalent of 1 cup of coffee (90 mg of caffeine).

Supplemental Table 2. Associations of maternal caffeine intake during pregnancy with childhood general body fat mass

	Body mass index (SDS)	Total body fat mass index (SDS)	Android/gynoid fat mass ratio (SDS)	Overweight/ obesity (OR)
	N=4,754	N=4,696	N=4,708	N=4,421
Maternal caffeine intake				
<2 units	Reference	Reference	Reference	Reference
2-3.9 units	-0.01 (-0.07, 0.05)	0.01 (-0.05, 0.06)	0.03 (-0.03, 0.09)	0.82 (0.69, 0.99)
4-5.9 units	0.12 (0.01, 0.24)	0.14 (0.04, 0.25)	0.16 (0.05, 0.27)	1.06 (0.76, 1.47)
≥6 units	0.24 (0.01, 0.47)	0.22 (0.02, 0.43)	0.22 (0.01, 0.44)	1.59 (0.92, 2.75)
P-value for trend	0.048	0.008	0.003	0.996

Values are regression coefficients (95% confidence intervals) from the confounder models that reflect the difference in childhood body mass index, total body fat mass index, android/gynoid fat mass ratio or odds ratios (95% confidence intervals) reflecting the risk of overweight/obesity in children of mothers who consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day, as compared to those whose mothers consumed <2 units of caffeine per day. 1 unit of caffeine represents the equivalent of 1 cup of coffee (90 mg). The models are adjusted for child's sex, child's age at follow-up measurement, maternal ethnicity, maternal education, maternal smoking, maternal alcohol use, folic acid supplementation and television watching time. P-values for trend were obtained from models in which the categorized caffeine intake variable (<2, 2-3.9, 4-5.9 and ≥6 units) was entered as continuous variable

Supplemental Table 3. Associations of maternal caffeine intake during pregnancy with childhood general body fat mass

	Body mass index (SDS)	Total body fat mass index (SDS)	Android/gynoid fat mass ratio (SDS)	Overweight/ obesity (OR)
	N=4,754	N=4,696	N=4,708	N=4,421
Maternal caffeine intake				
<2 units	Reference	Reference	Reference	Reference
2-3.9 units	-0.11 (-0.17, -0.04)	-0.12 (-0.18, -0.06)	-0.06 (-0.13, 0.00)	0.66 (0.55, 0.78)
4-5.9 units	0.02 (-0.10, 0.14)	0.00 (-0.11, 0.11)	0.06 (-0.05, 0.18)	0.91 (0.69, 1.19)
≥6 units	0.24 (0.00, 0.47)	0.20 (-0.02, 0.42)	0.24 (0.01, 0.46)	1.49 (1.28, 1.74)
P-value for trend	0.575	0.242	0.563	0.009

Values are regression coefficients (95% confidence intervals) from the basic models that reflect the difference in childhood body mass index, total body fat mass index, android/gynoid fat mass ratio or odds ratios (95% confidence intervals) reflecting the risk of overweight/obesity in children of mothers who consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day, as compared to those whose mothers consumed <2 units of caffeine per day. 1 unit of caffeine represents the equivalent of 1 cup of coffee (90 mg). The models are adjusted for child's sex and child's age at follow-up measurement. P-values for trend were obtained from models in which the categorized caffeine intake variable (<2, 2-3.9, 4-5.9 and ≥6 units) was entered as continuous variable.

Supplemental Table 4. Associations of maternal caffeine intake during pregnancy with childhood general body fat mass

	Body mass index (SDS)	Total body fat mass index (SDS)	Android/gynoid fat mass ratio (SDS)	Overweight/ obesity (OR)
	N=4,754	**N=4,696**	**N=4,708**	**N=4,421**
Maternal caffeine intake				
<2 units	Reference	Reference	Reference	Reference
2-3.9 units	-0.01 (-0.08, 0.05)	0.01 (-0.05, 0.07)	0.03 (-0.03, 0.09)	0.82 (0.68, 0.98)
4-5.9 units	0.11 (-0.01, 0.23)	0.14 (0.04, 0.25)	0.16 (0.05, 0.27)	1.03 (0.74, 1.44)
≥6 units	0.27 (0.04, 0.49)	0.23 (0.02, 0.43)	0.23 (0.01, 0.45)	1.63 (0.94, 2.83)
P-value for trend	0.053	0.007	0.002	0.929

Values are regression coefficients (95% confidence intervals) from the mediator models that reflect the difference in childhood body mass index, total body fat mass index, android/gynoid fat mass ratio or odds ratios (95% confidence intervals) reflecting the risk of overweight/obesity in children of mothers who consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day, as compared to those whose mothers consumed <2 units of caffeine per day. 1 unit of caffeine represents the equivalent of 1 cup of coffee (90 mg). The models are adjusted for child's sex, child's age at follow-up measurement, maternal ethnicity, maternal education, maternal smoking, maternal alcohol use, folic acid supplementation, television watching time, gestational age at birth weight and birth weight. P-values for trend were obtained from models in which the categorized caffeine intake variable (<2, 2-3.9, 4-5.9 and ≥6 units) was entered as continuous variable.

Supplemental Table 5. Associations of trimester-specific maternal caffeine intake with childhood general body fat mass

	Body mass index (SDS)	Total body fat mass index (SDS)	Android/gynoid fat mass ratio (SDS)
Maternal caffeine intake			
First trimester	**N=3,629**	**N=3,587**	**N=3,595**
<2 units	Reference	Reference	Reference
2-3.9 units	-0.04 (-0.12, 0.03)	-0.02 (-0.08, 0.05)	0.02 (-0.05, 0.09)
4-5.9 units	0.08 (-0.05, 0.20)	0.08 (-0.03, 0.19)	0.07 (-0.04, 0.19)
≥6 units	0.30 (0.10, 0.50)	0.26 (0.08, 0.43)	0.31 (0.12, 0.49)
P-value for trend	0.053	0.023	0.006
Second trimester	**N=4,035**	**N=3,985**	**N=3,995**
<2 units	Reference	Reference	Reference
2-3.9 units	-0.01 (-0.07, 0.06)	0.01 (-0.05, 0.07)	0.03 (-0.03, 0.10)
4-5.9 units	0.00 (-0.11, 0.11)	0.03 (-0.07, 0.12)	0.04 (-0.06, 0.14)
≥6 units	0.16 (-0.04, 0.36)	0.18 (0.00, 0.36)	0.18 (-0.02, 0.37)
P-value for trend	0.437	0.144	0.082
Third trimester	**N=3,834**	**N=3,792**	**N=3,801**
<2 units	Reference	Reference	Reference
2-3.9 units	0.04 (-0.03, 0.11)	0.04 (-0.03, 0.10)	0.03 (-0.04, 0.09)
4-5.9 units	0.07 (-0.05, 0.19)	0.07 (-0.03, 0.18)	0.11 (0.00, 0.22)
≥6 units	0.29 (0.07, 0.50)	0.27 (0.08, 0.46)	0.34 (0.14, 0.54)
P-value for trend	0.014	0.008	0.002

Values are regression coefficients (95% confidence intervals) from the confounder models that reflect the difference in childhood outcomes in children of mothers who consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day, as compared to those whose mothers consumed <2 units of caffeine per day. 1 unit of caffeine represents the equivalent of 1 cup of coffee (90 mg). The models are adjusted for child's sex, child's age at follow-up measurement, maternal ethnicity, maternal education, maternal smoking, maternal alcohol use, folic acid supplementation and television watching time. P-values for trend were obtained from models in which the categorized caffeine intake variable (<2, 2-3.9, 4-5.9 and ≥6 units) was entered as continuous variable.

Supplemental Table 6. Associations of maternal caffeine intake during pregnancy with childhood abdominal fat mass and liver fat fraction

	Abdominal subcutaneous fat mass index (SDS)	Abdominal visceral fat mass index (SDS)	Liver fat fraction (SDS)
Maternal caffeine intake			
A. Abdominal and organ-specific fat measure			
	N=2,391	**N=2,392**	**N=2,703**
<2 units	Reference	Reference	Reference
2-3.9 units	0.01 (-0.07, 0.09)	0.08 (-0.01, 0.16)	0.08 (0.00, 0.16)
4-5.9 units	0.15 (-0.01, 0.30)	0.14 (-0.03, 0.30)	0.24 (0.08, 0.40)
≥6 units	0.35 (0.04, 0.65)	0.43 (0.11, 0.76)	0.09 (-0.23, 0.40)
P-value for trend	0.023	0.003	0.004
B. Abdominal or liver fat measure, conditional on total fat mass index			
	N=2,378	**N=2,379**	**N=2,681**
<2 units	Reference	Reference	Reference
2-3.9 units	-0.07 (-0.16, 0.02)	0.08 (-0.01, 0.16)	0.08 (0.00, 0.16)
4-5.9 units	-0.01 (-0.18, 0.15)	0.04 (-0.13, 0.20)	0.20 (0.04, 0.36)
≥6 units	0.21 (-0.12, 0.54)	0.32 (0.00, 0.64)	-0.04 (-0.36, 0.27)
P-value for trend	0.774	0.044	0.023

Values are regression coefficients (95% confidence intervals) from the confounder models that reflect the difference in (A) childhood outcomes in SDS and (B) childhood outcomes in standardized residuals in children of mothers who consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day, as compared to those whose mothers consumed <2 units of caffeine per day. 1 unit of caffeine represents the equivalent of 1 cup of coffee (90 mg). The models are adjusted for child's sex, child's age at follow-up measurement, maternal ethnicity, maternal education, maternal smoking, maternal alcohol use, folic acid supplementation and television watching time. P-values for trend were obtained from models in which the categorized caffeine intake variable (<2, 2-3.9, 4-5.9 and ≥6 units) was entered as continuous variable.

Supplemental Table 7. Associations of maternal caffeine intake during pregnancy with childhood abdominal fat mass and liver fat fraction

	Abdominal subcutaneous fat mass index (SDS)	Abdominal visceral fat mass index (SDS)	Liver fat fraction (SDS)
Maternal caffeine intake			
A. Abdominal and organ-specific fat measure			
	N=2,391	**N=2,392**	**N=2,703**
<2 units	Reference	Reference	Reference
2-3.9 units	-0.09 (-0.18, -0.01)	0.04 (-0.05, 0.13)	0.02 (-0.06, 0.10)
4-5.9 units	0.03 (-0.13, 0.19)	0.11 (-0.05, 0.28)	0.18 (0.02, 0.33)
≥6 units	0.36 (0.04, 0.67)	0.48 (0.15, 0.81)	0.09 (-0.22, 0.41)
P-value for trend	0.910	0.011	0.070
B. Abdominal or liver fat measure, conditional on total fat mass index			
	N=2,378	**N=2,379**	**N=2,681**
<2 units	Reference	Reference	Reference
2-3.9 units	-0.05 (-0.13, 0.04)	0.14 (0.06, 0.23)	0.07 (-0.01, 0.16)
4-5.9 units	0.03 (-0.14, 0.20)	0.14 (-0.03, 0.30)	0.20 (0.04, 0.36)
≥6 units	0.26 (-0.07, 0.59)	0.39 (0.06, 0.72)	-0.02 (-0.34, 0.29)
P-value for trend	0.707	<0.001	0.021

Values are regression coefficients (95% confidence intervals) from the basic models that reflect the difference in (A) childhood outcomes in SDS and (B) childhood outcomes in standardized residuals in children of mothers who consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day, as compared to those whose mothers consumed <2 units of caffeine per day. 1 unit of caffeine represents the equivalent of 1 cup of coffee (90 mg). The models are adjusted for child's sex and child's age at follow-up measurement. P-values for trend were obtained from models in which the categorized caffeine intake variable (<2, 2-3.9, 4-5.9 and ≥6 units) was entered as continuous variable.

Supplemental Table 8. Associations of maternal caffeine intake during pregnancy with childhood abdominal fat mass and liver fat fraction

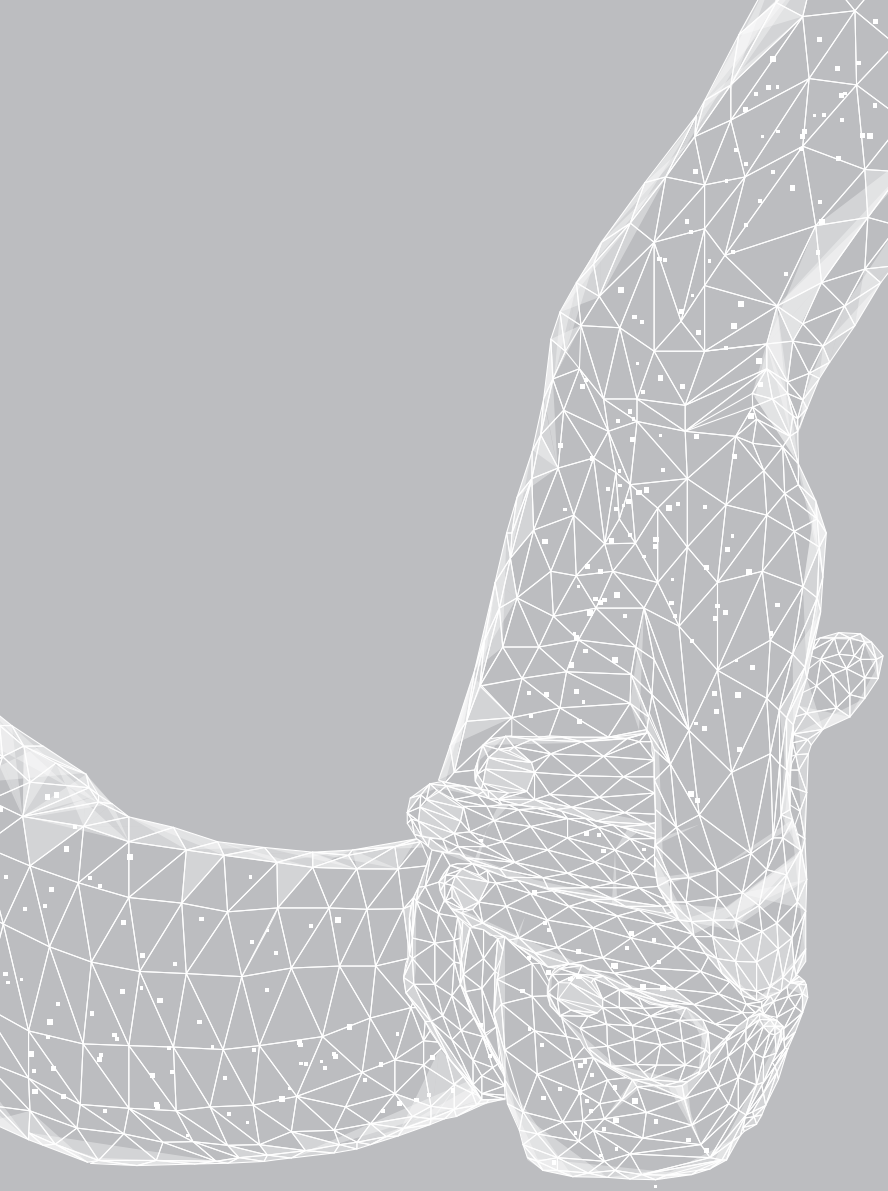
	Abdominal subcutaneous fat mass index (SDS)	Abdominal visceral fat mass index (SDS)	Liver fat fraction (SDS)
Maternal caffeine intake			
A. Abdominal and organ-specific fat measure			
	N=2,391	**N=2,392**	**N=2,703**
<2 units	Reference	Reference	Reference
2-3.9 units	0.02 (-0.07, 0.10)	0.08 (-0.01, 0.17)	0.08 (0.00, 0.16)
4-5.9 units	0.15 (0.00, 0.30)	0.14 (-0.03, 0.3)	0.25 (0.09, 0.40)
≥6 units	0.35 (0.05, 0.66)	0.44 (0.11, 0.76)	0.08 (-0.23, 0.39)
P-value for trend	0.020	0.002	0.003
B. Abdominal or liver fat measure, conditional on total fat mass index			
	N=2,378	**N=2,379**	**N=2,681**
<2 units	Reference	Reference	Reference
2-3.9 units	-0.07 (-0.16, 0.02)	0.08 (-0.01, 0.17)	0.08 (0.00, 0.17)
4-5.9 units	-0.01 (-0.18, 0.16)	0.04 (-0.12, 0.20)	0.21 (0.05, 0.37)
≥6 units	0.21 (-0.12, 0.55)	0.32 (0.00, 0.64)	-0.05 (-0.36, 0.27)
P-value for trend	0.806	0.041	0.020

Values are regression coefficients (95% confidence intervals) from the mediator models that reflect the difference in (A) childhood outcomes in SDS and (B) childhood outcomes in standardized residuals in children of mothers who consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day, as compared to those whose mothers consumed <2 units of caffeine per day. 1 unit of caffeine represents the equivalent of 1 cup of coffee (90 mg). The models are adjusted for child's sex, child's age at follow-up measurement, maternal ethnicity, maternal education, maternal smoking, maternal alcohol use, folic acid supplementation, television watching time, gestational age at birth weight and birth weight. P-values for trend were obtained from models in which the categorized caffeine intake variable (<2, 2-3.9, 4-5.9 and ≥6 units) was entered as continuous variable.

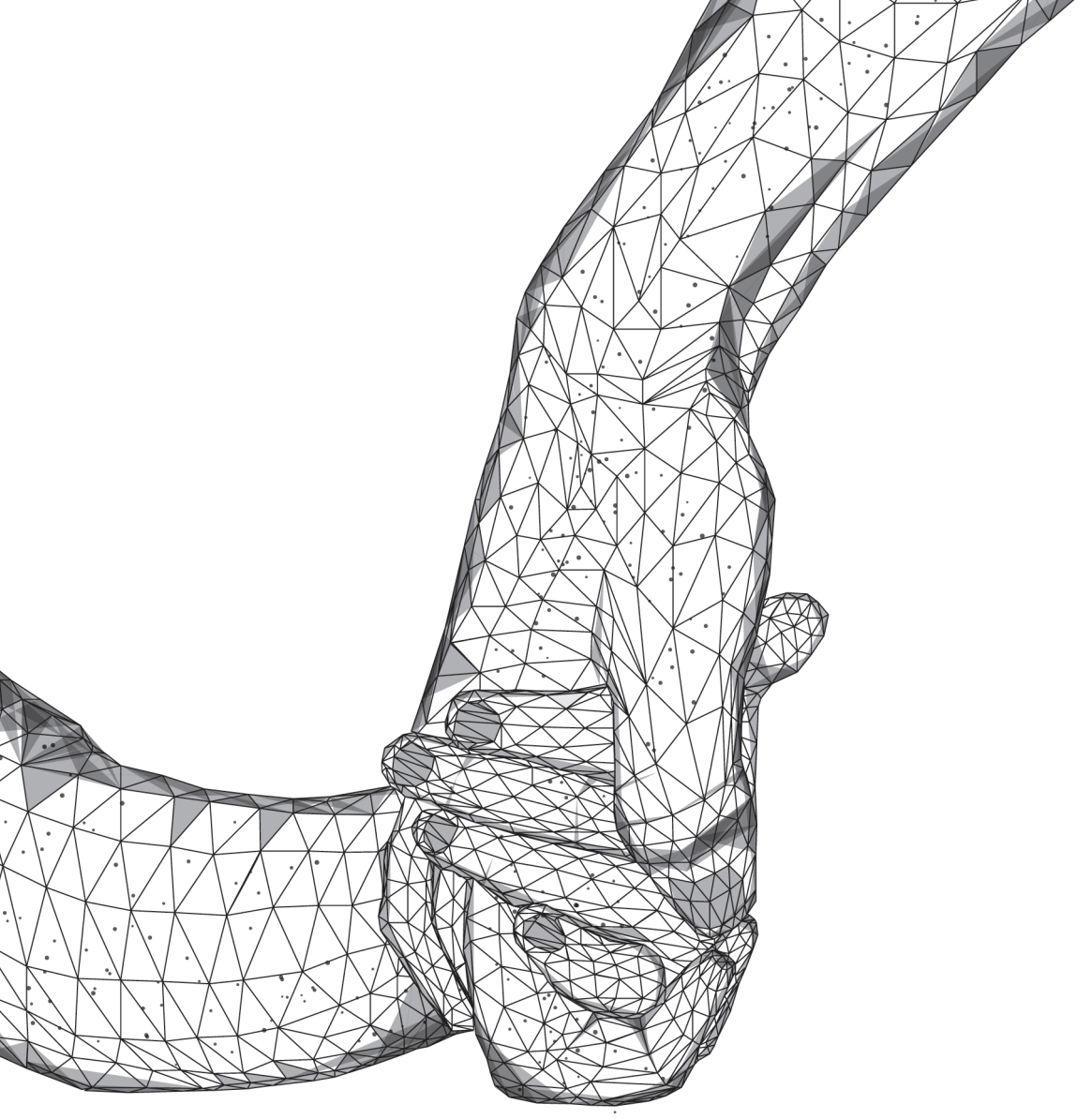
Supplemental Table 9. Associations of trimester-specific maternal caffeine intake with childhood abdominal fat mass and liver fat fraction

	Abdominal subcutaneous fat mass index (SDS)	Abdominal visceral fat mass index (SDS)	Liver fat fraction (SDS)
Maternal caffeine intake			
First trimester	**N=1,790**	**N=1,790**	**N=2,016**
<2 units	Reference	Reference	Reference
2-3.9 units	-0.03 (-0.12, 0.06)	0.03 (-0.07, 0.13)	0.01 (-0.08, 0.11)
4-5.9 units	0.03 (-0.12, 0.19)	0.00 (-0.17, 0.17)	0.12 (-0.04, 0.28)
≥6 units	0.24 (-0.04, 0.51)	0.46 (0.17, 0.76)	0.13 (-0.15, 0.40)
P-value for trend	0.383	0.066	0.159
Second trimester	**N=2,064**	**N=2,065**	**N=2,325**
<2 units	Reference	Reference	Reference
2-3.9 units	0.03 (-0.06, 0.12)	0.10 (0.01, 0.19)	0.08 (-0.01, 0.17)
4-5.9 units	0.06 (-0.07, 0.20)	0.04 (-0.11, 0.19)	0.15 (0.01, 0.29)
≥6 units	0.18 (-0.08, 0.44)	0.29 (0.02, 0.57)	0.16 (-0.11, 0.43)
P-value for trend	0.133	0.034	0.011
Third trimester	**N=1,938**	**N=1,939**	**N=2,184**
<2 units	Reference	Reference	Reference
2-3.9 units	0.02 (-0.07, 0.11)	0.10 (0.00, 0.19)	0.04 (-0.04, 0.13)
4-5.9 units	0.03 (-0.12, 0.19)	0.05 (-0.11, 0.22)	0.06 (-0.10, 0.21)
≥6 units	0.40 (0.14, 0.67)	0.41 (0.13, 0.69)	0.21 (-0.07, 0.48)
P-value for trend	0.050	0.008	0.114

Values are regression coefficients (95% confidence intervals) from the confounder models that reflect the difference in childhood outcomes in children of mothers who consumed 2-3.9, 4-5.9 and ≥6 units of caffeine per day, as compared to those whose mothers consumed <2 units of caffeine per day. 1 unit of caffeine represents the equivalent of 1 cup of coffee (90 mg). The models are adjusted for child's sex, child's age at follow-up measurement, maternal ethnicity, maternal education, maternal smoking, maternal alcohol use, folic acid supplementation and television watching time. P-values for trend were obtained from models in which the categorized caffeine intake variable (<2, 2-3.9, 4-5.9 and ≥6 units) was entered as continuous variable.



3 Maternal adiposity



3.1 Maternal body mass index, gestational weight gain and childhood overweight and obesity

Voerman E, Santos S, Patro Golab B, Amiano P, Ballester F, Barros H, Bergström A, Charles M, Chatzi L, Chevrier C, Chrousos GP, Corpeleijn E, Costet N, Crozier S, Devereux G, Eggesbø M, Ekström S, Fantini MP, Farchi S, Forastiere F, Georgiu V, Godfrey KM, Gori D, Grote V, Hanke W, Hertz-Picciotto I, Heude B, Hryhorczuk D, Huang R, Inskip H, Iszatt N, Karvonen AM, Kenny LC, Koletzko B, Küpers LK, Lagström H, Lehmann I, Magnus P, Majewska R, Mäkelä J, Manios Y, McAuliffe FM, McDonald SW, Mehegan J, Mommers M, Morgen CS, Mori TA, Moschonis G, Murray D, Chaoimh CN, Nohr EA, Nybo Andersen A, Oken E, Oostvogels AJJM, Pac A, Papadopoulou E, Pekkanen J, Pizzi C, Polanska K, Porta D, Richiardi L, Rifas-Shiman SL, Ronfani L, Santos AC, Standl M, Stoltenberg C, Thiering E, Thijs C, Torrent M, Tough SC, Trnovec T, Turner S, van Rossem L, von Berg A, Vrijheid M, Vrijkotte TGM, West J, Wijga A, Wright J, Zvinchuk O, Sørensen TIA, Lawlor DA, Gaillard R*, Jaddoe VWV*. Maternal body mass index, gestational weight gain and the risk of overweight and obesity across childhood: An individual participant data meta-analysis.

** Denotes shared last authors*

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ABSTRACT

Background: Maternal obesity and excessive gestational weight gain may have persistent effects on offspring fat development. However, it remains unclear whether these effects differ by severity of obesity, and whether these effects are restricted to the extremes of maternal body mass index (BMI) and gestational weight gain. We aimed to assess the separate and combined associations of maternal BMI and gestational weight gain with the risk of overweight/obesity throughout childhood, and their population impact.

Methods and findings: We conducted an individual participant data meta-analysis of data from 162,129 mothers and their children from 37 pregnancy and birth cohort studies from Europe, North America, and Australia. We assessed the individual and combined associations of maternal pre-pregnancy BMI and gestational weight gain, both in clinical categories and across their full ranges, with the risks of overweight/obesity in early (2.0–5.0 years), mid (5.0–10.0 years) and late childhood (10.0–18.0 years), using multilevel binary logistic regression models with a random intercept at cohort level adjusted for maternal sociodemographic and lifestyle related characteristics. We observed that higher maternal pre-pregnancy BMI and gestational weight gain both in clinical categories and across their full ranges were associated with higher risks of childhood overweight/obesity, with the strongest effects in late childhood (odds ratios [ORs] for overweight/obesity in early, mid, and late childhood, respectively: OR 1.66 [95% CI: 1.56, 1.78], OR 1.91 [95% CI: 1.85, 1.98], and OR 2.28 [95% CI: 2.08, 2.50] for maternal overweight; OR 2.43 [95% CI: 2.24, 2.64], OR 3.12 [95% CI: 2.98, 3.27], and OR 4.47 [95% CI: 3.99, 5.23] for maternal obesity; and OR 1.39 [95% CI: 1.30, 1.49], OR 1.55 [95% CI: 1.49, 1.60], and OR 1.72 [95% CI: 1.56, 1.91] for excessive gestational weight gain). The proportions of childhood overweight/obesity prevalence attributable to maternal overweight, maternal obesity, and excessive gestational weight gain ranged from 10.2% to 21.6%. Relative to the effect of maternal BMI, excessive gestational weight gain only slightly increased the risk of childhood overweight/obesity within each clinical BMI category (p-values for interactions of maternal BMI with gestational weight gain: $p = 0.038$, $p < 0.001$, and $p = 0.637$ in early, mid, and late childhood, respectively). Limitations of this study include the self-report of maternal BMI and gestational weight gain for some of the cohorts, and the potential of residual confounding. Also, as this study only included participants from Europe, North America, and Australia, results need to be interpreted with caution with respect to other populations.

Conclusions: In this study, higher maternal pre-pregnancy BMI and gestational weight gain were associated with an increased risk of childhood overweight/obesity, with the strongest effects at later ages. The additional effect of gestational weight gain in women who are overweight or obese before pregnancy is small. Given the large population impact, future intervention trials aiming to reduce the prevalence of childhood overweight and obesity should focus on maternal weight status before pregnancy, in addition to weight gain during pregnancy.

INTRODUCTION

Maternal pre-pregnancy obesity and excessive gestational weight gain are major public health problems. Maternal obesity is an important risk factor of gestational hypertensive and diabetic disorders, fetal death, pre-term birth, and macrosomia (1, 2). An accumulating body of evidence suggests that maternal obesity also has persistent effects on long-term health in offspring (3). A meta-analysis of published studies showed a 3-fold increased risk of overweight in children of mothers with pre-pregnancy obesity, as compared to those of mothers with a normal pre-pregnancy weight (4). It remains unclear whether these risks differ by severity of obesity, and whether these effects are restricted to the extremes of maternal BMI or are present across the full range. In addition to maternal pre-pregnancy obesity, excessive gestational weight gain also seems to be associated with increased risks of childhood overweight and obesity (2). Previous meta-analyses of published studies showed a 30%–40% increased risk of childhood overweight in children of mothers with excessive gestational weight gain (5-7). From a prevention perspective, insight into the combined effects of maternal BMI and gestational weight gain on offspring obesity risk and their population impact in different geographical regions is needed.

We conducted an individual participant data (IPD) meta-analysis among 162,129 mothers and their children from 37 pregnancy and birth cohorts from Europe, North America, and Australia, to assess the separate and combined associations of maternal pre-pregnancy body mass index (BMI) and gestational weight gain with the risk of overweight/obesity throughout childhood, and their population impact.

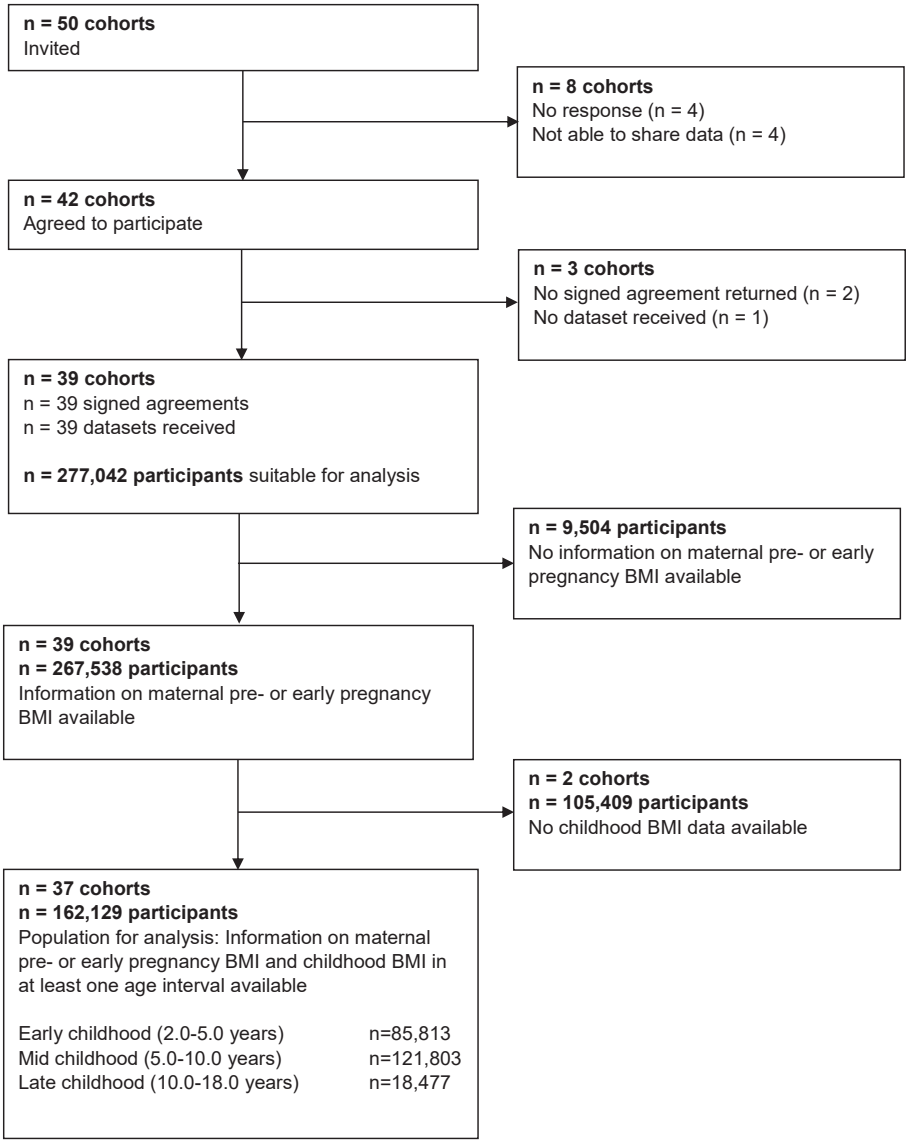
METHODS

Inclusion criteria and participating cohorts

This study was embedded in the international Maternal Obesity and Childhood Outcomes (MOCO) collaboration (8, 9). Pregnancy and birth cohort studies were eligible to participate if they included mothers with singleton live-born children born from January 1, 1989, onwards, had information available on maternal pre- or early pregnancy BMI and at least 1 offspring measurement (birth weight or childhood BMI), and were approved by their local institutional review boards. We invited 50 Western cohorts from Europe, North America, and Australia, selected from existing collaborations on childhood health (the EarlyNutrition project, the CHICOS project, and Birthcohorts.net assessed until July 2014), of which 39 agreed to participate. In total, 37 cohorts had data available on childhood BMI, corresponding to 162,129 mothers and their children eligible for analyses (**Figure 1**). All cohorts included were approved by their local institutional review boards, and all participants gave written informed consent. The plan for analyses given to the cohorts when inviting them to participate in the

MOCO collaboration is provided in **Supplemental Text 1**. Based on data availability and additional research questions, it was decided among the collaborators to refine the existing questions and to extend the project with additional questions to be addressed. Statistical analyses were adapted to these questions. Anonymized datasets were stored on a single central secured data server with access for the main analysts (EV, SS).

Figure 1. Flow chart of the cohorts and participants



Maternal pre-pregnancy BMI and gestational weight gain

Maternal BMI (kg/m^2) was measured, derived from clinical records, or self-reported (cohort-specific information in **Supplemental Table 1**). If available, we used information on maternal pre-pregnancy BMI for analyses. For participants without information on pre-pregnancy BMI (4.8% of the study population), we used BMI measured before 20 weeks of gestation. Maternal pre-pregnancy BMI was categorized into clinical categories according to World Health Organization (WHO) cutoffs (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]). The obesity category was further stratified into obesity grade 1 (30.0–34.9 kg/m^2), grade 2 (35.0–39.9 kg/m^2), and grade 3 (≥ 40.0 kg/m^2) (10). Maternal pre-pregnancy BMI was also categorized into 11 groups with a range of 2.5 kg/m^2 each. Data on gestational weight gain (kg), defined as the difference between the latest weight before delivery and pre-pregnancy weight, was provided by the cohorts, and was categorized as inadequate, adequate, or excessive weight gain in relation to maternal pre-pregnancy BMI according to the guidelines of the US Institute of Medicine (IOM) (11). We calculated *z*-scores of gestational weight gain based on maternal pre-pregnancy BMI-category-specific reference charts for gestational weight gain by gestational age created using the data of the cohorts participating in this collaboration (8), and categorized them into 6 categories (<–2.0 SD, –2.0 to –1.0 SD, –1.0 to –0.0 SD, 0.0 to 1.0 SD, 1.0 to 2.0 SD, and ≥2.0 SD).

Childhood overweight/obesity

Childhood BMI (kg/m^2) was measured, derived from clinical records, or reported by parents/caregivers or the child itself (cohort-specific information in **Supplemental Table 1**). BMI measurements were available in 3 age intervals: early childhood (≥ 2 to <5 years), mid childhood (≥ 5 to <10 years), and late childhood (≥ 10 to <18 years), hereafter referred to as 2.0–5.0, 5.0–10.0, and 10.0–18.0 years, respectively. If there were multiple measurements of a child available within the same age interval, we used the measurement at the highest age for our analyses. We calculated the sex- and age-adjusted standard deviation score (SDS) of childhood BMI based on WHO reference growth charts (Growth Analyser 4.0, Dutch Growth Research Foundation) (12, 13). We categorized childhood BMI into underweight, normal weight, overweight, and obesity, using WHO cutoffs (12, 13). For models focused on the risk of overweight/obesity, children with underweight were excluded, and overweight and obesity were combined. For models focused on the risk of underweight, children with overweight and obesity were excluded.

Covariates

Information on covariates was mostly assessed by questionnaires. We included as confounders the following: maternal age (<25.0 years, 25.0–30.0 years, 30–35.0 years, ≥35.0 years), maternal educational level (low, medium, high), maternal ethnicity (European/white, non-European/ non-white), parity (nulliparous, multiparous), and maternal smoking during pregnancy (yes, no).

Statistical analysis

We conducted 1-stage meta-analyses, analyzing IPD from all cohorts simultaneously in multilevel linear or binary logistic regression models, accounting for clustering of participants within cohorts (14). In these models, we included a random intercept at the cohort level, allowing intercepts to differ between cohorts. First, we examined the separate associations of maternal pre-pregnancy BMI and gestational weight gain, across their full ranges and in clinical categories, with BMI SDS, the risk of childhood underweight, and the risk of childhood overweight/ obesity in early, mid, and late childhood. Second, we calculated, both for the total study population and per country, the population attributable risk fraction (PAR), indicating the proportion of childhood underweight and childhood overweight/obesity attributable to each maternal BMI or gestational weight gain category. For these analyses, we used the adjusted odds ratio (OR) and the prevalence of the exposure in the study population (15). Country-specific analyses were performed for mid childhood only, based on available data. Third, we assessed the associations of the combinations of maternal pre-pregnancy BMI and gestational weight gain clinical categories with the outcomes. To assess whether the combined effects of maternal BMI and gestational weight gain on the outcomes were different from the separate effects, we tested for interaction between these 2 exposures.

Models were adjusted for maternal age, educational level, ethnicity, parity, and smoking during pregnancy. Models concerning gestational weight gain *z*-scores were additionally adjusted for maternal pre-pregnancy BMI. To examine whether any associations of maternal pre-pregnancy BMI with the risk of childhood overweight/obesity were explained by gestational diabetes, gestational hypertensive disorders, or gestational-age-adjusted birth weight, we added this information to the models. Covariates in the analyses were selected based on existing literature and data availability in participating cohorts. Findings from the unadjusted models were similar to the findings from the adjusted models and therefore are not presented separately. We did not observe consistent significant interactions of maternal pre-pregnancy BMI and gestational weight gain with child's sex. As sensitivity analyses, we conducted 2-stage random effects meta-analyses to study the associations of maternal pre-pregnancy BMI and gestational weight gain with the risk of childhood overweight/obesity in each cohort and to test for heterogeneity between estimates (14). All covariates were categorized. To deal with missing values of covariates, we used the missing values as an additional category, to prevent exclusion of non-complete cases. Exposures and outcomes were not imputed. If information on a covariate was available for less than 50% of the cohort sample used for each analysis, available information was not used and the corresponding data for that full cohort sample was assigned to the missing category. We also conducted a sensitivity analysis with complete cases only.

The statistical analyses were performed using the IBM SPSS Statistics version 21.0 for Windows (IBM, Armonk, NY, US), RevMan version 5.3 (Nordic Cochrane Centre, Copenhagen, Denmark), and R statistical software version 3.3.3.

RESULTS

Participants' characteristics

Table 1 shows that the median maternal pre-pregnancy BMI was 22.7 kg/m^2 (95% range: 18.1, 34.3) and the median gestational weight gain was 14.0 kg (95% range: 4.0, 26.0). Of all children, 6.5%, 20.1%, and 22.2% were overweight/obese in early, mid, and late childhood, respectively. The country-specific prevalences of maternal overweight and obesity, excessive gestational weight gain, and mid childhood overweight/obesity ranged 12.9%–53.1%, 22.2%–57.0%, and 10.6%–43.1%, respectively (**Supplemental Figure 1**). **Supplemental Table 2** shows cohort-specific information on covariates.

Maternal pre-pregnancy BMI and gestational weight gain clinical categories

Table 2 shows that, as compared to maternal normal weight, maternal underweight was associated with lower risks of overweight/obesity throughout childhood (p-values < 0.05). As compared to maternal normal weight, maternal overweight and obesity were associated with higher risks of overweight/obesity throughout childhood, with stronger associations at later ages (ORs for overweight/obesity in late childhood: 2.28 [95% CI: 2.08, 2.50] and 4.47 [95% CI: 3.99, 5.23] for maternal overweight and obesity, respectively). Among women with obesity, the risk of offspring overweight/obesity increased further for higher classes of maternal obesity (ORs for overweight/obesity in late childhood: 4.16 [95% CI: 3.56, 4.87], 5.98 [95% CI: 4.50, 7.94], and 5.55 [95% CI: 3.25, 9.45] for obesity grade 1, grade 2, and grade 3, respectively, as compared to normal weight; **Table 2**). These associations were not explained by gestational diabetes or gestational hypertensive disorders (**Supplemental Tables 3 and 4**). Additional adjustment for gestational-age-adjusted birth weight attenuated the associations only slightly (**Supplemental Table 5**). As compared to adequate gestational weight gain, inadequate gestational weight gain was associated with a lower risk of overweight/obesity in early and mid childhood (p-values < 0.05), but not in late childhood. As compared to adequate gestational weight gain, excessive gestational weight gain was associated with a higher risk of childhood overweight/obesity in early, mid, and late childhood (ORs 1.39 [95% CI: 1.30, 1.49], 1.55 [95% CI: 1.49, 1.60], and 1.72 [95% CI: 1.56, 1.91], respectively). **Supplemental Table 6** shows that, as compared to maternal normal weight, maternal underweight was associated with a higher risk of childhood underweight, whereas maternal overweight and obesity were associated with a lower risk of childhood underweight in early, mid, and late childhood. Similarly, as compared to adequate gestational weight gain, inadequate gestational weight gain was associated with higher risks of childhood underweight, and excessive gestational weight gain with lower risks. The associations of maternal BMI and gestational weight gain clinical categories with childhood BMI SDS are presented in **Table 3**.

Table 1. Cohort-specific description of exposures and outcomes

Cohort name, number of participants, birth years (country)	Maternal characteristics	
	Pre-/early pregnancy BMI (kg/m^2)	Gestational weight gain (kg)
ABCD, n=5,494, 2003-2004 (The Netherlands)	22.2 (17.9, 33.9)	NA
ALSPAC, n=8,435,1991-1992 (United Kingdom)	22.3 (18.0, 33.6)	12.5 (4.0, 22.0)
AOB/F, n=1,653, 2008-2010 (Canada)	23.0 (18.0, 38.2)	NA
BAMSE, n=2,930, 1994-1996 (Sweden)	22.3 (18.2, 31.6)[a]	13.0 (5.6, 25.0)
BIB, n=887, 2007-2010 (United Kingdom)	24.8 (17.6, 39.4)[a]	10.0 (0.0, 20.5)
CHOP, n=905, 2002-2004 (Multiple)	22.4 (17.6, 33.7)	NA
Co.N.ER, n=522, 2004-2005 (Italy)	21.2 (17.7, 30.4)	13.0 (6.0, 22.1)
DNBC, n=39,637, 1996-2002 (Denmark)	22.5 (18.1, 33.6)	15.0 (5.0, 28.0)
EDEN, n=1,331,2003-2005 (France)	22.1 (17.4, 35.0)	13.0 (4.0, 23.0)
FCOU, n=2,107, 1993-1996 (Ukraine)	21.8 (17.3, 32.1)	12.0 (3.5, 21.0)
GASPII, n=568, 2003-2004 (Italy)	21.3 (17.6, 31.1)	13.0 (6.0, 24.0)
GECKO Drenthe, n=1,963, 2006-2007 (The Netherlands)	23.7 (18.6, 36.8)	13.0 (4.0, 25.0)
GENERATION R, n=6,716, 2002-2006 (The Netherlands)	22.8 (18.1, 34.9)	13.0 (1.0, 25.0)
GENERATION XXI, n=5,940, 2005-2006 (Portugal)	23.0 (18.2, 34.7)	13.0 (3.0, 26.0)
GENESIS, n=1,898, 2003-2004 (Greece)	21.8 (17.6, 30.9)	13.0 (3.0, 28.6)
GINIplus, n=2,326, 1995-1998 (Germany)	22.1 (18.0, 31.4)	13.0 (5.0, 25.0)
HUMIS, n=945, 2003-2008 (Norway)	23.3 (18.4, 35.0)	14.0 (5.0, 27.0)
INMA, n=1,916, 1997-2008 (Spain)	22.5 (18.0, 34.6)	13.5 (4.2, 24.4)
KOALA, n=2,051, 2000-2002 (The Netherlands)	22.8 (18.5, 33.5)	14.0 (4.0, 25.0)
Krakow Cohort, n=422, 2000-2003 (Poland)	21.1 (17.3, 28.0)	15.0 (7.0, 28.0)

Early childhood characteristics (2.0-5.0 years)			Mid childhood characteristics (5.0-10.0 years)			Late childhood characteristics (10.0-18.0 years)		
Age (months)	BMI (SDS)	Overweight/ Obesity (n (%))	Age (months)	BMI (SDS)	Overweight/ obesity (n(%))	Age (months)	BMI (SDS)	Overweight/ obesity (n(%))
47.2 (25.5, 54.3)	0.28 (-1.52, 2.37)	212 (4.4)	68.2 (61.6, 82.2)	0.10 (-1.69, 2.38)	766 (17.1)	NA	NA	NA
48.7 (30.8, 49.7)	0.61 (-1.00, 2.45)	71 (6.4)	115.0 (88.0, 119.0)	0.24 (-1.60, 2.68)	2,001 (26.5)	165.0 (126.0, 171.0)	0.23 (-1.85, 2.53)	1,908 (26.0)
36.0 (35.0, 42.0)	0.23 (-2.27, 2.65)	94 (5.7)	NA	NA	NA	NA	NA	NA
51.3 (48.2, 57.6)	0.57 (-0.94, 2.47)	152 (6.0)	100.0 (89.0, 109.0)	0.51 (-1.19, 2.63)	695 (31.0)	201.2 (191.7, 210.2)	0.09 (-1.73, 2.03)	373 (16.9)
36.8 (35.8, 39.3)	0.49 (-1.40, 2.54)	64 (7.2)	NA	NA	NA	NA	NA	NA
48.6 (24.0, 60.0)	0.26 (-1.58, 2.51)	24 (5.8)	96.1 (65.9, 99.2)	0.32 (-1.72, 2.92)	201 (27.8)	NA	NA	NA
43.9 (34.8, 54.8)	0.27 (-2.28, 2.92)	47 (9.8)	94.9 (86.6, 111.2)	0.69 (-1.29, 2.82)	100 (35.3)	NA	NA	NA
NA	NA	NA	85.0 (75,6, 89.5)	0.01 (-1.95, 2.08)	6,138 (15.5)	NA	NA	NA
38.0 (36.9, 40.0)	0.29 (-1.46, 1.96)	26 (2.2)	67.6 (65.0, 72.4)	-0.02 (-1.52, 1.92)	140 (12.5)	NA	NA	NA
35.0 (24.0, 40.0)	0.55 (-1.93, 3.14)	134 (11.1)	84.0 (75.0, 93.4)	-0.02 (-2.01, 2.06)	111 (12.6)	194.0 (183.0, 209.0)	-0.09 (-2.06, 1.82)	70 (9.1)
50.0 (43.0, 53.0)	0.71 (-1.06, 2.94)	52 (9.7)	104.0 (98.0, 113.0)	0.70 (-1.37, 2.66)	171 (37.1)	NA	NA	NA
NA	NA	NA	70.4 (62.6, 78.6)	0.39 (-1.16, 2.43)	426 (21.7)	NA	NA	NA
45.8 (44.4, 48.6)	0.30 (-1.44, 2.48)	186 (4.9)	115.3 (69.4, 119.4)	0.36 (-1.52, 2.70)	1,661 (27.5)	122.1 (120.1, 137.8)	0.36 (-1.51, 2.62)	144 (29.8)
50.0 (46.0, 58.0)	0.52 (-1.27, 3.07)	483 (10.3)	85.0 (83.0, 95.0)	0.63 (-1.38, 3.23)	2,015 (37.9)	NA	NA	NA
43.6 (26.1, 57.8)	0.83 (-1.17, 3.57)	257 (14.2)	62.0 (60.1. 71.9)	0.93 (-1.44, 4.13)	38 (43.2)	NA	NA	NA
48.0 (44.0, 52.0)	0.08 (-1.72, 2.00)	54 (2.5)	62.9 (60.2, 75.0)	0.01 (-1.77, 1.94)	231 (10.7)	182.0 (177.0, 191.0)	0.01 (-1.88, 2.08)	366 (15.9)
25.7 (24.0, 37.4)	0.33 (-1.83, 2.39)	52 (6.1)	84.0 (60.0, 92.0)	0.04 (-2.02, 2.35)	62 (17.6)	NA	NA	NA
52.9 (49.0, 56.5)	0.50 (-1.21, 2.82)	142 (8.2)	83.8 (74.8, 94.5)	0.58 (-1.34, 3.32)	498 (37.7)	174.5 (172.0, 181.5)	0.32 (-1.59, 2.47)	76 (25.3)
55.5 (48.1, 59.7)	-0.07 (-2.00, 1.83)	16 (1.6)	106.0 (61.5, 119.3)	-0.17 (-2.16, 1.77)	198 (11.3)	121.4 (120.0, 126.7)	-0.16 (-2.06, 2.22)	19 (18.1)
48.0 (36.0, 51.3)	-0.06 (-2.24, 2.28)	11 (4.1)	108.0 (60.0, 111.0)	0.18 (-1.86, 2.56)	90 (26.5)	NA	NA	NA

Table 1. Cohort-specific description of exposures and outcomes (continued)

Cohort name, number of participants, birth years (country)	Maternal characteristics	
	Pre-/early pregnancy BMI (kg/m^2)	Gestational weight gain (kg)
LISAplus, n=2,334, 1997-1999 (Germany)	21.7 (17.9, 32.9)	14.0 (6.0, 24.5)
LUKAS, n=379, 2002-2005 (Finland)	24.0 (18.5, 36.6)	13.9 (3.9, 25.1)
MoBa, n=54,910, 1999-2009 (Norway)	23.1 (18.4, 34.7)	14.5 (4.0, 27.0)
NINFEA, n=1,753, 2005-2010 (Italy)[b]	21.4 (17.4, 31.9)	12.0 (3.0, 22.0)
PÉLAGIE, n=738, 2002-2005 (France)	21.7 (17.5, 32.4)	NA
PIAMA, n=2,324, 1996-1997 (The Netherlands)	22.2 (18.4, 31.5)	13.0 (5.0, 25.0)
Piccolipiù, n=687, 2011-2015 (Italy)	21.6 (17.6, 31.8)	13.0 (6.0, 21.2)
Project Viva, n=1,382, 1999-2002 (United States)	23.5 (18.3, 38.2)	15.5 (5.0, 27.3)
Raine Study, n=2,092, 1989-1992 (Australia)	21.3 (17.1, 34.0)	NA
REPRO_PL, n=283, 2007-2011 (Poland)	21.6 (17.2, 32.8)	12.5 (2.3, 23.0)
RHEA, n=748, 2007-2008 (Greece)	23.4 (18.1, 36.4)	13.0 (4.0, 26.0)
ROLO, n=290, 2007-2011 (Ireland)	25.3 (20.1, 38.7)[a]	12.1 (2.1, 22.7)
SCOPE BASELINE, n=1,045, 2008-2011 (Ireland)	24.0 (19.3, 34.8)[a]	14.3 (7.3, 23.3)
SEATON, n=933, 1998-1999 (United Kingdom)	24.0 (18.8, 37.9)[a]	NA
Slovak PCB study, n=480, 2002-2004 (Slovakia)	21.2 (16.7, 31.6)	14.0 (4.1, 24.8)
STEPS, n=484, 2008-2010 (Finland)	22.8 (18.3, 36.9)	14.1 (1.6, 25.5)
SWS, n=2,621, 1998-2007 (United Kingdom)	24.2 (18.9, 37.4)	11.9 (0.4, 25.2)
Total group	22.7 (18.1, 34.3)	14.0 (4.0, 26.0)

Values are expressed as medians (95% range) or numbers of participants (valid %). NA: Not available. SDS: Standard Deviation Score. [a]Only information available on BMI assessed in early pregnancy (<20 weeks of gestation).[b]Subset of participants with 4-years follow-up completed.

Early childhood characteristics (2.0-5.0 years)			Mid childhood characteristics (5.0-10.0 years)			Late childhood characteristics (10.0-18.0 years)		
Age (months)	BMI (SDS)	Overweight/ Obesity (n (%))	Age (months)	BMI (SDS)	Overweight/ obesity (n(%))	Age (months)	BMI (SDS)	Overweight/ obesity (n(%))
48.0 (44.0, 52.0)	0.07 (-1.83, 1.98)	53 (2.5)	62.7 (60.2, 74.0)	-0.09 (-1.92, 1.91)	207 (10.4)	181.0 (121.0, 191.0)	-0.02 (-1.88, 2.08)	293 (15.9)
48.2 (46.2, 50.0)	0.52 (-1.28, 2.81)	30 (7.9)	73.2 (68.6, 76.0)	0.52 (-1.08, 3.34)	112 (31.0)	NA	NA	NA
36.4 (25.5, 60.0)	0.37 (-1.84, 2.46)	2,447 (6.1)	86.9 (61.0, 100.9)	0.14 (-2.06, 2.30)	6,774 (19.4)	NA	NA	NA
49.7 (48.2, 57.1)	0.09 (-2.33, 2.52)	88 (5.1)	86.1 (84.8, 93.0)	-0.03 (-2.17, 2.43)	91 (21.0)	NA	NA	NA
24.4 (24.0, 26.5)	0.12 (-1.84, 1.95)	16 (2.2)	NA	NA	NA	NA	NA	NA
49.3 (44.2, 54.5)	0.69 (-1.20, 2.58)	105 (9.3)	97.5 (90.9, 110.6)	0.15 (-1.68, 2.35)	417 (20.5)	195.9 (192.5, 203.4)	-0.16 (-1.74, 1.80)	72 (9.5)
24.0 (24.0, 28.0)	0.36 (-2.16, 2.55)	40 (5.8)	NA	NA	NA	NA	NA	NA
37.9 (36.1, 50.2)	0.66 (-1.01, 2.69)	86 (7.0)	92.2 (82.5, 116.6)	0.44 (-1.43, 3.05)	326 (30.7)	123.8 (120.6, 131.1)	0.38 (-1.50, 3.76)	8 (25.8)
NA	NA	NA	71.0 (66.8, 77.1)	0.15 (-1.57, 2.75)	384 (20.0)	126.9 (125.0, 133.3)	0.45 (-1.62, 2.84)	566 (33.3)
25.0 (24.0, 31.0)	0.31 (-2.13, 2.51)	19 (7.1)	88.0 (84.3, 94.0)	0.64 (-1.55, 3.64)	19 (38.8)	NA	NA	NA
49.8 (48.0, 57.5)	0.60 (-1.13, 3.58)	92 (12.3)	NA	NA	NA	NA	NA	NA
24.7 (24.0, 34.0)	0.20 (-1.75, 2.62)	19 (6.6)	NA	NA	NA	NA	NA	NA
25.5 (24.5, 28.9)	0.65 (-1.02, 2.32)	62 (5.9)	NA	NA	NA	NA	NA	NA
58.6 (55.9, 59.9)	0.65 (-0.89, 2.68)	37 (7.8)	61.2 (60.0, 119.7)	0.59 (-1.10, 2.73)	58 (19.8)	180.1 (121.5, 186.0)	0.43 (-1.61, 2.60)	199 (31.6)
45.4 (44.8, 49.9)	1.95 (-2.46, 5.29)	212 (48.7)	93.0 (85.0, 100.0)	0.32 (-1.73, 3.22)	117 (32.1)	NA	NA	NA
36.8 (35.6, 38.4)	0.56 (-1.09, 2.18)	20 (4.1)	NA	NA	NA	NA	NA	NA
38.4 (35.6, 50.7)	0.49 (-1.27, 2.57)	155 (6.1)	80.4 (74.7, 87.2)	0.21 (-1.52, 2.54)	392 (22.0)	NA	NA	NA
38.2 (24.5, 60.0)	0.39 (-1.69, 2.58)	5,558 (6.5)	85.3 (61.0, 117.4)	0.14 (-1.85, 2.44)	24,439 (20.1)	168.0 (121.8, 203.7)	0.14 (-1.81, 2.41)	4,094 (22.2)

Table 2. Associations of maternal pre-pregnancy BMI and gestational weight gain clinical categories with the risk of childhood overweight/obesity

	Early childhood 2.0-5.0 years		Mid childhood 5.0-10.0 years		Late childhood 10.0-18.0 years	
	Overweight/obesity OR (95% CI)	PAR (%)	Overweight/obesity OR (95% CI)	PAR (%)	Overweight/obesity OR (95% CI)	PAR (%)
Maternal pre-pregnancy BMI						
Underweight (<18.5 kg/m^2)	0.57 (0.47, 0.69)	NA	0.44 (0.40, 0.49)	NA	0.44 (0.35, 0.55)	NA
	$n_{cases/total}$ =126/3,162		$n_{cases/total}$ =401/4,485		$n_{cases/total}$ =93/877	
Normal weight (18.5-24.9 kg/m^2)	Reference		Reference		Reference	
	$n_{cases/total}$ =3,092/57,293		$n_{cases/total}$ =13,870/82,438		$n_{cases/total}$ =2,505/13,497	
Overweight (25.0-29.9 kg/m^2)	1.66 (1.56, 1.78)	11.5	1.91 (1.85, 1.98)	15.1	2.28 (2.08, 2.50)	20.1
	$n_{cases/total}$ =1,476/17,013		$n_{cases/total}$ =6,556/23,359		$n_{cases/total}$ =968/2,799	
Obesity (≥30.0 kg/m^2)	2.43 (2.24, 2.64)	10.2	3.12 (2.98, 3,27)	14.4	4.47 (3.99, 5.23)	21.6
	$n_{cases/total}$ =864/7,058		$n_{cases/total}$ =3,612/9,248		$n_{cases/total}$ =528/1,000	
Obesity grade 1 (30.0-34.9 kg/m^2)	2.35 (2.14, 2.59)	7.3	2.89 (2.74, 3.05)	10.0	4.16 (3.56, 4.87)	15.6
	$n_{cases/total}$ =613/5,142		$n_{cases/total}$ =2,552/6,874		$n_{cases/total}$ =363/726	
Obesity grade 2 (35.0-39.9 kg/m^2)	2.57 (2.20, 3.02)	2.5	3.57 (3.24, 3.93)	3.9	5.98 (4.50, 7.94)	7.4
	$n_{cases/total}$ =190/1,489		$n_{cases/total}$ =782/1,836		$n_{cases/total}$ =129/215	
Obesity grade 3 (≥40.0 kg/m^2)	2.93 (2.22, 3.87)	0.9	5.17 (4.35, 6.15)	2.0	5.55 (3.25, 9.45)	2.1
	$n_{cases/total}$ =61/427		$n_{cases/total}$ =278/538		$n_{cases/total}$ =36/59	

Table 2. Associations of maternal pre-pregnancy BMI and gestational weight gain clinical categories with the risk of childhood overweight/obesity (continued)

	Early childhood 2.0-5.0 years		Mid childhood 5.0-10.0 years		Late childhood 10.0-18.0 years	
	Overweight/obesity OR (95% CI)	PAR (%)	Overweight/obesity OR (95% CI)	PAR (%)	Overweight/obesity OR (95% CI)	PAR (%)
Gestational weight gain						
Inadequate weight gain	0.86 (0.78, 0.93)	NA	0.90 (0.84, 0.92)	NA	0.91 (0.82, 1.02)	NA
	$n_{cases/total}$ =853/15,484		$n_{cases/total}$=3,515/20,596		$n_{cases/total}$=705/3,897	
Adequate weight gain	Reference		Reference		Reference	
	$n_{cases/total}$ =1,542/25,418		$n_{cases/total}$=6,404/36,391		$n_{cases/total}$=944/5,152	
Excessive weight gain	1.39 (1.30, 1.49)	11.4	1.55 (1.49, 1.60)	15.4	1.72 (1.56, 1.91)	19.2
	$n_{cases/total}$=2,265/27,590		$n_{cases/total}$=9,884/40,201		$n_{cases/total}$=1,061/3,703	

Values are odds ratios (95% confidence intervals) from multilevel binary logistic regression models that reflect the risk of childhood overweight/obesity in early childhood (2.0-5.0 years), mid childhood (5.0-10.0 years) and late childhood (10.0-18.0 years) in children of mothers in the different pre-pregnancy BMI groups or gestational weight gain groups, as compared with the reference group (normal weight for pre-pregnancy BMI and adequate weight gain for gestational weight gain) or population attributable risk fractions (PAR), indicating the proportion of childhood overweight/obesity cases attributable to each maternal BMI or gestational weight gain category. The models are adjusted for maternal age, education level, ethnicity, parity, and smoking during pregnancy. NA: not applicable

Table 3. Associations of maternal pre-pregnancy BMI and gestational weight gain clinical categories with childhood BMI SDS

	Childhood BMI (SDS)		
	Early childhood 2.0-5.0 years	**Mid childhood 5.0-10.0 years**	**Late childhood 10.0-18.0 years**
Maternal pre-pregnancy BMI			
Underweight (<18.5 kg/m^2)	-0.29 (-0.33, -0.26)	-0.41 (-0.45, -0.38)	-0.48 (-0.54, -0.41)
	n =3,240	n =4,673	n=917
Normal weight (18.5-24.9 kg/m^2)	Reference	Reference	Reference
	n =58,177	n =84,119	n=13,737
Overweight (25.0-29.9 kg/m^2)	0.19 (0.17, 0.21)	0.33 (0.32, 0.35)	0.45 (0.41, 0.49)
	n=17,258	n =23,671	n=2,819
Obesity (≥30.0 kg/m^2)	0.34 (0.32, 0.37)	0.62 (0.60, 0.64)	0.92 (0.86, 0.99)
	n =7,138	n =9,340	n=1,004
Obesity grade 1 (30.0-34.9 kg/m^2)	0.32 (0.29, 0.35)	0.57 (0.54, 0.59)	0.85 (0.76, 0.93)
	n =5,197	n =6,944	n =730
Obesity grade 2 (35.0-39.9 kg/m^2)	0.39 (0.33, 0.44)	0.72 (0.67, 0.77)	1.09 (0.96, 1.23)
	n =1,509	n =1,854	n =215
Obesity grade 3 (≥40.0 kg/m^2)	0.45 (0.35, 0.55)	0.94 (0.85, 1.03)	1.18 (0.92, 1.44)
	n =343	n =542	n =59
Gestational weight gain			
Inadequate weight gain	-0.10 (-0.12, -0.08)	-0.09 (-0.11, -0.07)	-0.09 (-0.13, -0.05)
	n =15,782	n =21,094	n=3,998
Adequate weight gain	Reference	Reference	Reference
	n =25,829	n =37,142	n=5,247
Excessive weight gain	0.14 (0.12, 0.16)	0.22 (0.21, 0.24)	0.28 (0.24, 0.32)
	n=27,965	n =40,879	n=3,742

Values are regression coefficients (95% confidence intervals) from multilevel linear regression models that reflect differences in early childhood (2.0-5.0 years), mid childhood (5.0-10.0 years) and late childhood (10.0-18.0 years) in children of mothers in the different pre-pregnancy BMI groups or gestational weight gain groups, as compared with the reference group (normal weight for pre-pregnancy BMI and adequate weight gain for gestational weight gain). The models are adjusted for maternal age, education level, ethnicity, parity, and smoking during pregnancy.

The estimated proportions of childhood overweight/obesity attributable to maternal pre-pregnancy overweight and obesity were respectively 11.5% and 10.2% in early childhood, 15.1% and 14.4% in mid childhood, and 20.1% and 21.6% in late childhood (**Table 2**). The estimated proportions of childhood overweight/obesity attributable to excessive gestational weight gain were 11.4%, 15.4%, and 19.2%, in early, mid, and late childhood, respectively (**Table 2**). The country-specific proportions of mid childhood overweight/obesity are given in **Supplemental Figure 2**.

Maternal pre-pregnancy BMI and gestational weight gain across their full ranges

Figures 2A and 2B show that higher maternal pre-pregnancy BMI was across the full range associated with higher risk of offspring overweight/obesity and higher offspring BMI SDS throughout childhood. The ORs for childhood overweight/obesity per kg/m^2 increase in maternal pre-pregnancy BMI were 1.08 (95% CI: 1.07, 1.09), 1.12 (95% CI: 1.11, 1.12), and 1.16 (95% CI: 1.15, 1.17), in early, mid, and late childhood, respectively. Similarly, higher maternal gestational weight gain across its full range was associated with a higher risk of overweight/obesity and higher childhood BMI in early, mid, and late childhood (**Figures 2C and 2D**). The ORs for childhood overweight/obesity per SD increase in gestational weight gain were 1.14 (95% CI: 1.11, 1.17), 1.16 (95% CI: 1.14, 1.18), and 1.14 (95% CI: 1.09, 1.20), in early, mid, and late childhood, respectively. Similar results were observed when performing 2-stage random effects meta-analyses, with low to moderate heterogeneity (**Supplemental Figures 3 and 4**).

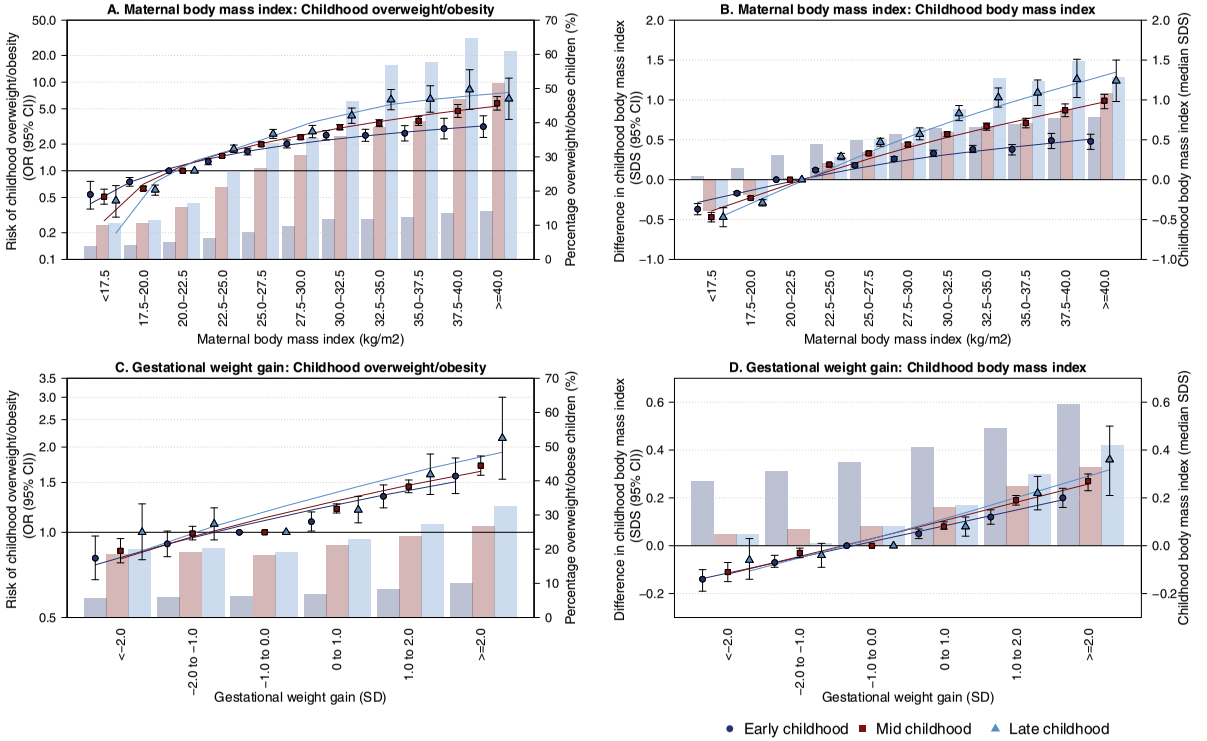
Combined effects of maternal pre-pregnancy BMI and gestational weight gain clinical categories

Table 4 shows the combined effect of clinical categories of maternal pre-pregnancy BMI and gestational weight gain on childhood overweight/obesity. Regardless of their mothers' gestational weight gain, children of mothers with underweight tended to have a lower risk of overweight/ obesity, whereas children of mothers with overweight or obesity had a higher risk of overweight/obesity, as compared to children whose mothers had normal weight and adequate gestational weight gain. Within each maternal BMI category, excessive gestational weight gain tended to increase the risk of overweight/obesity in early and mid childhood only slightly. The combined associations of clinical categories of maternal pre-pregnancy BMI and gestational weight gain with childhood BMI SDS are given in **Table 5**.

DISCUSSION

In this IPD meta-analysis, we observed that higher maternal pre-pregnancy BMI and gestational weight gain were across their full ranges associated with higher risks of offspring overweight/ obesity throughout childhood. The effects tended to be stronger at older ages. However, the effect of gestational weight gain in addition to that of pre-pregnancy BMI was small. At the population level, 21.7% to 41.7% of childhood overweight/obesity prevalence was estimated to be attributable to maternal overweight and obesity together, whereas 11.4% to 19.2% was estimated to be attributable to excessive gestational weight gain.

Figure 2. Associations of maternal pre-pregnancy BMI and gestational weight gain with the risk of childhood overweight/obesity and childhood BMI



The circles, squares and triangles represent odds ratios (ORs) (A and C) or regression coefficients (B and D) (95% confidence intervals) obtained from multilevel binary logistic or linear regression models that reflect the risk of overweight/obesity or differences in early, mid, and late childhood BMI standard deviation score (SDS) in the different maternal pre-pregnancy BMI or gestational weight gain groups, as compared to the reference group (20.0–22.5 kg/m2 for maternal BMI, -1.0 to 0.0 SD for gestational weight gain [largest groups], primary y-axis). The lines are trendlines through the estimates. The models are adjusted for maternal age, education level, ethnicity, parity, and smoking during pregnancy. The bars represent the percentage overweight/obese children (A and C) or the median childhood BMI SDS (B and D) in early (2.0–5.0 years, violet bars), mid (5.0–10.0 years, brown bars), and late childhood (10.0–18.0 years, light blue bars) in the study population (secondary y-axis).

Table 4. Combined associations of maternal pre-pregnancy BMI and gestational weight gain clinical categories with the risk of childhood overweight/obesity

	Childhood overweight/obesity (OR (95% CI))		
Maternal pre-pregnancy BMI	**Early childhood 2.0-5.0 years**	**Mid childhood 5.0-10.0 years**	**Late childhood 10.0-18.0 years**
Underweight (<18.5 kg/m^2)			
Inadequate weight gain	0.58 (0.42, 0.81)	0.39 (0.31, 0.48)	0.36 (0.22, 0.58)
	$n_{cases/total}$ =40/970	$n_{cases/total}$ =97/1,284	$n_{cases/total}$ =19/266
Adequate weight gain	0.58 (0.42, 0.80)	0.46 (0.38, 0.56)	0.37 (0.22, 0.62)
	$n_{cases/total}$ =44/1,087	$n_{cases/total}$ =126/1,538	$n_{cases/total}$ =16/226
Excessive weight gain	0.75 (0.49, 1.15)	0.75 (0.60, 0.95)	1.28 (0.67, 2.42)
	$n_{cases/total}$=25/455	$n_{cases/total}$=85/635	$n_{cases/total}$=13/56
Normal weight (18.5-24.9 kg/m^2)			
Inadequate weight gain	0.87 (0.78, 0.96)	0.91 (0.86, 0.96)	0.95 (0.84, 1.08)
	$n_{cases/total}$ =583/12,027	$n_{cases/total}$ =2,462/16,163	$n_{cases/total}$ =536/3,255
Adequate weight gain	Reference	Reference	Reference
	$n_{cases/total}$ =1,032/19,502	$n_{cases/total}$ =4,326/28,316	$n_{cases/total}$ =677/4,173
Excessive weight gain	1.28 (1.17, 1.41)	1.36 (1.30, 1.43)	1.30 (1.14, 1.49)
	$n_{cases/total}$ =1,010/14,927	$n_{cases/total}$ =4,381/22,400	$n_{cases/total}$ =446/2,162
Overweight (25-29.9 kg/m^2)			
Inadequate weight gain	1.49 (1.21, 1.82)	1.61 (1.44, 1.81)	2.04 (1.52, 2.74)
	$n_{cases/total}$ =118/1,417	$n_{cases/total}$ =447/1,767	$n_{cases/total}$ =74/239
Adequate weight gain	1.62 (1.41, 1.86)	1.87 (1.73, 2.01)	1.94 (1.58, 2.38)
	$n_{cases/total}$=295/3,451	$n_{cases/total}$=1,224/4,656	$n_{cases/total}$=163/574
Excessive weight gain	1.85 (1.68, 2.04)	2.25 (2.13, 2.37)	2.65 (2.28, 3.08)
	$n_{cases/total}$=828/9,010	$n_{cases/total}$=3,660/12,786	$n_{cases/total}$=395/1,119
Obesity (≥30 kg/m^2)			
Inadequate weight gain	2.04 (1.66, 2.52)	2.95 (2.62, 3.31)	5.62 (3.94, 8.02)
	$n_{cases/total}$ =112/1,070	$n_{cases/total}$ =509/1,382	$n_{cases/total}$ =76/137
Adequate weight gain	2.49 (2.09, 2.97)	3.45 (3.12, 3.82)	4.64 (3.39, 6.34)
	$n_{cases/total}$ =171/1,378	$n_{cases/total}$ =728/1,881	$n_{cases/total}$ =88/179
Excessive weight gain	2.63 (2.32, 2.98)	3.70 (3.44, 3.97)	6.02 (4.79, 7.56)
	$n_{cases/total}$ =402/3,198	$n_{cases/total}$ =1,758/4,380	$n_{cases/total}$ =207/366

Values are odds ratios (95% confidence intervals) from multilevel binary logistic regression models that reflect the risk of childhood overweight/obesity in early childhood (2.0-5.0 years), mid childhood (5.0-10.0 years) and late childhood (10.0-18.0 years) in children of mothers in the different BMI and gestational weight gain categories, as compared to the reference group (normal weight mothers with adequate gestational weight gain). The models are adjusted for maternal age, education level, ethnicity, parity, and smoking during pregnancy. P-values for interaction between maternal BMI and gestational weight gain for the risk of childhood overweight/obesity: p=0.038, p<0.001 and p=0.637, in early-, mid- and late childhood, respectively.

Table 5. Combined associations of maternal pre-pregnancy BMI and gestational weight gain clinical categories with childhood BMI SDS

	Childhood BMI (SDS (95%CI))		
Maternal pre-pregnancy BMI	**Early childhood 2.0-5.0 years**	**Mid childhood 5.0-10.0 years**	**Late childhood 10.0-18.0 years**
Underweight (<18.5 kg/m^2)			
Inadequate weight gain	-0.32 (-0.39, -0.25)	-0.52 (-0.58, -0.47)	-0.53 (-0.65, -0.41)
	n =991	n =1,351	n= 280
Adequate weight gain	-0.27 (-0.34, -0.21)	-0.37 (-0.42, -0.31)	-0.43 (-0.57, -0.30)
	n =1,118	n =1598	n= 236
Excessive weight gain	-0.14 (-0.24, -0.05)	-0.19 (-0.26, -0.11)	-0.21 (-0.47, 0.04)
	n =469	n =666	n =60
Normal weight (18.5-24.9 kg/m^2)			
Inadequate weight gain	-0.10 (-0.12, -0.07)	-0.07 (-0.09, -0.05)	-0.06 (-0.11, -0.01)
	n =12,264	n =16,549	n= 3,340
Adequate weight gain	Reference	Reference	Reference
	n =19,815	n =28,928	n =4,249
Excessive weight gain	0.10 (0.08, 0.13)	0.14 (0.13, 0.16)	0.12 (0.06, 0.17)
	n =15,121	n =22,825	n =2,187
Overweight (25-29.9 kg/m^2)			
Inadequate weight gain	0.06 (0.00, 0.12)	0.22 (0.17, 0.27)	0.32 (0.19, 0.45)
	n = 1,445	n =1,803	n =241
Adequate weight gain	0.13 (0.09, 0.17)	0.29 (0.26, 0.32)	0.34 (0.25, 0.43)
	n =3,501	n=4,715	n=581
Excessive weight gain	0.23 (0.20, 0.25)	0.41 (0.39, 0.43)	0.55 (0.49, 0.62)
	n =9,137	n =12,962	n =1,127
Obesity (≥30 kg/m^2)			
Inadequate weight gain	0.26 (0.20, 0.26)	0.58 (0.52, 0.63)	0.94 (0.77, 1.11)
	n =1,082	n =1,391	n =137
Adequate weight gain	0.36 (0.30, 0.41)	0.64 (0.59, 0.69)	0.88 (0.73, 1.03)
	n=1,395	n =1,901	n =181
Excessive weight gain	0.36 (0.33, 0.40)	0.69 (0.66, 0.72)	1.01 (0.90, 1.11)
	n =3,238	n =4,426	n=368

Values are regression coefficients (95% confidence intervals) from multilevel linear regression models that reflect differences in early childhood (2.0 – 5.0 years), mid childhood (5.0 – 10.0 years) and late childhood (10.0 – 18.0 years) BMI SDS between children of mothers in the different BMI and total gestational weight gain groups compared with the reference group (normal weight and adequate gestational weight gain). The models are adjusted for maternal age, education level, ethnicity, parity, and smoking during pregnancy. P-values for interaction between maternal BMI and gestational weight gain for childhood BMI SDS: p=0.016, p=0.002 and p=0.406, in early-, mid- and late childhood, respectively.

Interpretation of main findings

Maternal obesity does not only affect pregnancy outcomes, but may also have persistent effects on offspring fat development. Previous studies showed consistently that maternal overweight and obesity were positively associated with offspring BMI (2-4). In this IPD meta-analysis, we observed not only that maternal overweight and obesity were associated with higher risks of childhood overweight/obesity, but that these risks were progressively higher among children of mothers with grade 1, grade 2, and grade 3 obesity, respectively. In addition, we observed that this association was not limited to the extremes of maternal BMI, but was present across the full range. We observed a stronger association of maternal BMI with the risk of childhood overweight/obesity at later ages. Although we used a different reference chart in early childhood and did not correct for correlations between BMI measurements due to the large sample size, the observed association is unlikely to be explained by methodological issues as the observed risk also increased between mid and late childhood, where we used the same reference charts. Also, 2 recent studies among 1,494 Australian and 3,805 Dutch participants observed stronger associations of maternal BMI with childhood growth and obesity risk with increasing age, while accounting for correlated repeated measures (16, 17). This increasing strength of the association with age might reflect an intra-uterine programming mechanism becoming more apparent when children get older, or might be explained by a stronger influence of lifestyle characteristics of the child at later ages. We estimated that about 10% to 20% of overweight/obesity cases throughout childhood are attributable to maternal pre-pregnancy overweight and obesity, with the highest proportions explained by maternal overweight. Thus, our results suggest that high maternal BMI has a considerable population impact, and can be used as a target for preventive strategies. Importantly, the risk of childhood overweight and obesity is not confined to maternal obesity, but increases gradually over the full range of maternal pre-pregnancy BMI.

In addition to pre-pregnancy BMI, the amount of weight gain during pregnancy also seems to be associated with offspring obesity (18, 19). Previous meta-analyses of published studies showed a 33% to 40% increased risk of overweight or obesity in children of mothers with excessive gestational weight gain (6, 7). In line with these studies, we observed that excessive gestational weight gain was related to a 39%–72% higher risk of overweight throughout childhood. On a population level, 11% to 19% of childhood overweight/obesity could be attributed to excessive gestational weight gain. Also, gestational weight gain *z*-scores (specific for maternal BMI and gestational age) across the full range tended to be associated with an increased risk of offspring overweight/obesity. Thus, higher gestational weight gain across the full range, rather than only lower or higher gestational weight gain than recommended by the IOM, seems to be related to offspring weight status.

For the prevention of childhood overweight and obesity, insight into the combined effects of maternal BMI and gestational weight gain is important. Only 2 previous studies assessed the combined associations of maternal pre-pregnancy BMI and gestational weight

gain with childhood adiposity (20, 21). A study among 100,612 participants from China reported that, compared to normal maternal weight and adequate weight gain, normal weight or overweight/ obesity and excessive gestational weight gain was associated with an increased risk of overweight at 3–6 years of age in children (20). In a study in the US among 4,436 participants describing trajectories of maternal weight from pre-pregnancy until the postpartum period, the trajectory with the highest risk of offspring obesity at ages 6–11 and 12–19 years consisted almost entirely of women who were overweight or obese at the start of pregnancy, but only half of this group had excessive gestational weight gain (21). We observed that, compared to children of women with normal weight and adequate gestational weight gain, children of overweight and obese mothers had a higher risk of overweight/obesity, regardless of gestational weight gain. Within the BMI categories, there was only a small effect of gestational weight gain on offspring overweight/obesity. These findings suggest that the effects of gestational weight gain add only to a limited extent to the effects of maternal pre-pregnancy BMI.

Our results strongly suggest that maternal pre-pregnancy BMI and gestational weight gain are associated with increased risk of overweight and obesity throughout childhood. It remains unclear whether these associations are causal and which mechanisms are underlying these associations. Maternal pre-pregnancy obesity and excessive gestational weight gain are complex traits, which reflect multiple components. Maternal pre-pregnancy obesity reflects maternal genetic predisposition, nutritional status, fat accumulation, and low-grade inflammation, whereas maternal weight gain during pregnancy also reflects fluid expansion and growth of the fetus, placenta, and uterus (22, 23). Both may, at least partly, be explained by intra-uterine programming mechanisms. The fetal over-nutrition hypothesis suggests that increased fetal exposure to nutrients may lead to persistent adaptations in the structure and function of adipose tissue, appetite regulation, and energy metabolism, leading to an increased susceptibility to later obesity (24, 25). Epigenetic processes may play an important role in these adaptations (26). The associations may also reflect genetic predisposition to obesity (27, 28) or sociodemographic or lifestyle factors shared by mother and child. Common pregnancy disorders, including gestational diabetes and gestational hypertensive disorders, have also been related to offspring obesity risk (29-31). However, using data from the same studies as our current analyses, we previously reported that these associations were not independent of maternal BMI (9). In the current analysis, the associations of maternal BMI with the risk of childhood overweight/ obesity were independent of gestational diabetes and gestational hypertensive disorders. Unfortunately, no information on maternal glucose concentrations was available to assess the role of maternal glycemic status in further detail. Previous research has shown that there are sex differences in weight and body fat development in childhood (32, 33). We hypothesized that maternal BMI and gestational weight gain would influence the risk of childhood overweight/obesity differently or with a different timing in boys and girls. However, we did not observe sex differences in the observed associations,

possibly due to the fact that BMI does not distinguish between fat and lean mass. Further research is needed into the causality and underlying mechanisms of these associations (22).

Maternal pre-pregnancy BMI and, to a smaller extent, gestational weight gain are important modifiable risk factors of childhood weight status with a considerable population impact. Thus far, intervention trials have been focused on maternal weight during mid or late pregnancy, mostly showing reductions in gestational weight gain, but not showing any effect on birth outcomes or infant body composition (34-36). Only 1 small Swedish study including a lifestyle intervention reported results on childhood adiposity and showed no difference in BMI at age 5 years (37). We observed that the effect of excessive gestational weight gain was small in women with pre-pregnancy overweight and obesity. We also observed that the highest proportion of childhood overweight/obesity on a population level could be attributed to maternal pre-pregnancy overweight. Future intervention studies should shift their focus to preconceptional weight management, targeting women of reproductive age to achieve a normal weight.

Strengths and limitations

We meta-analyzed original data of different pregnancy and birth cohorts, limiting the potential of publication bias and enabling a consistent definition of exposures and outcomes and adjustment for potential confounders. Due to the large sample size, we were able to study effects in people with relatively rare conditions, such as severely obese women. We had data available at different childhood ages, enabling us to study the effects throughout childhood. We did not assess the associations of maternal BMI and gestational weight gain with the risk of childhood overweight/obesity over time in longitudinal analyses, as the data needed for such analyses were only available in a small subgroup of children and cohorts. Further studies are needed specifically exploring the development of childhood overweight and obesity over time in response to maternal BMI and gestational weight gain. Cohorts were initiated between 1989 and 2011. Given the rise in obesity prevalence in the past decades (38), it is likely that the current obesity prevalence and consequently the proportions of childhood overweight/obesity attributable to maternal overweight and obesity are underestimated. In our study, fewer cohorts reached the age for the late childhood analyses than for the early or mid childhood analyses. Our results for the late childhood analyses may be biased by cohort effects. We consider that the bias would most likely lead to an underestimation of the age differences because of the higher prevalences of childhood obesity in more recently started cohorts with younger children. As this study only included cohorts from Europe, North America, and Australia, and demographic characteristics in other continents may be different, results can only be generalized to participants from these continents. Further studies are needed to address similar research questions in other populations. We performed sensitivity analyses based on 2-stage random effects meta-analyses, which gave similar results with low to moderate heterogeneity between the cohorts. We used a missing value category to

deal with missing data for covariates. Due to data availability and the size of the dataset, we were not able to apply more advanced imputation strategies. We observed similar results when we conducted a complete case analysis (**Supplemental Tables 7 and 8**). Because maternal BMI was self-reported in some cohorts, some misclassification and underestimation of the effect estimates might be present. For the cohorts with no information on maternal pre-pregnancy BMI available, we used BMI measured in early pregnancy, which might have led to an overestimation of BMI as a result of gestational weight gain. For the country-specific analyses, it is important to note that countries are represented by 1 to 5 cohorts of different sample sizes per country and that not all cohorts are population-based, affecting the representativeness of the results for the full country. Although we adjusted the models for potential confounders, residual confounding by, for example, physical activity and dietary intake might still be an issue.

Conclusions

Higher maternal pre-pregnancy BMI and gestational weight gain are across their full ranges associated with an increased risk of offspring overweight/obesity throughout childhood and have a considerable population impact. The effect of gestational weight gain in addition to the effect of maternal pre-pregnancy BMI was small. Future intervention trials aiming to reduce childhood overweight and obesity should focus on maternal weight status before pregnancy, in addition to weight gain during pregnancy.

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SUPPLEMENTAL MATERIAL

Supplemental Text 1. Can be found online.

Supplemental Table 1. Cohort-specific methods of data collection

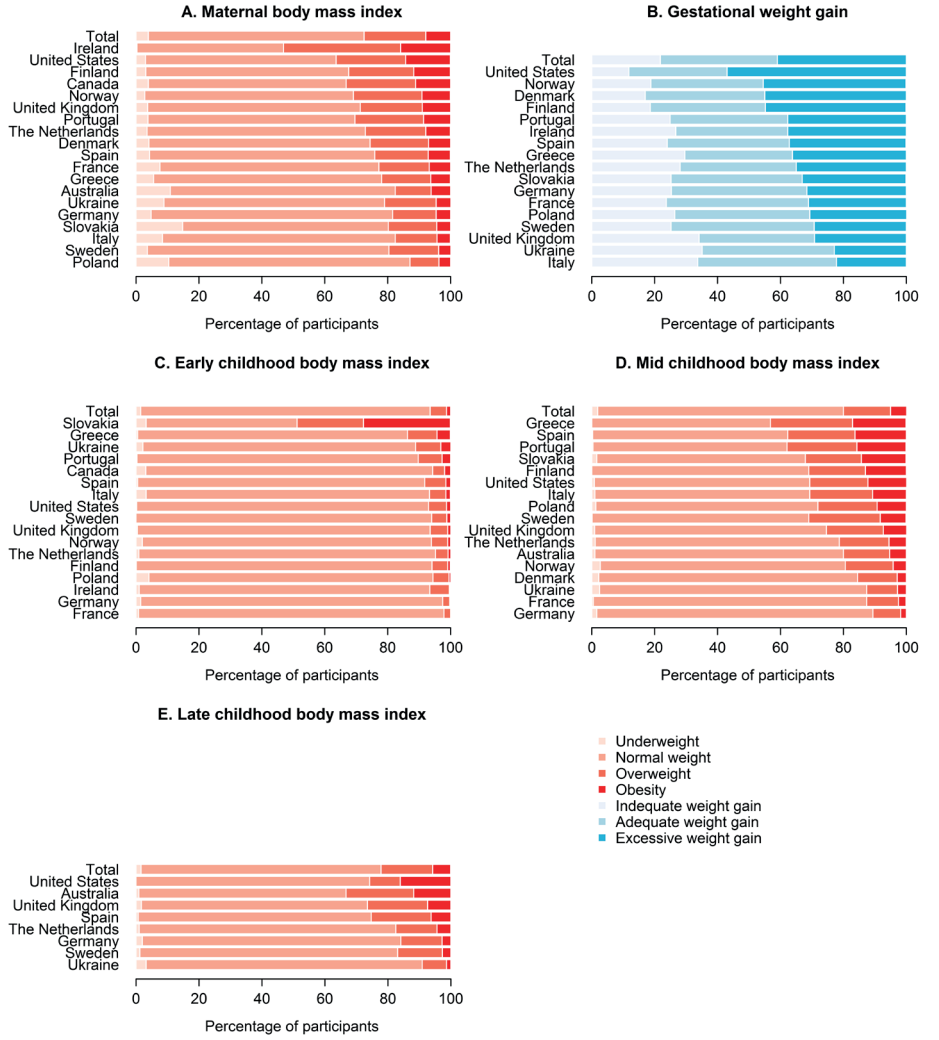
Cohort name (country)	Maternal height	Maternal pre-/ early pregnancy weight	Maternal latest weight before delivery or gestational weight gain	Childhood weight and height
ABCD (The Netherlands)	Self-reported	Self-reported	NA	Measured
ALSPAC (United Kingdom)	Self-reported	Self-reported	Clinical records	Measured
AOB/F (Canada)	Self-reported	Self-reported	NA	Reported
BAMSE (Sweden)	Medical Birth Registry	Medical Birth Registry	Medical Birth Registry	Measured
BIB (United Kingdom)	Measured	Measured	Clinical records	Measured
CHOP (Multiple)	Measured	Self-reported	NA	Measured
Co.N.ER (Italy)	Self-reported	Self-reported	Self-reported	Reported
DNBC (Denmark)	Self-reported	Self-reported	Self-reported	Reported or measured
EDEN (France)	Measured	Self-reported	Clinical records	Measured or clinical records
FCOU (Ukraine)	Clinical records	Clinical records	Clinical records	Clinical records
GASPII (Italy)	Self-reported	Self-reported	Self-reported	Measured
GECKO Drenthe (The Netherlands)	Self-reported	Self-reported	Self-reported	Measured
GENERATION R (The Netherlands)	Measured	Self-reported	Self-reported	Measured
GENERATION XXI (Portugal)	Measured or ID card	Self-reported	Self-reported	Measured
GENESIS (Greece)	Self-reported	Self-reported	Self-reported	Measured
GINIplus (Germany)	Self-reported	Self-reported	Self-reported	Clinical records at 4y, measured and reported at 10 and 15y
HUMIS (Norway)	Self-reported	Self-reported	Self-reported	Reported
INMA (Spain)	Measured or self-reported	Self-reported	Clinical records	Measured
KOALA (The Netherlands)	Self-reported	Self-reported	Self-reported	Reported
Krakow Cohort (Poland)	Self-reported	Self-reported	Self-reported	Measured
LISAplus (Germany)	Self-reported	Self-reported	Self-reported	Clinical records at 4y, measured and reported at 10 and 15y

Supplemental Table 1. Cohort-specific methods of data collection (continued)

Cohort name (country)	Maternal height	Maternal pre-/ early pregnancy weight	Maternal latest weight before delivery or gestational weight gain	Childhood weight and height
LUKAS (Finland)	Self-reported	Self-reported	Self-reported or clinical records	Reported
MoBa (Norway)	Self-reported	Self-reported	Self-reported	Reported
NINFEA (Italy)	Self-reported	Self-reported	Self-reported	Reported
PÉLAGIE (France)	Self-reported	Self-reported	NA	Reported
PIAMA (The Netherlands)	Self-reported	Self-reported	Self-reported	Reported and measured (4 and 8y)
Piccolipiù (Italy)	Self-reported	Self-reported	Self-reported	Measured
Project Viva (United States)	Self-reported	Self-reported	Clinical records	Measured
Raine Study (Australia)	Measured	Self-reported	NA	Measured
REPRO_PL (Poland)	Measured	Self-reported	Measured	Measured
RHEA (Greece)	Measured	Self-reported	Measured	Clinical records or measured
ROLO (Ireland)	Measured	Measured	Measured	Measured
SCOPE BASELINE (Ireland)	Measured	Measured	Measured	Measured
SEATON (United Kingdom)	Measured	Measured	NA	Measured
Slovak PCB study (Slovakia)	Self-reported	Self-reported	Self-reported	Measured
STEPS (Finland)	Self-reported	Self-reported	Self-reported	Measured
SWS (United Kingdom)	Measured	Measured	Measured	Measured

NA: Not available or not applicable

Supplemental Figure 1. Country-specific description of exposures and outcomes



Values are valid percentages. The CHOP cohort was excluded from the country-specific analyses, as participants come from multiple countries.

Supplemental Table 2. Cohort-specific description of available covariates

Cohort name, number of participants, (country)	Maternal age, (years)	Parity, n (%)		Maternal education level, n (%)				Ethnicity, n (%)		Smoking during pregnancy, n (%)		Child's sex, n (%)	
	Median (95% range)	Nulliparous	Missings	Low	Medium	High	Missings	European/ White	Missings	Yes	Missings	Male	Missings
ABCD , n=5,494, 2003-2004 (The Netherlands)	32.0 (20.0, 40.0)	3,030 (55.2)	–	1,144 (20.8)	2,018 (36.7)	2,291 (41.7)	41 (0.7)	4,083 (74.3)	10 (0.2)	602 (11.0)	269 (4.9)	2,727 (49.6)	–
ALSPAC, n=8,435, 1991-1992 (United Kingdom)	29.0 (20.0, 38.0)	3,761 (44.6)	217 (2.6)	4,853 (57.5)	2,101 (24.9)	1,239 (14.7)	242 (2.9)	8,018 (95.1)	262 (3.1)	1,701 (20.2)	99 (1.2)	4,266 (50.6)	–
AOB/F, n=1,653, 2008-2010 (Canada)	31.0 (22.0, 40.0)	832 (50.3)	–	131 (7.9)	1,248 (75.5)	271 (16.4)	3 (0.2)	1,356 (82.0)	2 (0.1)	143 (8.7)	89 (5.4)	867 (53.0)	–
BAMSE, n=2,930, 1994-1996 (Sweden)	30.0 (22.0, 40.0)	1,641 (56.0)	–	981 (33.5)	747 (25.5)	1,185 (40.4)	17 (0.6)	2,549 (87.0)	212 (7.2)	372 (12.7)	–	1,477 (50.4)	–
BIB, n=887, 2007-2010 (United Kingdom)	27.0 (18.0, 39.0)	349 (39.3)	15 (1.7)	211 (23.8)	340 (38.3)	334 (37.7)	2 (0.2)	368 (41.5)	-	130 (14.7)	1 (0.1)	413 (46.6)	–
CHOP, n=905, 2002-2004 (Multiple)	30.7 (20.3, 39.9)	451 (49.8)	2 (0.2)	192 (21.2)	460 (50.8)	251 (27.7)	2 (0.2)	NA	–	208 (23.0)	0 (0.2)	429 (47.4)	–
Co.N.ER, n=522, 2004-2005 (Italy)	33.9 (25.5, 42.1)	234 (44.8)	1 (0.2)	79 (15.1)	237 (45.5)	205 (39.3)	1 (0.2)	518 (99.2)	–	66 (12.6)	–	264 (50.6)	–
DNBC, n=39,637, 1996-2002 (Denmark)	30.4 (22.9, 39.4)	19,601 (49.5)	22 (0.1)	3,039 (7.7)	14,410 (36.4)	22,071 (55.7)	117 (0.3)	NA	–	9,432 (23.8)	14 (0)	20,396 (51.5)	–
EDEN, n=1,331, 2003-2005 (France)	29.9 (21.2, 39.7)	709 (53.3)	2 (0.2)	305 (22.9)	246 (18.5)	777 (58.4)	3 (0.2)	NA	–	302 (22.7)	6 (0.5)	705 (53.0)	–
FCOU, n=2,107, 1993-1996 (Ukraine)	23.0 (17.0, 36.0)	1,401 (66.5)	75 (3.6)	107 (5.1)	1,443 (68.5)	518 (24.6)	39 (1.9)	2,107 (100.0)	–	167 (7.9)	128 (6.1)	1,103 (52.3)	–

Supplemental Table 2. Cohort-specific description of available covariates (continued)

Cohort name, number of participants, (country)	Maternal age, (years)	Parity, n (%)		Maternal education level, n (%)				Ethnicity, n (%)		Smoking during pregnancy, n (%)		Child's sex, n (%)	
	Median (95% range)	Nulliparous	Missings	Low	Medium	High	Missings	European/ White	Missings	Yes	Missings	Male	Missings
GASPII, n=568, 2003-2004 (Italy)	33.0 (24.0, 41.0)	326 (57.4)	–	77 (13.6)	283 (49.8)	208 (36.6)	–	563 (99.1)	1 (0.2)	64 (11.3)	–	294 (51.8)	–
GECKO Drenthe, n=1,963, 2006-2007 (The Netherlands)	31.0 (23.0, 39.0)	730 (37.2)	128 (6.5)	1,213 (61.8)	734 (37.4)	0 (0)	16 (0.8)	1,919 (97.8)	1 (0.1)	280 (14.3)	1 (0.1)	991 (50.5)	–
GENERATION R, n=6,716, 2002-2006 (The Netherlands)	30.8 (19.8, 39.3)	3,760 (56.0)	37 (0.6)	616 (9.2)	2,823 (42.0)	2,887 (43.0)	390 (5.8)	3,978 (59.2)	147 (2.2)	1,575 (23.5)	232 (3.5)	3,358 (50.0)	–
GENERATION XXI, n=5,940, 2005-2006 (Portugal)	30.0 (18.0, 40.0)	3,407 (57.4)	89 (1.5)	1,773 (29.8)	2,596 (43.7)	1,542 (26.0)	29 (0.5)	NA	–	1,293 (21.8)	58 (1.0)	3,038 (51.1)	–
GENESIS, n=1,898, 2003-2004 (Greece)	30.2 (21.0, 39.0)	975 (51.4)	–	89 (4.7)	961 (50.6)	791 (41.7)	57 (3.0)	NA	–	343 (18.1)	1 (0.1)	981 (51.7)	–
GINIplus, n=2,326, 1995-1998 (Germany)	31.0 (24.0, 40.0)	NA	–	273 (11.7)	982 (42.2)	1,065 (45.8)	6 (0.3)	NA	–	257 (11.0)	29 (1.2)	1,140 (49.0)	–
HUMIS, n=945, 2003-2008 (Norway)	30.0 (22.0, 39.0)	414 (43.8)	–	93 (9.8)	150 (15.9)	559 (59.2)	143 (15.1)	710 (75.1)	149 (15.8)	92 (9.7)	40 (4.2)	473 (50.1)	–
INMA, n=1,916, 1997-2008 (Spain)	30.0 (22.0, 39.0)	1,052 (54,9)	2 (0.1)	570 (29.7)	732 (38.2)	595 (31.1)	19 (1.0)	1,830 (95.5)	3 (0.2)	338 (17.6)	18 (0.9)	983 (51.3)	–
KOALA, n=2,051, 2000-2002 (The Netherlands)	32.0 (25.0, 40.0)	896 (43.7)	43 (2.1)	177 (8.6)	755 (36.8)	1,024 (49.9)	95 (4.6)	1,983 (96.7)	6 (0.3)	122 (5.9)	4 (0.2)	1,058 (51.6)	–
Krakow Cohort, n=422, 2000-2003 (Poland)	28.0 (20.0, 34.0)	269 (63.7)	–	39 (9.2)	160 (37.9)	223 (52.8)	–	422 (100.0)	–	NA	–	215 (50.9)	–

Supplemental Table 2. Cohort-specific description of available covariates (continued)

Cohort name, number of participants, (country)	Maternal age, (years)	Parity, n (%)		Maternal education level, n (%)				Ethnicity, n (%)		Smoking during pregnancy, n (%)		Child's sex, n (%)	
	Median (95% range)	Nulliparous	Missings	Low	Medium	High	Missings	European/ White	Missings	Yes	Missings	Male	Missings
LISAplus, n=2,334, 1997-1999 (Germany)	31.0 (23.0, 40.0)	1,020 (43.7)	8 (0.3)	172 (7.4)	867 (37.1)	1,267 (54.3)	28 (2.1)	NA	–	337 (14.4)	11 (0.5)	1,214 (52.0)	–
LUKAS, n=379, 2002-2005 (Finland)	31.0 (21.2, 42.1)	132 (34.8)	–	13 (3.4)	283 (74.7)	83 (21.9)	–	379 (100.0)	–	60 (15.8)	–	189 (49.9)	–
MoBa, n=54,910, 1999-2009 (Norway)	30.0 (22.0, 39.0)	25,455 (46.4)	–	14,895 (27.1)	24,242 (44.1)	14,725 (26.8)	1,048 (1.9)	NA	–	3,947 (7.2)	5,435 (9.9)	28,153 (51.3)	–
NINFEA, n=1,753, 2005-2010 (Italy)[a]	33.0 (25.0, 41.0)	1,183 (67.5)	-	64 (3.7)	585 (33.4)	1,100 (62.7)	4 (0.2)	1,728 (98.6)[b]	–	143 (8.2)	9 (0.5)	905 (51.6)	–
PÉLAGIE, n=738, 2002-2005 (France)	30.1 (22.8, 39.5)	325 (44.0)	–	104 (14.1)	127 (17.2)	506 (68.6)	1 (0.1)	NA	–	197 (26.7)	1 (0.1)	380 (51.5)	–
PIAMA, n=2,324, 1996-1997 (The Netherlands)	31.0 (23.0, 38.0)	1,156 (49.7)	–	457 (19.7)	967 (41.6)	900 (38.7)	-	2,213 (95.2)	45 (1.9)	361 (15.5)	16 (0.7)	1,173 (50.5)	–
Piccolipiù, n=687, 2011-2015 (Italy)	34.0 (24.0, 43.0)	430 (62.6)	-	68 (9.9)	265 (38.6)	353 (51.4)	1 (0.1)	678 (98.7)	1 (0.1)	156 (22.7)	-	360 (52.4)	–
Project Viva, n=1,382, 1999-2002 (United States)	32.4 (18.9, 41.2)	661 (47.8)	–	431 (31.2)	489 (35.4)	457 (33.1)	5 (0.4)	952 (68.9)	5 (0.4)	144 (10.4)	30 (2.2)	706 (51.1)	–
Raine Study, n=2,092, 1989-1992 (Australia)	29.0 (18.0, 40.1)	989 (47.3)	–	524 (25.0)	885 (42.3)	379 (18.1)	304 (14.5)	1,886 (90.2)	–	634 (30.3)	144 (6.9)	1,085 (51.9)	–
REPRO_PL, n=283, 2007-2011 (Poland)	28.0 (20.0, 37.0)	162 (57.2)	-	32 (11.3)	98 (34.6)	153 (54.1)	-	283 (100.0)	–	33 (11.7)	–	134 (47.3)	–

Supplemental Table 2. Cohort-specific description of available covariates (continued)

Cohort name, number of participants, (country)	Maternal age, (years)	Parity, n (%)		Maternal education level, n (%)				Ethnicity, n (%)		Smoking during pregnancy, n (%)		Child's sex, n (%)	
	Median (95% range)	Nulliparous	Missings	Low	Medium	High	Missings	European/ White	Missings	Yes	Missings	Male	Missings
RHEA, n=748, 2007-2008 (Greece)	30.0 (20.0, 40.0)	NA	–	108 (14.4)	390 (52.1)	248 (33.2)	2 (0.3)	747 (99.9)	1 (0.1)	255 (34.1)	1 (0.1)	397 (53.1)	–
ROLO, n=290, 2007-2011 (Ireland)	33.3 (24.6, 40.3)	0 (0)	–	0 (0)	51 (17.6)	207 (71.4)	32 (11.0)	287 (99.0)	–	6 (2.1)	–	132 (45.5)	–
SCOPE BASELINE, n=1,045, 2008-2011 (Ireland)	31.0 (22.0, 39.0)	1,045 (100.0)	–	0 (0)	121 (11.6)	921 (88.1)	3 (0.3)	1,032 (98.8)	–	229 (21.9)	–	531 (50.8)	–
SEATON, n=933, 1998-1999 (United Kingdom)	30.5 (19.5, 40.2))	344 (36.9)	–	196 (21.0)	273 (29.3)	362 (38.8)	102 (10.9)	NA	–	342 (36.7)	-	469 (50.3)	–
Slovak PCB study, n=480, 2002-2004 (Slovakia)	26.0 (19.0, 39.0)	199 (41.5)	1 (0.2)	200 (41.7)	246 (51.3)	31 (6.5)	3 (0.6)	411 (85.6)	–	72 (15.0)	12 (2.5)	236 (49.2)	–
STEPS, n=484, 2008-2010 (Finland)	31.2 (23.4, 40.7)	297 (61.4)	–	31 (6.4)	128 (26.4)	311 (64.3)	14 (2.9)	NA	–	12 (2.5)	182 (37.6)	255 (52.7)	–
SWS, n=2,621, 1998-2007 (United Kingdom)	30.3 (22.8, 36.5)	1,385 (52.8)	2 (0.1)	290 (11.1)	1564 (59.7)	761 (29.0)	6 (0.2)	2,522 (96.2)	–	329 (12.6)	281 (10.7)	1354 (51.7)	–
Total group	30.2 (21.0, 39.3)	78,621 (48.9)	3,720 (2.3)	33,547 (20.7)	65,007 (40.1)	6,800 (37.5)	2,775 (1.7)	43,522 (26.8)	112,281 (69.3)	24,744 (15.3)	7535 (4.6)	82,860 (51.1)	-

Values are expressed as medians (95% range) or numbers of participants (%).[a]Subset of participants with 4-years follow-up completed. [b] Distinguishes between those born in Italy and those born outside Italy.

Supplemental Table 3. Associations of maternal pre-pregnancy BMI clinical categories with the risk of childhood overweight/obesity, additionally adjusted for gestational diabetes

	Early childhood 2.0-5.0 years	Mid childhood 5.0-10.0 years	Late childhood 10-18.0 years
	Overweight/obesity OR (95% CI)	Overweight/obesity OR (95% CI)	Overweight/obesity OR (95% CI)
Maternal pre-pregnancy BMI			
Underweight (<18.5 kg/m^2)	0.57 (0.47, 0.69)	0.44 (0.40, 0.49)	0.44 (0.35, 0.55)
	$n_{cases/total}$ =126/3,162	$n_{cases/total}$ =401/4,485	$n_{cases/total}$ =93/877
Normal weight (18.5-24.9 kg/m^2)	Reference	Reference	Reference
	$n_{cases/total}$ =3,092/57,293	$n_{cases/total}$ =13,870/82,438	$n_{cases/total}$ =2,505/13,497
Overweight (25.0-29.9 kg/m^2)	1.66 (1.55, 1.77)	1.91 (1.84, 1.98)	2.28 (2.08, 2.50)
	$n_{cases/total}$ =1,476/17,013	$n_{cases/total}$ =6,556/23,359	$n_{cases/total}$ =968/2,799
Obesity (≥30.0 kg/m^2)	2.40 (2.21, 2.61)	3.11 (2.97, 3.26)	4.57 (3.99, 5.24)
	$n_{cases/total}$ =864/7,058	$n_{cases/total}$ =3,612/9,248	$n_{cases/total}$ =528/1,000
Obesity grade 1 (30.0-34.9 kg/m^2)	2.33 (2.12, 2.56)	2.88 (2.73, 3.04)	4.17 (3.56, 4.87)
	$n_{cases/total}$ =613/5,142	$n_{cases/total}$ =2,552/6,874	$n_{cases/total}$ =363/726
Obesity grade 2 (35.0-39.9 kg/m^2)	2.53 (2.16, 2.97)	3.55 (3.23, 3.91)	5.98 (4.50, 7.95)
	$n_{cases/total}$ =190/1,489	$n_{cases/total}$ =782/1,836	$n_{cases/total}$ =129/215
Obesity grade 3 (≥40.0 kg/m^2)	2.85 (2.16, 3.77)	5.14 (4.32, 6.12)	5.54 (3.25, 9.45)
	$n_{cases/total}$ =61/427	$n_{cases/total}$ =278/538	$n_{cases/total}$ =36/59

Values are odds ratios (95% confidence intervals) from multilevel binary logistic regression models that reflect the risk of childhood overweight in early childhood (2.0-5.0 years), mid childhood (5.0-10.0 years) and late childhood (10.0-18.0 years) in children of mothers in the different pre-pregnancy BMI groups, as compared with the reference group (normal weight). The models are adjusted for maternal age, education level, ethnicity, parity, smoking during pregnancy, and gestational diabetes.

Supplemental Table 4. Associations of maternal pre-pregnancy BMI clinical categories with the risk of childhood overweight/obesity, additionally adjusted for gestational hypertensive disorders

	Early childhood 2.0-5.0 years	Mid childhood 5.0-10.0 years	Late childhood 10-18.0 years
	Overweight/obesity OR (95% CI)	Overweight/obesity OR (95% CI)	Overweight/obesity OR (95% CI)
Maternal pre-pregnancy BMI			
Underweight (<18.5 kg/m^2)	0.57 (0.47, 0.69)	0.44 (0.40, 0.49)	0.44 (0.35, 0.55)
	$n_{cases/total}$ =126/3,162	$n_{cases/total}$ =401/4,485	$n_{cases/total}$ =93/877
Normal weight (18.5-24.9 kg/m^2)	Reference	Reference	Reference
	$n_{cases/total}$ =3,092/57,293	$n_{cases/total}$ =13,870/82,438	$n_{cases/total}$ =2,505/13,497
Overweight (25.0-29.9 kg/m^2)	1.66 (1.56, 1.78)	1.91 (1.84, 1.98)	2.26 (2.06, 2.48)
	$n_{cases/total}$ =1,476/17,013	$n_{cases/total}$ =6,556/23,359	$n_{cases/total}$ =968/2,799
Obesity (≥30.0 kg/m^2)	2.45 (2.25, 2.66)	3.12 (2.98, 3.26)	4.46 (3.89, 5.11)
	$n_{cases/total}$ =864/7,058	$n_{cases/total}$ =3,612/9,248	$n_{cases/total}$ =528/1,000
Obesity grade 1 (30.0-34.9 kg/m^2)	2.37 (2.15, 2.60)	2.89 (2.74, 3.05)	4.08 (3.49, 4.77)
	$n_{cases/total}$ =613/5,142	$n_{cases/total}$ =2,552/6,874	$n_{cases/total}$ =363/726
Obesity grade 2 (35.0-39.9 kg/m^2)	2.59 (2.21, 3.04)	3.56 (3.24, 3.93)	5.82 (4.37, 7.73)
	$n_{cases/total}$ =190/1,489	$n_{cases/total}$ =782/1,836	$n_{cases/total}$ =129/215
Obesity grade 3 (≥40.0 kg/m^2)	2.96 (2.24, 3.91)	5.16 (4.34, 6.14)	5.27 (3.09, 9.00)
	$n_{cases/total}$ =61/427	$n_{cases/total}$ =278/538	$n_{cases/total}$ =36/59

Values are odds ratios (95% confidence intervals) from multilevel binary logistic regression models that reflect the risk of childhood overweight in early childhood (2.0-5.0 years), mid childhood (5.0-10.0 years) and late childhood (10.0-18.0 years) in children of mothers in the different pre-pregnancy BMI groups, as compared with the reference group (normal weight). The models are adjusted for maternal age, education level, ethnicity, parity, smoking during pregnancy, and gestational hypertensive disorders (gestational hypertension and pre-eclampsia).

Supplemental Table 5. Associations of maternal pre-pregnancy BMI clinical categories with the risk of childhood overweight/obesity, additionally adjusted for gestational-age-adjusted birth weight

	Early childhood 2.0-5.0 years	Mid childhood 5.0-10.0 years	Late childhood 10-18.0 years
	Overweight/obesity OR (95% CI)	Overweight/obesity OR (95% CI)	Overweight/obesity OR (95% CI)
Maternal pre-pregnancy BMI			
Underweight (<18.5 kg/m^2)	0.60 (0.49, 0.72)	0.46 (0.42, 0.51)	0.45 (0.36, 0.56)
	$n_{cases/total}$ =126/3,162	$n_{cases/total}$ =401/4,485	$n_{cases/total}$ =93/877
Normal weight (18.5-24.9 kg/m^2)	Reference	Reference	Reference
	$n_{cases/total}$ =3,092/57,293	$n_{cases/total}$ =13,870/82,438	$n_{cases/total}$ =2,505/13,497
Overweight (25.0-29.9 kg/m^2)	1.61 (1.50, 1.72)	1.86 (1.80, 1.93)	2.25 (2.05, 2.46)
	$n_{cases/total}$ =1,476/17,013	$n_{cases/total}$ =6,556/23,359	$n_{cases/total}$ =968/2,799
Obesity (≥30.0 kg/m^2)	2.29 (2.11, 2.49)	2.98 (2.84, 3.12)	4.42 (3.86, 5.07)
	$n_{cases/total}$ =864/7,058	$n_{cases/total}$ =3,612/9,248	$n_{cases/total}$ =528/1,000
Obesity grade 1 (30.0-34.9 kg/m^2)	2.23 (2.03, 2.45)	2.77 (2.62, 2.92)	4.02 (3.44, 4.71)
	$n_{cases/total}$ =613/5,142	$n_{cases/total}$ =2,552/6,874	$n_{cases/total}$ =363/726
Obesity grade 2 (35.0-39.9 kg/m^2)	2.40 (2.05, 2.82)	3.37 (3.06, 3.72)	5.82 (4.38, 7.74)
	$n_{cases/total}$ =190/1,489	$n_{cases/total}$ =782/1,836	$n_{cases/total}$ =129/215
Obesity grade 3 (≥40.0 kg/m^2)	2.69 (2.04, 3.55)	4.85 (4.07, 5.77)	5.35 (3.13, 9.13)
	$n_{cases/total}$ =61/427	$n_{cases/total}$ =278/538	$n_{cases/total}$ =36/59

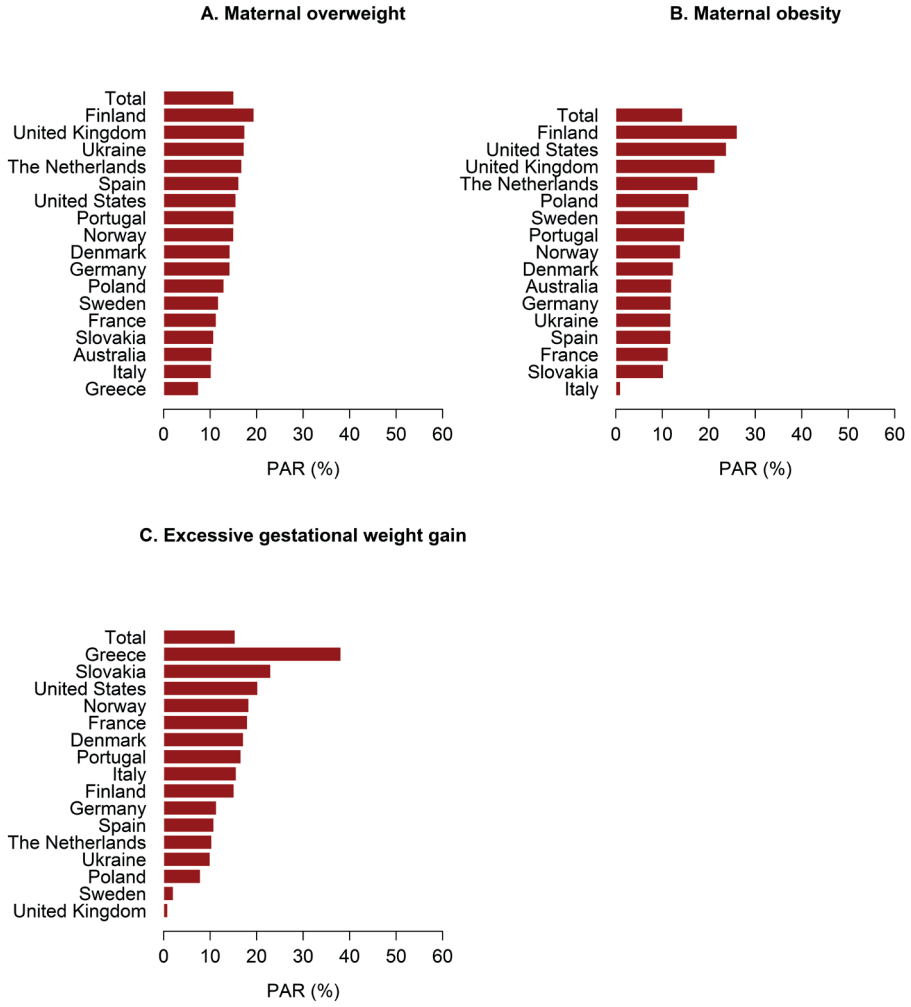
Values are odds ratios (95% confidence intervals) from multilevel binary logistic regression models that reflect the risk of childhood overweight in early childhood (2.0-5.0 years), mid childhood (5.0-10.0 years) and late childhood (10.0-18.0 years) in children of mothers in the different pre-pregnancy BMI groups, as compared with the reference group (normal weight). The models are adjusted for maternal age, education level, ethnicity, parity, smoking during pregnancy, and gestational-age-adjusted birth weight.

Supplemental Table 6. Associations of maternal pre-pregnancy BMI and gestational weight gain clinical categories with the risk of childhood underweight

	Early childhood 2.0-5.0 years		Mid childhood 5.0-10.0 years		Late childhood 10-18.0 years	
	Underweight		Underweight		Underweight	
	OR (95% CI)	PAR (%)	OR (95% CI)	PAR (%)	OR (95% CI)	PAR (%)
Maternal pre-pregnancy BMI						
Underweight (<18.5 kg/m^2)	1.46 (1.15, 1.86)	1.8	1.94 (1.66, 2.27)	3.5	2.40 (1.69, 3.39)	5.2
	$n_{cases/total}$ =78/3,114		$n_{cases/total}$ =188/4,272		$n_{cases/total}$ =40/824	
Normal weight (18.5-24.9 kg/m^2)	Reference		Reference		Reference	
	$n_{cases/total}$ =884/55,085		$n_{cases/total}$ =1,681/70,249		$n_{cases/total}$ =240/11,232	
Overweight (25.0-29.9 kg/m^2)	0.93 (0.81, 1.08)	NA	0.71 (0.63, 0.81)	NA	0.49 (0.31, 0.78)	NA
	$n_{cases/total}$ =245/15,782		$n_{cases/total}$ =6,556/23,359		$n_{cases/total}$ =20/1,851	
Obesity (≥30.0 kg/m^2)	0.74 (0.58. 0.93)	NA	0.60 (0.49, 0.75)	NA	0.38 (0.14, 1.03)	NA
	$n_{cases/total}$ =80/6,274		$n_{cases/total}$ =312/17,115		$n_{cases/total}$ =4/367	
Obesity grade 1 (30.0-34.9 kg/m^2)	0.69 (0.53, 0.91)	NA	0.60 (0.47, 0.76)	NA	0.49 (0.18, 1.34)	NA
	$n_{cases/total}$ =55/4,584		$n_{cases/total}$ =70/4,392		$n_{cases/total}$ =4/476	
Obesity grade 2 (35.0-39.9 kg/m^2)	0.88 (0.56, 1.38)	NA	0.64 (0.40, 1.03)	NA	NA	NA
	$n_{cases/total}$ =20/1,319		$n_{cases/total}$ =18/1,072		$n_{cases/total}$ =0/86	
Obesity grade 3 (≥40.0 kg/m^2)	0.77 (0.32, 1.88)	NA	0.55 (0.20, 1.47)	NA	NA	NA
	$n_{cases/total}$ =5/371		$n_{cases/total}$ =4/264		$n_{cases/total}$ =0/23	
Gestational weight gain						
Inadequate weight gain	1.24 (1.06, 1.44)	4.1	1.25 (1.11, 1.40)	4.2	1.15 (0.92, 1.43)	2.6
	$n_{cases/total}$ =298/14,929		$n_{cases/total}$=489/17,579		$n_{cases/total}$=101/3,293	
Adequate weight gain	Reference		Reference		Reference	
	$n_{cases/total}$ =411/24,287		$n_{cases/total}$=751/30,738		$n_{cases/total}$=95/4,303	
Excessive weight gain	0.84 (0.73, 0.97)	NA	0.83 (0.74, 0.92)	NA	0.88 (0.69, 1.14)	NA
	$n_{cases/total}$=375/25,700		$n_{cases/total}$=678/30,995		$n_{cases/total}$=39/2,681	

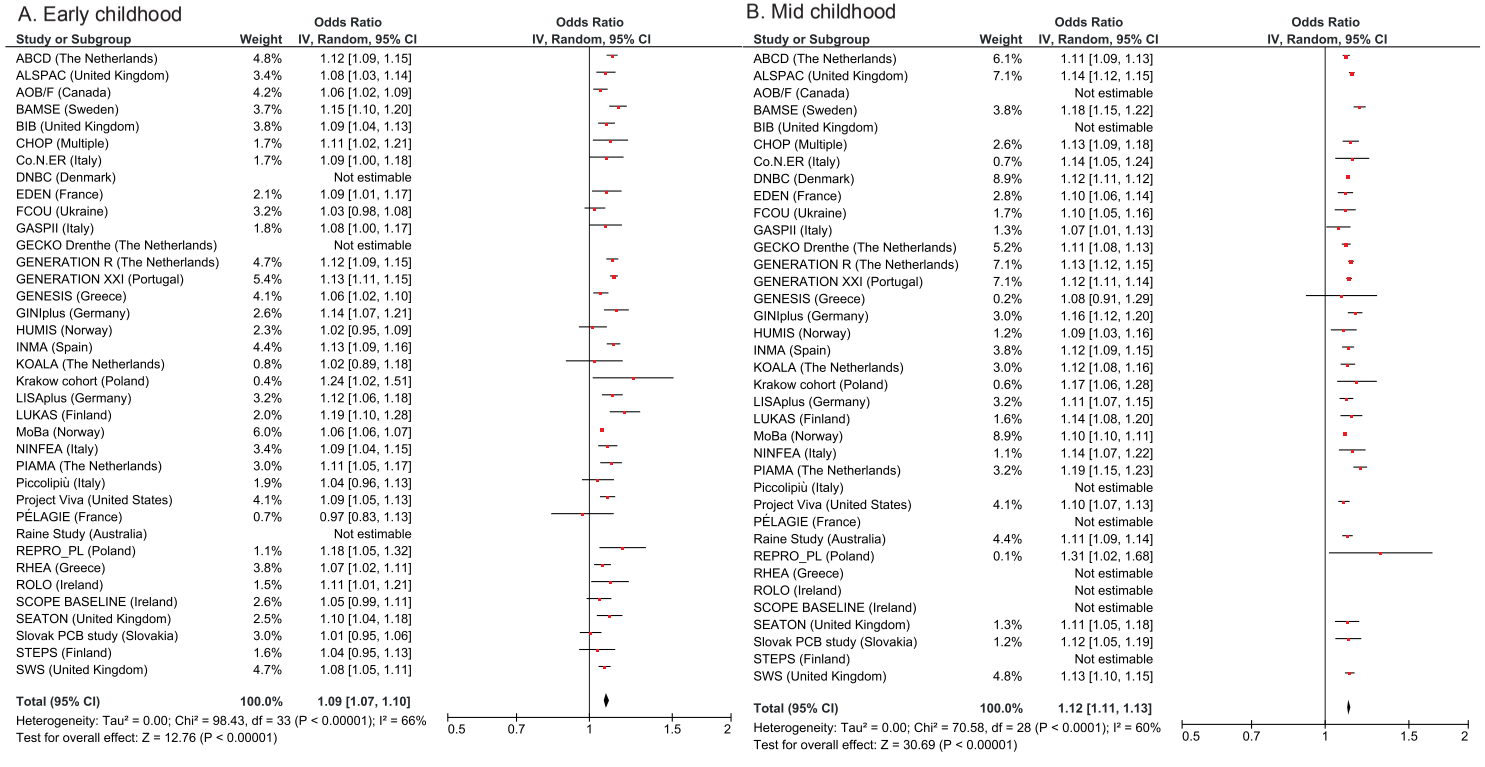
Values are odds ratios (95% confidence intervals) from multilevel binary logistic regression models that reflect the risk of childhood underweight in early childhood (2.0-5.0 years), mid childhood (5.0-10.0 years) and late childhood (10.0-18.0 years) in children of mothers in the different pre-pregnancy BMI groups or gestational weight gain groups, as compared with the reference group (normal weight for pre-pregnancy BMI and adequate weight gain for gestational weight gain) or population attributable risk fractions (PAR), indicating the proportion of childhood underweight cases attributable to each maternal BMI or gestational weight gain category. The models are adjusted for maternal age, education level, ethnicity, parity, and smoking during pregnancy. NA: not applicable.

Supplemental Figure 2. Overall and country-specific population attributable risk fractions of maternal overweight, obesity and excessive gestational weight gain for mid childhood overweight/obesity



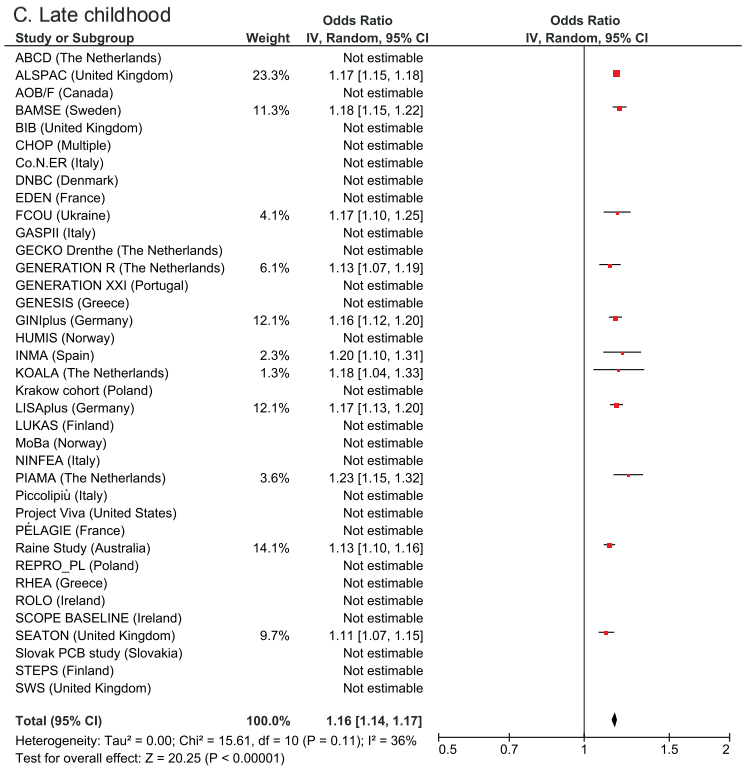
Values are population attributable risk fractions (PAR) indicating the proportion of mid childhood overweight/obesity cases attributable to (A) maternal overweight, (B) maternal obesity and (C) excessive gestational weight gain. The CHOP cohort was excluded from the country-specific analyses, as participants come from multiple countries.

Supplemental Figure 3. Associations of maternal pre-pregnancy BMI with the risk of childhood overweight/obesity assessed by 2-stage IPD meta-analysis



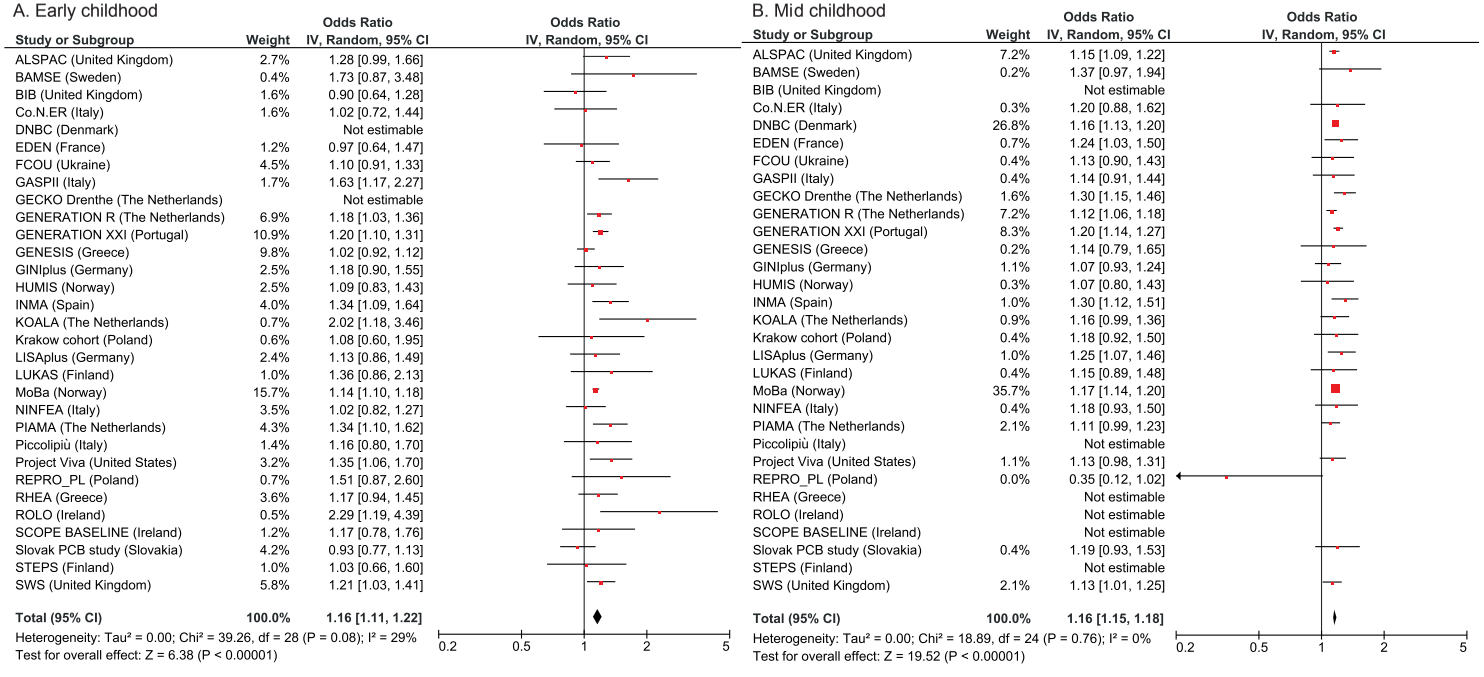
Values are pooled odds ratios (95% confidence intervals) that reflect the risk of childhood overweight/obesity in (A) early childhood (2.0-5.0 years), (B) mid childhood (5.0-10.0 years) and (C) late childhood (10.0-18.0 years) per kg/m^2 increase in maternal pre-pregnancy BMI. The cohorts for which no estimate was provided had no or not sufficient data available for that particular analysis. The models are adjusted for maternal age, education level, ethnicity, parity, and smoking during pregnancy.

Supplemental Figure 3. Associations of maternal pre-pregnancy BMI with the risk of childhood overweight/obesity assessed by 2-stage IPD meta-analysis (continued)



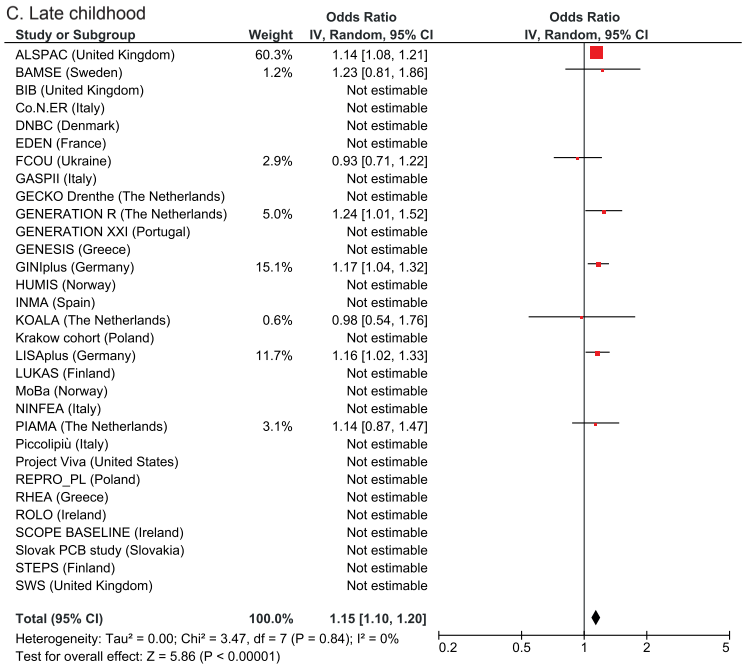
Values are pooled odds ratios (95% confidence intervals) that reflect the risk of childhood overweight/obesity in (A) early childhood (2.0-5.0 years), (B) mid childhood (5.0-10.0 years) and (C) late childhood (10.0-18.0 years) per kg/m^2 increase in maternal pre-pregnancy BMI. The cohorts for which no estimate was provided had no or not sufficient data available for that particular analysis. The models are adjusted for maternal age, education level, ethnicity, parity, and smoking during pregnancy.

Supplemental Figure 4. Associations of gestational weight gain with the risk of overweight/obesity assessed by 2-stage IPD meta-analysis



Values are pooled odds ratios (95% confidence intervals) that reflect the risk of childhood overweight/obesity in (A) early childhood (2.0-5.0 years), (B) mid childhood (5.0-10.0 years) and (C) late childhood (10.0-18.0 years) per SD increase in gestational weight gain. The cohorts for which no estimate was provided had no or not sufficient data available for that particular analysis. The models are adjusted for maternal age, education level, ethnicity, parity, and smoking during pregnancy.

Supplemental Figure 4. Associations of gestational weight gain with the risk of overweight/obesity assessed by 2-stage IPD meta-analysis (continued)



Values are pooled odds ratios (95% confidence intervals) that reflect the risk of childhood overweight/obesity in (A) early childhood (2.0-5.0 years), (B) mid childhood (5.0-10.0 years) and (C) late childhood (10.0-18.0 years) per SD increase in gestational weight gain. The cohorts for which no estimate was provided had no or not sufficient data available for that particular analysis. The models are adjusted for maternal age, education level, ethnicity, parity, and smoking during pregnancy.

Supplemental Table 7. Associations of maternal pre-pregnancy BMI and gestational weight gain clinical categories with the risk of childhood overweight/obesity, complete case analysis

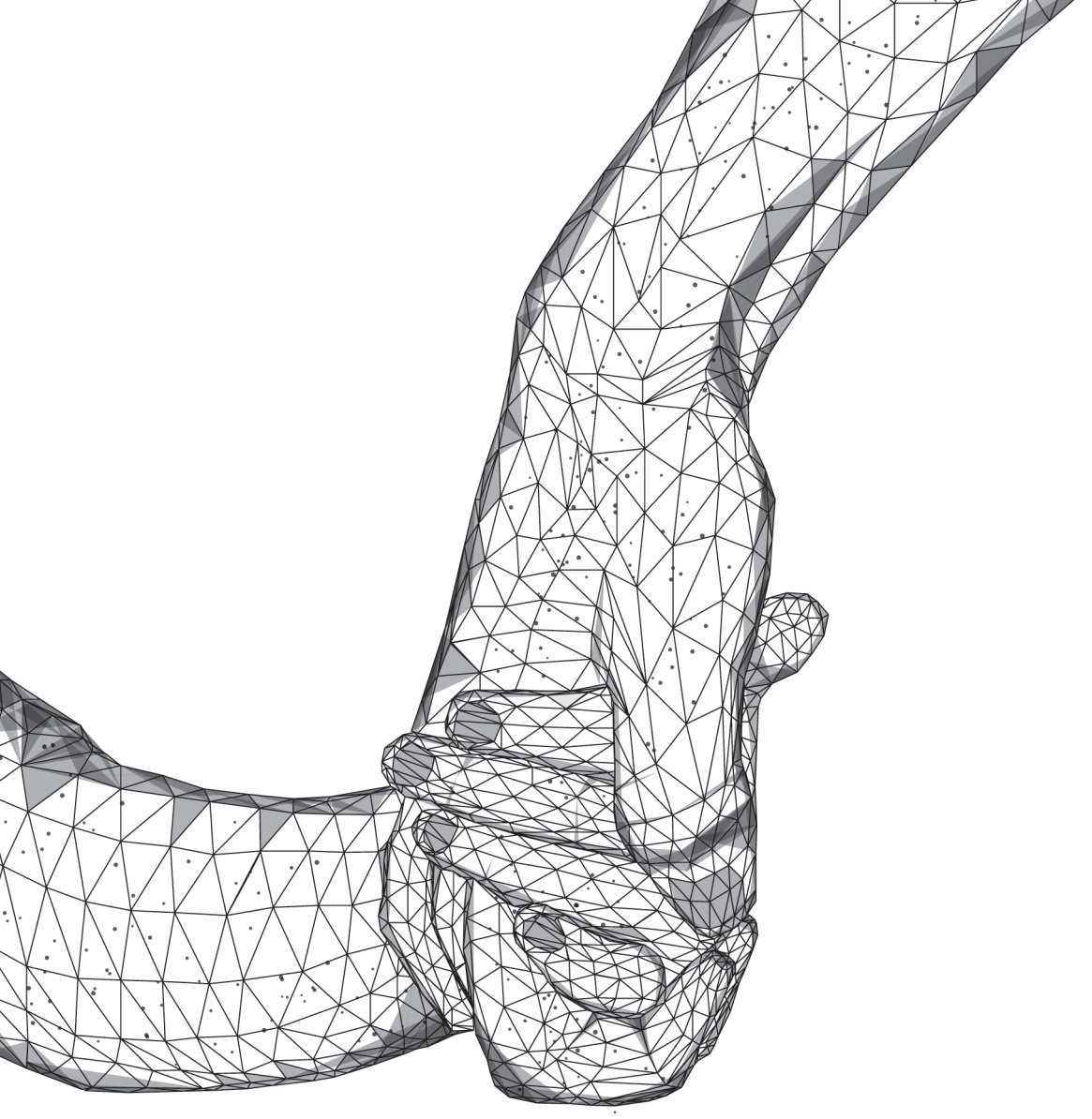
	Early childhood 2.0-5.0 years	Mid childhood 5.0-10.0 years	Late childhood 10-18.0 years
	overweight/obesity	overweight/obesity	overweight/obesity
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Maternal pre-pregnancy BMI			
Underweight (<18.5 kg/m^2)	0.65 (0.49, 0.85)	0.42 (0.35, 0.51)	0.48 (0.37, 0.61)
	$n_{cases/total}$ =68/1,266	$n_{cases/total}$ =146/1,356	$n_{cases/total}$ =73/601
Normal weight (18.5-24.9 kg/m^2)	Reference	Reference	Reference
	$n_{cases/total}$ =1,055/19,057	$n_{cases/total}$ =4,625/22,872	$n_{cases/total}$ =1,791/9,176
Overweight (25.0-29.9 kg/m^2)	1.73 (1.54, 1.95)	2.07 (1.94, 2.21)	2.36 (2.12, 2.64)
	$n_{cases/total}$ =469/5,289	$n_{cases/total}$ =1,997/5,832	$n_{cases/total}$ = 691/1,908
Obesity (≥30.0 kg/m^2)	2.82 (2.43, 3.26)	3.72 (3.40, 4.08)	4.84 (4.09, 5.72)
	$n_{cases/total}$ =295/2,197	$n_{cases/total}$ =1,115/2,325	$n_{cases/total}$ =359/649
Obesity grade 1 (30.0-34.9 kg/m^2)	2.66 (2.27, 3.18)	3.53 (3.18, 3.92)	4.47 (3.69, 5.42)
	$n_{cases/total}$ =204/1,568	$n_{cases/total}$ =785/1,689	$n_{cases/total}$ =252/476
Obesity grade 2 (35.0-39.9 kg/m^2)	3.29 (2.52, 4.30)	3.92 (3.26, 4.72)	5.50 (3.85, 7.87)
	$n_{cases/total}$ =71/477	$n_{cases/total}$ =243/491	$n_{cases/total}$ =79/134
Obesity grade 3 (≥40.0 kg/m^2)	2.77 (1.71, 4.48)	5.88 (4.18, 8.28)	8.56 (4.22, 17.36)
	$n_{cases/total}$ =20/152	$n_{cases/total}$ =87/145	$n_{cases/total}$ =28/39
Gestational weight gain			
Inadequate weight gain	0.84 (0.72, 0.98)	0.84 (0.78, 0.91)	0.92 (0.80, 1.05)
	$n_{cases/total}$ = 303/4,974	$n_{cases/total}$= 1,315/6,355	$n_{cases/total}$= 511/2,614
Adequate weight gain	Reference	Reference	Reference
	$n_{cases/total}$ = 475/6,676	$n_{cases/total}$= 1,938/8,425	$n_{cases/total}$= 646/3,207
Excessive weight gain	1.49 (1.30, 1.70)	1.53 (1.42, 1.64)	1.75 (1.55, 1.99)
	$n_{cases/total}$= 567/5,939	$n_{cases/total}$= 2,341/7,471	$n_{cases/total}$= 731/2,321

Values are odds ratios (95% confidence intervals) from multilevel binary logistic regression models with complete cases that reflect the risk of childhood overweight/obesity in early childhood (2.0-5.0 years), mid childhood (5.0-10.0 years) and late childhood (10.0-18.0 years) in children of mothers in the different pre-pregnancy BMI groups or gestational weight gain groups, as compared with the reference group (normal weight for pre-pregnancy BMI and adequate weight gain for gestational weight gain). The models are adjusted for maternal age, education level, ethnicity, parity, and smoking during pregnancy.

Supplemental Table 8. Associations of maternal pre-pregnancy BMI and gestational weight gain clinical categories with childhood BMI SDS, complete case analysis

	Childhood BMI (SDS)		
	Early childhood	**Mid childhood**	**Late childhood**
	2.0-5.0 years	**5.0-10.0 years**	**10.0-18.0 years**
Maternal pre-pregnancy BMI			
Underweight (<18.5 kg/m^2)	-0.29 (-0.34, -0.23)	-0.43 (-0.34, -0.23)	-0.45 (-0.53, -0.37)
	n =1,272	n =1,370	n=619
Normal weight (18.5-24.9 kg/m^2)	Reference	Reference	Reference
	n =19,127	n =23,007	n=9,280
Overweight (25.0-29.9 kg/m^2)	0.22 (0.19, 0.25)	0.39 (0.36, 0.42)	0.47 (0.42, 0.52)
	n=5,303	n =5,852	n=1,918
Obesity (≥30.0 kg/m^2)	0.34 (0.36, 0.45)	0.76 (0.72, 0.80)	0.94 (0.86, 1.02)
	n =2,200	n =2,330	n=653
Obesity grade 1 (30.0-34.9 kg/m^2)	0.38 (0.33, 0.43)	0.71 (0.66, 0.76)	0.88 (0.78, 0.97)
	n =1,570	n =1,693	n=480
Obesity grade 2 (35.0-39.9 kg/m^2)	0.45 (0.36, 0.54)	0.81 (0.72, 0.90)	1.05 (0.88, 1.23)
	n =478	n =492	n =134
Obesity grade 3 (≥40.0 kg/m^2)	0.453 (0.38, 0.69)	1.15 (0.98, 1.31)	1.38 (1.06, 1.70)
	n =152	n =145	n =39
Gestational weight gain			
Inadequate weight gain	-0.10 (-0.14, -0.06)	-0.12 (-0.16, -0.09)	-0.10 (-0.15, -0.04)
	n =5,064	n =6,447	n=2,676
Adequate weight gain	Reference	Reference	Reference
	n =6,768	n =8,515	n=3,264
Excessive weight gain	0.18 (0.14, 0.21)	0.24 (0.21, 0.27)	0.30 (0.25, 0.36)
	n=5,981	n =7,526	n=2,345

Values are regression coefficients (95% confidence intervals) from multilevel linear regression models with complete cases that reflect differences in early childhood (2.0-5.0 years), mid childhood (5.0-10.0 years) and late childhood (10.0-18.0 years) in children of mothers in the different pre-pregnancy BMI groups or gestational weight gain groups, as compared with the reference group (normal weight for pre-pregnancy BMI and adequate weight gain for gestational weight gain). The models are adjusted for maternal age, education level, ethnicity, parity, and smoking during pregnancy.



3.2 Gestational weight gain and maternal and infant adverse outcomes

Voerman E, Santos S, Inskip H, Amiano P, Barros H, Charles M, Chatzi L, Chrousos GP, Corpeleijn E, Crozier S, Doyon M, Eggesbø M, Fantini MP, Farchi S, Forastiere F, Georgiu V, Gori D, Hanke W, Hertz-Picciotto I, Heude B, Hivert M, Hryhorczuk D, Iñiguez C, Karvonen AM, Küpers LK, Lagström H, Lawlor DA, Lehmann I, Magnus P, Majewska R, Mäkelä J, Manios Y, Mommers M, Morgen CS, Moschonis G, Nohr EA, Nybo Andersen A, Oken E, Pac A, Papadopoulou E, Pekkanen J, Pizzi C, Polanska K, Porta D, Richiardi L, Rifas-Shiman SL, Roeleveld N, Ronfani L, Santos AC, Standl M, Stigum H, Stoltenberg C, Thiering E, Thijs C, Torrent M, Trnovec T, van Gelder MMHJ, van Rossem L, von Berg A, Vrijheid M, Wijga A, Zvinchuk O, Sørensen TIA, Godfrey KM, Jaddoe VWV*, Gaillard R*. Association of gestational weight gain with adverse maternal and infant outcomes.

** Denotes shared last authors*

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ABSTRACT

Importance: Both low and high gestational weight gain have been associated with adverse maternal and infant outcomes, but optimal gestational weight gain remains uncertain and not well defined for all prepregnancy weight ranges.

Objectives: To examine the association of ranges of gestational weight gain with risk of adverse maternal and infant outcomes and estimate optimal gestational weight gain ranges across prepregnancy body mass index categories.

Design, setting and participants: Individual participant-level meta-analysis using data from 196 670 participants within 25 cohort studies from Europe and North America (main study sample). Optimal gestational weight gain ranges were estimated for each prepregnancy body mass index (BMI) category by selecting the range of gestational weight gain that was associated with lower risk for any adverse outcome. Individual participant-level data from 3505 participants within 4 separate hospital-based cohorts were used as a validation sample. Data were collected between 1989 and 2015. The final date of follow-up was December 2015.

Exposures: Gestational weight gain.

Main outcomes and measures: The main outcome termed *any adverse outcome* was defined as the presence of 1 or more of the following outcomes: preeclampsia, gestational hypertension, gestational diabetes, cesarean delivery, preterm birth, and small or large size for gestational age at birth.

Results: Of the 196 670 women (median age, 30.0 years [quartile 1 and 3, 27.0 and 33.0 years] and 40 937 were white) included in the main sample, 7809 (4.0%) were categorized at baseline as underweight (BMI <18.5); 133 788 (68.0%), normal weight (BMI, 18.5-24.9); 38 828 (19.7%), overweight (BMI, 25.0-29.9); 11 992 (6.1%), obesity grade 1 (BMI, 30.0-34.9); 3284 (1.7%), obesity grade 2 (BMI, 35.0-39.9); and 969 (0.5%), obesity grade 3 (BMI, ≥40.0). Overall, any adverse outcome occurred in 37.2% (n = 73 161) of women, ranging from 34.7%(2706 of 7809) among women categorized as underweight to 61.1%(592 of 969) among women categorized as obesity grade 3. Optimal gestational weight gain ranges were 14.0 kg to less than 16.0 kg for women categorized as underweight; 10.0 kg to less than 18.0 kg for normal weight; 2.0 kg to less than 16.0 kg for overweight; 2.0 kg to less than 6.0 kg for obesity grade 1; weight loss or gain of 0 kg to less than 4.0 kg for obesity grade 2; and weight gain of 0 kg to less than 6.0 kg for obesity grade 3. These gestational weight gain ranges were associated with low to moderate discrimination between those with and those without adverse outcomes (range for area under the receiver operating characteristic curve, 0.55-0.76). Results for discriminative performance in the validation sample were similar to the corresponding results in the main study sample (range for area under the receiver operating characteristic curve, 0.51-0.79).

Conclusions and relevance: In this meta-analysis of pooled individual participant data from 25 cohort studies, the risk for adverse maternal and infant outcomes varied by gestational weight gain and across the range of prepregnancy weights. The estimates of optimal gestational weight gain may inform prenatal counseling; however, the optimal gestational weight gain ranges had limited predictive value for the outcomes assessed.

INTRODUCTION

Gestational weight gain has been found to be related to the risk of pregnancy complications, maternal postpartum weight retention, and obesity in offspring (1-3). Gestational weight gain reflects multiple characteristics, including maternal fat accumulation, fluid expansion, and the growth of the fetus, placenta, and uterus (4). Gestational weight gain is necessary to ensure a healthy fetus, but excessive gestational weight gain has been associated with adverse outcomes.

Higher prepregnancy body mass index (BMI; calculated as weight in kilograms divided by height in meters squared) also has been associated with lower gestational weight gain and increased risk for adverse maternal and infant outcomes. Therefore, optimal gestational weight gain ranges should account for prepregnancy BMI (5, 6). Existing guidelines for gestational weight gain from the US National Academy of Medicine (NAM; formerly the Institute of Medicine) have limitations such as the reliance on a limited number of observational studies relating gestational weight gain to 5 maternal and offspring outcomes and insufficient information about important pregnancy outcomes (eg, gestational hypertension and gestational diabetes) (7). In addition, the NAM guidelines do not include recommendations for obesity grade 1, 2, and 3 separately even though the prevalence of extreme obesity is increasing in Western populations. Information regarding optimal gestational weight gain across a range of maternal BMI categories is important for the identification of groups at increased risk.

This study pooled individual participant data from 25 pregnancy and birth cohorts from Europe and North America to assess associations of the amount of gestational weight gain with maternal and infant outcomes according to baseline weight status of underweight, normal weight, overweight, obesity grade 1, obesity grade 2, and obesity grade 3.

METHODS

Inclusion criteria and participating cohorts

This study was part of an international LifeCycle Project collaboration on maternal obesity and childhood outcomes (8, 9). A pregnancy or birth cohort study was eligible for inclusion if it included mothers with singleton live-born children who were born between 1989 and 2015, had information on maternal prepregnancy or early-pregnancy BMI, and had at least 1 offspring measurement (birth weight or childhood BMI). The final date of follow-up was December 2015. No exclusions were made based on previous pregnancy or birth complications.

The cohorts included had received institutional review board approval and written informed consent had been obtained. We invited 50 Western cohorts from Europe, North America, and Oceania that had been selected from existing collaborations on childhood health (the

EarlyNutrition Project, the CHICOS Project, and Birthcohorts.net, which was accessed until July 2014), of which 39 cohorts agreed to participate. Only participants with information on maternal prepregnancy BMI, gestational weight gain, and at least 1 maternal or infant outcome of interest were included.

Of the 29 cohorts with the required data, 25 were population based cohorts and were included in the main study sample. The remaining 4 hospital-based cohorts were included as the external validation sample (**Supplemental Figure 1**). The included cohorts and the data collection methods appear in **Supplemental Table 1**. Women could be included more than once in the analyses if they had multiple singleton pregnancies during the study period. Anonymized data sets were stored on a single central secure data server that was only accessible by the main investigator analysts (E.V. and R.G.).

Maternal prepregnancy BMI and gestational weight gain

Maternal prepregnancy BMI was grouped into categories by 2 BMI units and clinical BMI groups according to World Health Organization definitions (10). Data on total gestational weight gain in kilograms, which was defined as the difference between the latest weight before delivery and the prepregnancy weight, were provided by the cohorts. Gestational weight gain was grouped into categories of 2 kg each, ranging from weight loss to weight gain of 28 kg or greater. Smaller increments of gestational weight gain were not used because of insufficient statistical power among underweight and severely obese women. Categories at the extremes of gestational weight gain were combined for maternal underweight, obesity grade 2, and obesity grade 3. To be included, women were required to have data for maternal prepregnancy BMI, total gestational weight gain, and any adverse outcome (defined below).

Adverse maternal and infant outcomes

The main outcome of the analyses was the composite any adverse outcome, which was defined as the presence of at least 1 of the following outcomes: preeclampsia, gestational hypertension, gestational diabetes, cesarean delivery, preterm birth, and small or large size for gestational age at birth. Preterm birth was defined as gestational age at birth of less than 37 weeks. Sex- and gestational age–adjusted SD scores for birth weight were calculated using a Northern European reference chart (11). Small and large sizes for gestational age at birth were defined as sex- and gestational age–adjusted birth weight less than the10th percentile and greater than the 90th percentile, respectively, within each cohort.

For the sensitivity analyses, sex- and age-adjusted SD scores were calculated for childhood BMI based on reference growth charts from the World Health Organization (12, 13). The SD scores were obtained using data from the highest age available for each child (median age, 84.9 months [quartile 1 and 3, 61.9 and 95.9 months]) and categorized as underweight, normal weight, and overweight or obesity (referred to as overweight) using World Health Organization cutoffs (12, 13).

Statistical analysis

Exploratory multilevel linear regression models were used to assess associations of maternal baseline characteristics with total gestational weight gain. The absolute risk for any adverse outcome was estimated across the full range of maternal prepregnancy BMI and gestational weight gain. Absolute risks were calculated as the percentage of women with any adverse outcome within each combination of BMI and gestational weight gain categories. Similarly, the absolute risks were estimated for any adverse outcome and for each individual outcome across the range of gestational weight gain categories within each clinical BMI group.

The optimal gestational weight gain ranges per clinical BMI group were constructed. The odds ratios (ORs) for any adverse outcome were calculated for each gestational weight gain category within the particular clinical BMI group vs all other women within that BMI group. The individual-level data from all cohorts were analyzed simultaneously using multilevel models. The models followed a 2-level hierarchical structure with participants (level 1) nested within cohorts (level 2).We used a generalized linear mixed model with a binominal distribution and logit link. A random intercept at the cohort level was included to allow variation in the baseline risk for each cohort. Allowing a random slope for gestational weight gain did not improve the models. Model assumptions regarding linearity, independent errors, and influential values were met. Optimal gestational weight gain was defined as all weight gain categories with a statistically significant protective association (OR <1) for any adverse outcome (14). If a gestational weight gain category with a nonsignificant association was between 2 significant estimates with an OR of less than 1, that category was included in the optimal gestational weight gain range. To construct easily interpretable optimal gestational weight gain ranges directly applicable for clinical practice, the main analyses were not adjusted for maternal age or parity. We also assessed continuous associations of maternal prepregnancy BMI and total gestational weight gain in SDs with any adverse outcome and compared the strength of these associations by using *Z* tests for the difference in ORs.

The following sensitivity analyses were performed: (1) we redefined the gestational weight gain ranges based on protective associations only (OR <1) regardless of statistical significance; (2) we adjusted the models for gestational age at birth and excluded preterm births because gestational weight gain depends on length of gestation; (3) we excluded participants with missing data on separate adverse maternal and infant outcomes; (4) we adjusted for maternal age and parity to explore whether optimal gestational weight gain ranges would change when maternal age and parity were taken into account; (5) we excluded cesarean delivery as an adverse outcome and included childhood underweight and overweight as adverse outcomes to explore whether optimal gestational weight gain ranges would change depending on the definition of the composite outcome; and (6) we excluded preeclampsia and gestational diabetes as outcomes to address possible reverse causation. We also constructed optimal gestational weight gain ranges during the first half of pregnancy, which

were defined as the difference between weight at median gestational age of 15.4 weeks (quartile 1 and 3, 13.2 and 17.1 weeks) and prepregnancy weight using a similar approach.

The clinical performance of the gestational weight gain ranges in this study were assessed as secondary analyses and compared with the NAM guidelines by assessing the number of participants classified as having inadequate or excessive weight gain, the associations with adverse outcomes using binary logistic multilevel models, and the discriminative performance for both classification systems. The discriminative performance of the classification (the ability of the classification to discriminate between those with and those without the outcome) from this study and the NAM guidelines was assessed based on the area under the receiver operating characteristic curve (AUROC) (15). Predicted probabilities were obtained from binary logistic multilevel models assessing the associations of inadequate and excessive gestational weight gain with the outcomes. The predicted probabilities were used to calculate the AUROC. To assess the associations of the optimal gestational weight gain ranges with clinically relevant outcomes not used for the construction of the ranges, we also assessed low and high birth weight (≤2500 g or ≥4000 g). In addition, the clinical performance of both classification systems was assessed in the external validation sample (n = 3505).

All statistical tests were 2-sided with a significance threshold of .05. However, the secondary analyses were not adjusted for multiple testing; therefore, these findings should be considered exploratory. All statistical analyses were performed using SPSS Statistics version 24.0 (IBM) and R version 3.3.3 (R Foundation for Statistical Computing).

RESULTS

Participant characteristics in main sample

Of the 29 cohorts with the required data (n = 200 175 participants), 25 were population-based cohorts (n = 196 670 women) and were included as the main study sample (median age, 30.0 years [quartile 1 and 3, 27.0 and 33.0 years] and 40 937 were white). At baseline, 7809 women (4.0%) were categorized as underweight (BMI <18.5); 133 788 (68.0%), normal weight (BMI, 18.5-24.9); 38 828 (19.7%), overweight (BMI, 25.0- 29.9); 11 992 (6.1%), obesity grade 1 (BMI, 30.0-34.9); 3284 (1.7%), obesity grade 2 (BMI, 35.0-39.9); and 969 (0.5%), obesity grade 3 (BMI, ≥40.0) (**Table 1**). Overall, any adverse outcome occurred in 37.2% (n = 73 161) of women, ranging from 34.7% (2706 of 7809) among women categorized as underweight to 61.1% (592 of 969) among women categorized as obesity grade 3.

Women who gained more gestational weight had a lower maternal prepregnancy BMI and were slightly younger and more often nulliparous than multiparous (**Supplemental Table 2**). There were no missing data for any individual adverse outcome for 169 437 women (86.2%). Of the remainder, 17093 women (8.7%) were missing data for gestational hypertensive disorders (including preeclampsia and gestational hypertension), 6898 (3.5%) for gestational

diabetes, 9786 (5.0%) for cesarean delivery, 8541 (4.3%) for preterm birth, and 6453 (3.3%) for size (small or large) for gestational age at birth (**Supplemental Table 3**). Based on the profiles of all included cohorts, the percentage of women included with multiple singleton pregnancies is about 1%.

Participant characteristics in validation sample

There were 3505 women included in the validation sample. They had a median age of 31.0 years (quartile 1 and 3, 27.7 and 34.7 years) and 1696 were white. There were 277 women (7.9%) categorized as underweight; 2400 (68.5%), normal weight; 577 (16.5%), overweight; 188 (5.4%), obesity grade 1; 53 (1.5%), obesity grade 2; and 10 (0.3%), obesity grade 3. Any adverse outcome occurred in 1423 women (40.6%; **Supplemental Table 4**).

There were no missing data for any individual adverse outcome for 3059 women (87.3%). Of the remainder, 423 women (12.1%) were missing data for gestational hypertensive disorders (including preeclampsia and gestational hypertension), 421 (12.0%) for gestational diabetes, 15 (0.4%) for cesarean delivery, 426 (12.2%) for preterm birth, and 7 (0.2%) for size (small or large) for gestational age at birth (**Supplemental Table 3**). **Supplemental Tables 5 and 6** provide cohort specific information for both the main sample and the validation sample.

Maternal prepregnancy BMI, gestational weight gain, and absolute risk for any adverse outcome

The absolute risk for any adverse outcome increased across the full range of maternal prepregnancy BMI and was largely independent of gestational weight gain (**Figure 1**). The lowest absolute risks were observed among women with low to normal BMI and a moderate to high total gestational weight gain. The lowest risk was 26.7% (16 of 60) for women with a BMI of less than 18.0 and gestational weight gain of 26.0 kg to 27.9 kg. The highest absolute risks were observed among women with a high BMI and a high gestational weight gain. The highest risk was 94.4% (17 of 18) for women with a BMI of 40.0 or greater and gestational weight gain of 20.0 kg to 21.9 kg.

Among women categorized as underweight, the absolute risk for any adverse outcome ranged from 29.2%(387 of 1326) for gestational weight gain of 14.0 kg to 15.9 kg to 50.2% (203 of 404) for gestational weight gain of less than 8.0 kg (**Figure 2**). Of all outcomes separately, the absolute risk was highest for small size for gestational age (highest risk: 32.1% [125 of 390] for gestational weight gain <8 kg).

Among women categorized as normal weight, the absolute risk for any adverse outcome ranged from 31.7% (7314 of 23 073) for gestational weight gain of 14.0 kg to 15.9 kg to 46.9% (1256 of 2679) for gestational weight gain of 28.0 kg or greater and was highest at both extremes of gestational weight gain.

Table 1. Characteristics of the study population

	Total group	Underweight	Normal weight	Overweight	Obesity grade 1	Obesity grade 2	Obesity grade 3
		(<18.5 kg/m^2)	(18.5-24.9 kg/m^2)	(25.0-29.9 kg/m^2)	(30.0-34.9 kg/m^2)	(35.0-39.9 kg/m^2)	(≥40.0 kg/m^2)
	n= 196670	n =7809	n= 133788	n=38828	n=11992	n=3284	n=969
Pre-pregnancy body mass index, median (Q1, Q3)[a]	22.7 (20.8, 25.5)	17.9 (17.4, 18.3)	21.8 (20.5, 23.2)	26.8 (25.8, 28.0)	31.8 (30.8, 33.1)	36.7 (35.8, 38)	41.8 (40.8, 43.4)
Total gestational weight gain (kg)							
Median (Q1, Q3)	14.0 (11.0, 18.0)	14.0 (11.0, 17.0)	14.4 (11.6, 18.0)	14.0 (10.0, 18.0)	11.0 (7.0, 16.0)	9.0 (4.5, 13.7)	7.0 (2.0, 12.0)
P2.5, P97.5	4.0, 27.0	6.0, 26.0	6.0, 27.0	2.3, 28.0	0.0, 27.0	-2.4, 25.0	-6.0, 25.0
Maternal age (years), median (Q1, Q3)	30.0 (27.0, 33.0)	29.0 (25.1, 32.0)	30.0 (27.0, 33.0)	30.0 (27.0, 33.3)	30.0 (27.0, 33.0)	30.0 (27.0, 33.3)	30 (27.0, 33.1)
Education [b,c]							
Low, n (%)	42192 (21.9)	1756 (23.0)	25241 (19.2)	9802 (25.7)	3848 (32.8)	1166 (36.5)	379 (40.7)
Medium, n (%)	78924 (40.9)	3109 (40.7)	52394 (39.9)	16533 (43.4)	5101 (43.5)	1378 (43.2)	409 (43.9)
High, n (%)	71819 (37.2)	2780 (36.4)	53724 (40.9)	11736 (30.8)	2786 (23.7)	649 (20.3)	144 (15.5)
Country							
Norway, n (%)	74507 (37.9)	2154 (27.6)	49388 (36.9)	16224 (41.8)	5013 (41.8)	1360 (41.4)	368 (38.0)
Denmark, n (%)	60963 (31.0)	2583 (33.1)	41344 (30.9)	11930 (30.7)	3762 (31.4)	1024 (31.2)	320 (33.0)
The Netherlands, n (%)	14861 (7.6)	531 (6.8)	10329 (7.7)	2841 (7.3)	860 (7.2)	235 (7.2)	65 (6.7)
United Kingdom, n (%)	12610 (6.4)	521 (6.7)	8948 (6.7)	2232 (5.7)	659 (5.5)	191 (5.8)	59 (6.1)
Portugal, n (%)	7220 (3.7)	293 (3.8)	4783 (3.6)	1525 (3.9)	454 (3.8)	129 (3.9)	36 (3.7)
Italy, n (%)	5307 (2.7)	428 (5.5)	3893 (2.9)	725 (1.9)	209 (1.7)	50 (1.5)	2 (0.2)
Germany, n (%)	5099 (2.6)	269 (3.4)	3889 (2.9)	699 (1.8)	183 (1.5)	46 (1.4)	13 (1.3)
Ukraine, n (%)	3261 (1.7)	303 (3.9)	2360 (1.8)	479 (1.2)	102 (0.9)	16 (0.5)	1 (0.1)
Greece, n (%)	2872 (1.5)	163 (2.1)	2088 (1.6)	463 (1.2)	118 (1.0)	33 (1.0)	7 (0.7)
Spain, n (%)	1933 (1.0)	89 (1.1)	1351 (1.0)	344 (0.9)	99 (0.8)	36 (1.1)	14 (1.4)
United States, n (%)	2021 (1.0)	78 (1.0)	1192 (0.9)	440 (1.1)	195 (1.6)	74 (2.3)	42 (4.3)
Poland, n (%)	1702 (0.9)	163 (2.1)	1299 (1.0)	191 (0.5)	41 (0.3)	7 (0.2)	1 (0.1)
Finland, n (%)	1406 (0.7)	39 (0.5)	945 (0.7)	254 (0.7)	119 (1.0)	31 (0.9)	18 (1.9)

Table 1. Characteristics of the study population (continued)

	Total group	Underweight	Normal weight	Overweight	Obesity grade 1	Obesity grade 2	Obesity grade 3
		(<18.5 kg/m^2)	(18.5-24.9 kg/m^2)	(25.0-29.9 kg/m^2)	(30.0-34.9 kg/m^2)	(35.0-39.9 kg/m^2)	(≥40.0 kg/m^2)
	n= 196670	n =7809	n= 133788	n=38828	n=11992	n=3284	n=969
Slovakia, n (%)	983 (0.5)	119 (1.5)	681 (0.5)	130 (0.3)	44 (0.4)	9 (0.3)	0 (0.0)
Canada, n (%)	844 (0.4)	37 (0.5)	494 (0.4)	166 (0.4)	86 (0.7)	38 (1.2)	23 (2.4)
No information available, n (%)	1081 (0.5)	39 (0.5)	804 (0.4)	185 (0.5)	48 (0.4)	5 (0.2)	0 (0.0)
Pre-eclampsia, n (%)[d]	5996 (3.5)	112 (1.7)	3067 (2.6)	1637 (4.8)	781 (7.6)	287 (10.4)	112 (13.9)
Gestational hypertension, n (%)[e]	6683 (3.9)	151 (2.2)	3583 (3.0)	1776 (5.2)	807 (7.8)	284 (10.3)	82 (10.5)
Gestational diabetes, n (%)[f]	2946 (1.6)	57 (0.8)	1407 (1.1)	818 (2.2)	420 (3.6)	183 (5.8)	61 (6.6)
Caesarean delivery, n (%)	29567 (15.8)	927 (12.6)	17825 (14.1)	6944 (18.7)	2685 (23.3)	882 (27.8)	304 (32.7)
Preterm birth, n (%)[g]	8250 (4.4)	383 (5.3)	5314 (4.2)	1664 (4.4)	643 (5.5)	177 (5.5)	69 (7.2)
Small size-for-gestational-age, n (%)[h]	19030 (10.0)	1336 (17.9)	13527 (10.5)	2963 (7.8)	900 (7.7)	224 (7.0)	80 (8.5)
Large size-for-gestational-age, n (%)[h]	2542 (10.0)	256 (3.4)	10789 (8.4)	5099 (13.5)	1995 (17.0)	649 (20.3)	217 (23.0)
Childhood underweight, n (%)[i]	2542 (2.0)	196 (4.2)	1865 (2.2)	367 (1.5)	88 (1.2)	20 (1.0)	6 (1.1)
Childhood overweight, n (%)[i]	21718 (17.2)	348 (7.5)	12263 (14.2)	5814 (23.4)	2328 (31.6)	722 (37.0)	243 (43.2)
Any adverse outcome, n (%)[j]	73161 (37.2)	2706 (34.7)	45687 (34.1)	16292 (42.0)	6019 (50.2)	1865 (56.8)	592 (61.1)

a. Calculated as weight in kilograms divided by height in meters squared.

b. Based on cohort-specific criteria. Each cohort used their own country-specific criteria to define low, medium, and high educational level. These 3 categories were subsequently used in the meta-analysis.

c. These rows have missing data.

d. Defined as gestational hypertension plus proteinuria.

e. Defined as systolic blood pressure of 140mmHg or higher, diastolic blood pressure of 90mmHg or higher, or both after 20 weeks of gestation in previously normotensive women.

f. Defined as either a random glucose level greater than 11.0 mmol/L, a fasting glucose level of 7.0 mmol/L or greater, or a fasting glucose level between 6.1 and 6.9 mmol/L with a subsequent abnormal glucose tolerance test (glucose level >7.8 mmol/L after glucose intake).

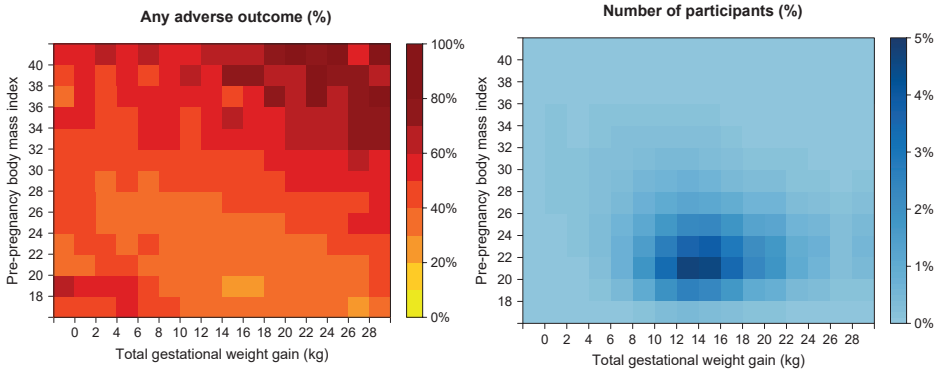
g. Defined as gestational age at birth of less than 37 weeks.

h. Small was defined as sex- and gestational age–adjusted birth weight less than the 10th percentile; large, greater than the 90th percentile.

i. Weight was included at the highest age available for each child (median, 84.9 [quartile 1 and quartile 3, 61.9 and 95.9] months). Underweight based on sex- and age-adjusted SD scores of less than –2.0 for children aged 2 to 5 years and for those older than 5 years; overweight, SD scores greater than 2.0 for children aged 2 to 5 years and greater than 1.0 for those older than 5 years.

j. Includes preeclampsia, gestational hypertension, gestational diabetes, cesarean delivery, preterm birth, and small or large size for gestational age at birth.

Figure 1. Heatmap of absolute risk of any adverse maternal or infant outcome

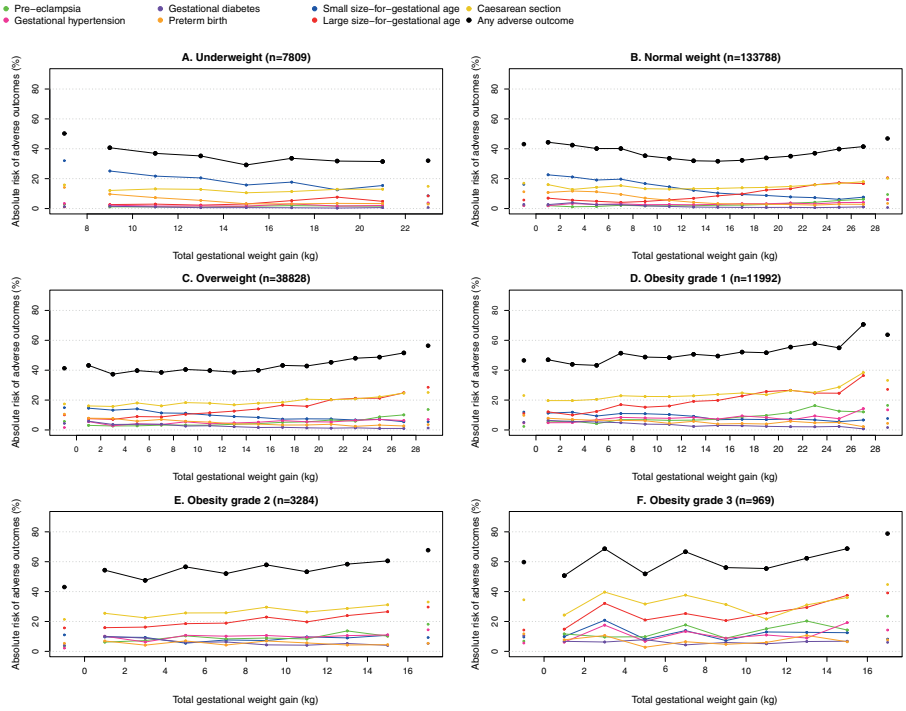


Values represent the absolute risks of any adverse maternal and infant outcome (left panel) and the percentages of participants (right panel) for each combination of body mass index and gestational weight gain. Absolute risk was calculated as No. of participants (any adverse outcome)/No. of participants (body mass index and gestational weight gain category) × 100. The percentages of participants were calculated as the number of participants with each combination of body mass index and gestational weight gain as a percentage of the total study sample. The total study sample size was 196 670. Participants in the extreme categories of prepregnancy body mass index (calculated as weight in kilograms divided by height in meters squared) and gestational weight gain had values beyond the most extreme labeled tick marks. Any adverse outcome includes preeclampsia (gestational hypertension plus proteinuria), gestational hypertension (systolic blood pressure ≥140mmHg, diastolic blood pressure ≥90mmHg, or both after 20weeks of gestation in previously normotensive women), gestational diabetes (a random glucose level >11.0 mmol/L, a fasting glucose level ≥7.0 mmol/L, or a fasting glucose level between 6.1 and 6.9 mmol/L with a subsequent abnormal glucose tolerance test [glucose level >7.8 mmol/L after glucose intake]), cesarean delivery, preterm birth (gestational age at birth <37weeks), and small or large size for gestational age at birth (sex- and gestational age–adjusted birthweight <10th percentile and >90th percentile, respectively.

Among women categorized as overweight, the absolute risk for any adverse outcome increased from 37.3% (249 of 667) for gestational weight gain of 2.0 kg to 3.9 kg to 56.4% (624 of 1107) for gestational weight gain of 28.0 kg or greater. Of all outcomes separately, the absolute risk was highest for cesarean delivery (highest risk: 25.1% [272 of 1084] for gestational weight gain of ≥28.0 kg).

Among women categorized as obesity grade 1, 2, or 3, the absolute risk for any adverse outcome increased across the range of gestational weight gain. The highest absolute risks were 63.7% (160 of 251) for gestational weight gain of 28.0 kg or greater in women categorized as obesity grade 1,67.7% (384 of 567) for gestational weight gain of 16.0 kg or greater in women categorized as obesity grade 2, and 78.8% (93 of 118) for gestational weight gain of 16.0 kg or greater in women categorized as obesity grade 3. The association of maternal prepregnancy BMI with the risk for any adverse outcomes was stronger than the association of gestational weight gain. The ORs for the risk of any adverse outcome were 1.28 (95%CI, 1.27-1.29) and 1.04 (95%CI, 1.03-1.05) per 1-SD increase in maternal prepregnancy BMI and gestational weight gain, respectively (*P*<.001 for comparison).The absolute data for each gestational weight gain category appear in **Supplemental Table 7.**

Figure 2. Absolute risk for adverse maternal or infant outcomes



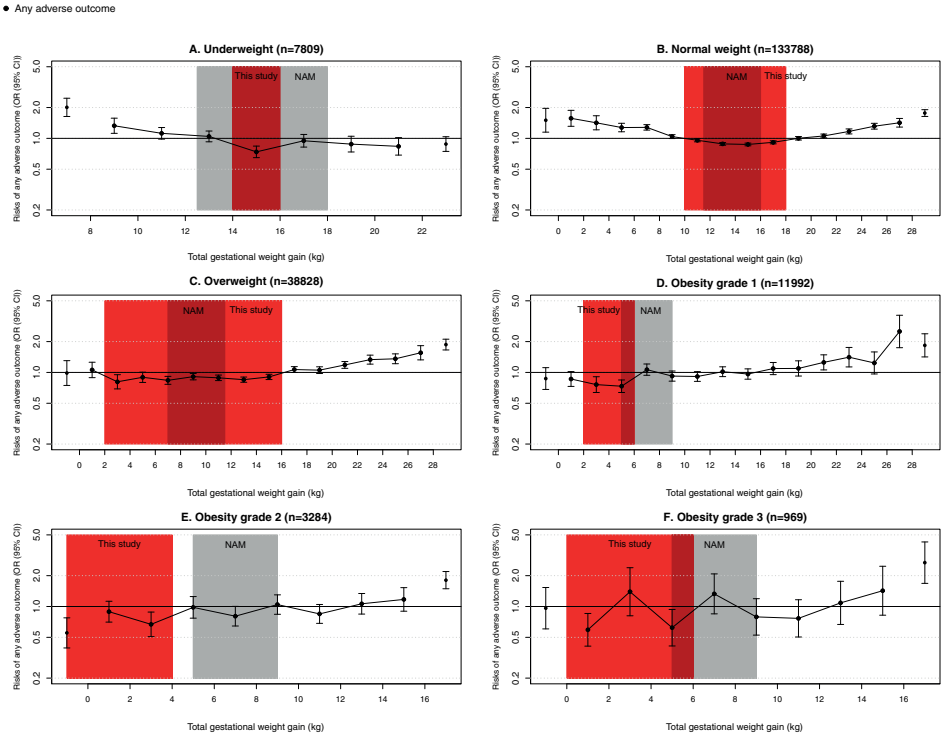
Absolute risk was calculated as (No. of women with adverse outcome/No. of women in gestational weight gain category within body mass index group) × 100. The symbols represent the absolute risk for women in each gestational weight gain category. The gestational weight gain categories were 2 kg each. Participants in the extreme categories of gestational weight gain had values beyond the most extreme labeled tick marks. The maternal body mass index (calculated as weight in kilograms divided by height in meters squared) categories were underweight (<18.5), normal weight (18.5-24.9), overweight (25.0-29.9), obesity grade 1(30.0-34.9), obesity grade 2 (35.0-39.9), and obesity grade 3 (≥40.0). Any adverse outcome includes preeclampsia (gestational hypertension plus proteinuria), gestational hypertension (systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or both after 20 weeks of gestation in previously normotensive women), gestational diabetes (a random glucose level >11.0 mmol/L, a fasting glucose level ≥7.0 mmol/L, or a fasting glucose level between 6.1 and 6.9 mmol/L with a subsequent abnormal glucose tolerance test [glucose level >7.8 mmol/L after glucose intake]), cesarean delivery, preterm birth (gestational age at birth <37weeks), and small or large size for gestational age at birth (sex- and gestational age–adjusted birthweight <10th percentile and >90th percentile, respectively). The odds ratios for the risk of any adverse outcome were 1.28 (95%CI, 1.27-1.29) and 1.04 (95%CI, 1.03-1.05) per 1-SD increase in maternal prepregnancy body mass index and gestational weight gain, respectively (P < .001 for comparison). The number of cases for each outcome and the total number of participants in each gestational weight gain category appears in **Supplemental Table 7**.

Optimal gestational weight gain per clinical BMI group

The optimal gestational weight gain ranges associated with the lowest risks for any adverse outcome appear in **Figure 3**. Among women categorized as underweight, the optimal gestational weight gain range was 14.0 kg to less than 16.0 kg, with corresponding OR and absolute risk reduction (ARR; the percentage reduction in absolute risk of any adverse outcome) of 0.74 (95% CI, 0.65-0.84) and 0.07% (95% CI, 0.04%-0.09%), respectively. Among women categorized as normal weight, the optimal gestational weight gain range was 10.0 kg to less than 18.0 kg (ORs at the outer ends of this range, 0.96 [95%CI, 0.93-0.99] and 0.91 [95%CI, 0.88-0.95]; ARRs, 0.01% [95%CI, 0%- 0.01%] and 0.02% [95%CI, 0.01%-0.03%]).Among women categorized as overweight, the optimal gestational weight gain range was 2.0 kg to less than 16.0 kg (ORs at the outer ends of this range, 0.81 [95% CI, 0.69-0.95] and 0.90 [95% CI, 0.85- 0.96]; ARRs, 0.05% [95%CI, 0.01%-0.08%] and 0.02% [95% CI, 0.01%-0.04%]). Among women categorized as obesity grade 1, the optimal gestational weight gain range was 2.0 kg to less than 6.0 kg (ORs at the outer ends of this range, 0.76 [95%CI, 0.64-0.91] and 0.73 [95%CI, 0.64-0.84]; ARRs, 0.07% [95% CI, 0.02%-0.11%] and 0.08% [95% CI, 0.04%-0.11%]). Among women categorized as obesity grade 2, the optimal gestational weight gain range was weight loss or gain of 0 kg to less than 4.0 kg (median weight loss: 3.0 kg; ORs at the outer ends of this range, 0.55 [95%CI, 0.39-0.78] and 0.67 [95%CI, 0.51-0.88]; ARRs, 0.14% [95% CI, 0.06%-0.22%] and 0.10% [95% CI, 0.03%-0.17%]). Among women categorized as obesity grade 3, the optimal gestational weight gain range was 0 kg to less than 6.0 kg (ORs for the outer ends of this range, 0.59 [95%CI, 0.41-0.85] and 0.62 [95%CI, 0.41-0.94]; ARRs, 0.12% [95%CI, 0.03%-0.21%] and 0.10% [95%CI, 0%-0.20%]). The ORs and ARRs for each gestational weight gain category used to determine the optimal ranges appear in **Supplemental Tables 8 and 9**, respectively. The gestational weight gain ranges defined in this study and the NAM ranges appear in **Supplemental Table 10**.

The gestational weight gain ranges in this study were roughly comparable with the NAM ranges for underweight, normal weight, and overweight, and were lower for all obesity grades. This study classified 11.3% of women (n = 22 236) in the main sample as having inadequate gestational weight gain and 33.8% of women (n = 66 463) as having excessive gestational weight gain. The NAM categories classified 21.5%of women (n = 42 323) as having inadequate gestational weight gain and 42.0% of women (n = 82 544) as having excessive gestational weight gain. Gestational weight gain outside the ranges from the current study and the NAM ranges was associated with adverse outcomes (**Supplemental Figures 2 and 3**). Each classification system had a low to moderate ability to distinguish between those with and those without adverse outcomes (range for AUROC, 0.55-0.77; **Supplemental Figure 4**).

Figure 3. Associations of gestational weight gain categories with any adverse outcome per maternal clinical body mass index group, used to determine optimal weight gain ranges



OR indicates odds ratio and it reflects the risk for any adverse outcome per gestational weight gain category for women with underweight, normal weight, overweight, obesity grade 1, obesity grade 2, and obesity grade 3, parts A-F, respectively, compared with all other gestational weight gain categories in that specific group for clinical maternal body mass index (BMI; calculated as weight in kilograms divided by height in meters squared). The solid circles represent the OR for all participants in each gestational weight gain category. The error bars indicate 95%CIs. The red area represents the optimal gestational weight gain range according to the current analysis, the gray area represents the gestational weight gain ranges recommended by the US National Academy of Medicine (NAM; formerly the Institute of Medicine). The gestational weight gain categories were 2 kg each. Participants in the extreme categories of gestational weight gain had values beyond the most extreme labeled tick marks. The maternal BMI categories were underweight (<18.5), normal weight (18.5-24.9), overweight (25.0-29.9), obesity grade 1 (30.0-34.9), obesity grade 2 (35.0-39.9), and obesity grade 3 (≥40.0). Any adverse outcome includes preeclampsia (gestational hypertension plus proteinuria), gestational hypertension (systolic blood pressure ≥140mmHg, diastolic blood pressure ≥90mmHg, or both after 20 weeks of gestation in previously normotensive women), gestational diabetes (a random glucose level >11.0 mmol/L, a fasting glucose level 7.0 mmol/L, or a fasting glucose level between 6.1 and 6.9 mmol/L with a subsequent abnormal glucose tolerance test [glucose level >7.8 mmol/L after glucose intake]), cesarean delivery, preterm birth (gestational age at birth <37weeks), and small or large size for gestational age at birth (sex- and gestational age–adjusted birthweight <10th percentile and >90th percentile, respectively). For the gestational weight gain ranges defined in this study, a statistically significant OR lower than 1 for a gestational weight gain category was considered the optimal weight gain. If a non-significant association (either with an OR >1, <1, or of 1) for a gestational weight gain category was surrounded by 2 significant estimates with an OR below 1, that gestational weight gain category was included in the optimal gestational weight gain range. The number of cases for each outcome and the total number of participants in each gestational weight gain category appear in **Supplemental Table 7**.The optimal gestational weight gain ranges based only on protective associations appear in **Supplemental Figure 5**.

Sensitivity analyses

The sensitivity analyses, in which optimal gestational weight gain was determined based on protective associations regardless of statistical significance, resulted in broader ranges of optimal gestational weight gain (**Supplemental Figure 5**). Optimal gestational weight gain ranges similar to those from the main analyses were observed when length of gestation was considered and when participants with missing individual outcome data were excluded (**Supplemental Table 11**). In addition, the sensitivity analyses showed that optimal weight gain definitions were not altered by including or excluding preterm birth, cesarean delivery, childhood underweight or overweight, gestational diabetes, and preeclampsia as adverse outcomes or by adjusting for maternal age and parity (**Supplemental Table 11**).

Of all the women classified as having excessive gestational weight gain during the full pregnancy, 84.6% also would be classified as having excessive weight gain during the first half of the pregnancy (**Supplemental Figure 6** and **Supplemental Tables 12 and 13**). Results for the validation sample showed that the discriminative performance of the optimal gestational weight gain ranges developed in this study and the weight gain ranges from the NAM guidelines were consistent with findings in the main study sample (range for AUROC, 0.50-0.79; **Supplemental Table 14** and **Supplemental Figures 7 and 8**).

DISCUSSION

Maternal prepregnancy BMI, and to a lesser extent gestational weight gain, are associated with risks of adverse maternal and infant adverse outcomes. Gestational weight gain ranges that were associated with lower risks for adverse outcomes were 14.0 kg to less than 16.0 kg for women categorized as being underweight; 10.0 kg to less than 18.0 kg for normal weight; 2.0 kg to less than 16.0 kg for overweight; 2.0 kg to less than 6.0 kg for obesity grade1; weight loss or gain of 0 kg to less than 4.0 kg for obesity grade 2; and weight gain of 0 kg to less than 6.0 kg for obesity grade 3.

Gestational weight gain outside these ranges was associated with adverse outcomes. However, discriminative performance of gestational weight gain with adverse maternal and infant outcomes was low to moderate. Prepregnancy BMI was more strongly associated with adverse maternal and infant outcomes than the amount of gestational weight gain.

Prepregnancy BMI is significantly associated with pregnancy complications and offspring obesity and also is associated with gestational weight gain (5, 6). Results from this study suggest that maternal prepregnancy BMI was more strongly associated with adverse maternal and infant outcomes than gestational weight gain. Therefore, prepregnancy BMI may be an important focus for preconception counseling.

Previous studies that attempted to define optimal gestational weight gain associated with fewer adverse outcomes differed considerably among study populations, statistical ap-

proaches, outcomes, and conclusions regarding optimal gestational weight gain ranges (14, 16-22). Only 1 study of 120 251 obese US women defined optimal gestational weight gain ranges according to maternal obesity grade 1 (4.5 kg-11.3 kg), obesity grade 2 (0 kg-4.1 kg),and obesity grade 3 (weight loss <4 kg), and that study used data from term births only (21).

Compared with prior work, the present study focused on common and important adverse maternal and infant outcomes, included women from multiple Western countries, and compared the associations of gestational weight gain and prepregnancy BMI with adverse outcomes. Consistent with the NAM guidelines, this study used total gestational weight gain to identify optimal gestational weight gain ranges instead of gestational weight gain per week because gestational weight gain does not have a linear pattern (7, 8). Total gestational weight gain is dependent in part on pregnancy duration. The observed results were similar after adjustment for gestational age at birth and after excluding preterm births. Consistent with the NAM guidelines, this study showed that among women with higher prepregnancy BMI, lower gestational weight gain was associated with fewer adverse outcomes. Gestational weight gain ranges for women categorized as obesity grade 1, 2, or 3 were lower than the NAM guidelines and even involved weight loss for severely obese women, although neither classification was predictive for adverse outcomes. However, the results for severely obese women should be interpreted with caution because the optimal gestational weight gain ranges for obesity grades 1 through 3 associated with better outcomes fluctuate and do not follow a clear linear trend. These results may represent the relatively small sample size of obese women and lack of statistical power rather than biological plausibility. Future studies should evaluate the effect and safety of weight loss during pregnancy in severely obese women.

Gestational weight gain guidelines are used in several Western countries for preconception counseling. The gestational weight gain ranges developed in this study classified fewer women as having suboptimal weight gain compared with the NAM guidelines. However, the discriminative performance, as indicated by the AUROC, was weak for both classification systems. This suggests that the use of gestational weight gain guidelines may need to be reconsidered for individual prediction of the risk for adverse outcomes.

Future research should assess whether optimal gestational weight gain ranges combined with other maternal and fetal pregnancy characteristics are useful for prediction of adverse outcomes. The findings from this study suggest that prepregnancy weight might be a more important target for interventions than gestational weight gain. Previous studies of dietary and physical activity interventions for pregnant women have not shown an effect on pregnancy outcomes (23-26). Based on current evidence, future clinical trials designed to reduce weight-related maternal and infant adverse outcomes should focus on maternal weight before or at the start of pregnancy.

Limitations

This study has several limitations. First, not all invited cohorts were able to participate in the current analyses. Second, the analyses did not measure changes in the association of gestational weight gain with adverse outcomes over time. The results may be biased if the association of gestational weight gain with adverse outcomes changed over time. Third, data on prepregnancy weight was mainly self-reported, and the latest weight during pregnancy was either self-reported or measured. This may have led to misclassification of gestational weight gain. Fourth, the composite outcome of any adverse outcome might have been misclassified as a result of some missing data for individual outcomes. Fifth, all outcomes were considered equally important and the analyses did not account for the differences in outcome severity. Sixth, cesarean delivery may be due to many factors and may not be an appropriate outcome for studying associations of weight change with adverse maternal outcomes (7). Seventh, information on stillbirth was not available. Eighth, optimal gestational weight gain was defined as a protective association with the risk for any adverse outcome, reflecting the best outcome possible and limiting the number of participants incorrectly classified as having adequate gestational weight gain. The ranges would be slightly broader if optimal gestational weight gain was defined as no increased risk for adverse outcomes, which includes both a protective association and a null association. Ninth, the analyses were not adjusted for multiple testing. Tenth, as a result of the limited sample sizes for underweight and severely obese women, heterogeneity was not assessed. Eleventh, based on the profiles of all the included cohorts, about 1% of women were included more than once for multiple pregnancies. Twelfth, for some outcomes, discriminative performance in the validation sample was lower than in the main sample, potentially resulting from overfitting of the models in the main sample.

Conclusions

In this meta-analysis of pooled individual participant data from 25 cohort studies, the risk for adverse maternal and infant outcomes varied by gestational weight gain and across the range of prepregnancy weights. The estimates of optimal gestational weight gain may inform prenatal counseling; however, the optimal gestational weight gain ranges had limited predictive value for the outcomes assessed.

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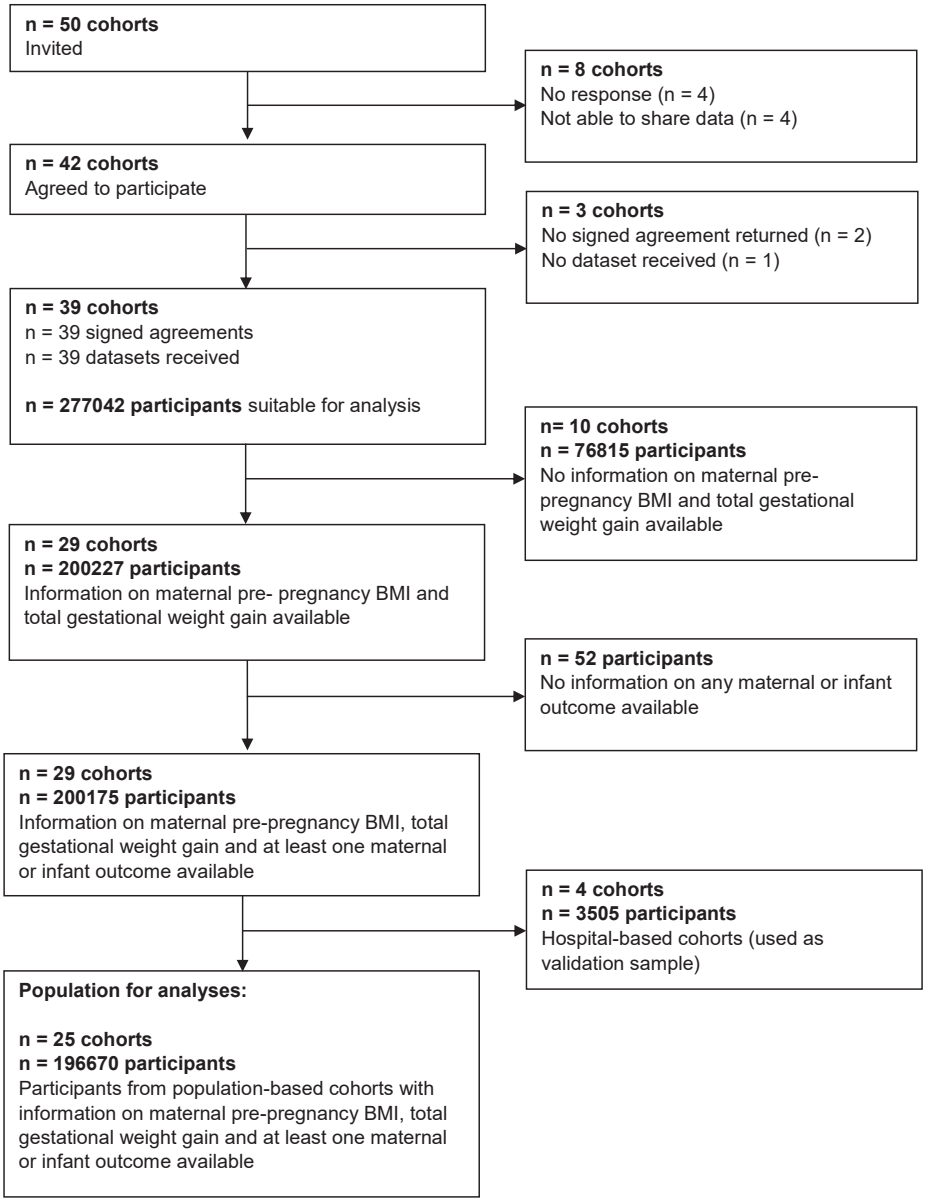
SUPPLEMENTAL MATERIAL

Supplemental Figure 1. Flow chart of the cohorts and participants



Supplemental Table 1. Cohort-specific methods of data collection[a]

Cohort name (country)	Maternal height	Maternal pre-pregnancy weight	Maternal latest weight before delivery or gestational weight gain	Pre-eclampsia	Gestational hyperten-sion	Gestational diabetes	Mode of delivery	Gestational age at birth	Birth weight	Childhood weight and height
Main analysis										
ALSPAC (United Kingdom)	Self-reported	Self-reported	Clinical records	Clinical records	Clinical records	Clinical records	Clinical records	Clinical records	Measured	Measured
DNBC (Denmark)	Self-reported	Self-reported	Self-reported	Clinical records	Self-reported	Clinical records	National Medical Birth Registry	Parental report or National Medical Birth Registry	National Medical Birth Registry	Reported or Measured
FCOU (Ukraine)	Clinical records	Clinical records	Clinical records	Clinical records	Clinical records	Clinical records	Clinical records	NA	Clinical records	Clinical records
GECKO Drenthe (The Netherlands)	Self-reported	Self-reported	Self-reported	Self-reported	Self-reported	Self-reported	Clinical records or Self-reported	Clinical records	Clinical records	Measured
Gen3G (Canada)	Measured	Self-reported	Clinical records	Clinical records	Clinical records	Oral glucose tolerance test	Clinical records	Clinical records	Clinical records	NA
GENESIS (Greece)	Self-reported	Self-reported	Self-reported	NA	NA	Self-reported	NA	Clinical records	Clinical records	Measured
GENERATION R (The Netherlands)	Measured	Self-reported	Self-reported	Clinical records or Self-reported	Clinical records or Self-reported	Clinical records or Self-reported	Clinical records	Clinical records	Clinical records	Measured
GENERATION XXI (Portugal)	Measured or ID card	Self-reported	Self-reported	Clinical records	Clinical records	Clinical records	Clinical records	Clinical records	Clinical records	Measured

Supplemental Table 1. Cohort-specific methods of data collection[a] (continued)

Cohort name (country)	Maternal height	Maternal pre-pregnancy weight	Maternal latest weight before delivery or gestational weight gain	Pre-eclampsia	Gestational hyperten-sion	Gestational diabetes	Mode of delivery	Gestational age at birth	Birth weight	Childhood weight and height
GINIplus (Germany)	Self-reported	Self-reported	Self-reported	NA	NA	Self-reported	Self-reported	NA	NA	Clinical records at 4y, Measured and Reported at 10 and 15y
HUMIS (Norway)	Self-reported	Self-reported	Self-reported	Medical birth registry	Medical birth registry	Medical birth registry	Medical birth registry	Clinical records	Clinical records	Reported
INMA (Spain)	Measured or Self-reported	Self-reported	Clinical records	NA	NA	Clinical records	Clinical records	Clinical records	Clinical records	Measured
KOALA (The Netherlands)	Self-reported	Self-reported	Self-reported	Clinical records or Self-reported	Clinical records or Self-reported	Clinical records or Self-reported	Self-reported	Clinical records	Clinical records	Reported
Krakow Cohort (Poland)	Self-reported	Self-reported	Self-reported	Self-reported	Self-reported	Self-reported	Clinical records	Clinical records	Clinical records	Measured
LISAplus (Germany)	Self-reported	Self-reported	Self-reported	NA	NA	Self-reported	Self-reported	NA	NA	Clinical records at 4y, Measured and Reported at 10 and 15y
MoBa (Norway)	Self-reported	Self-reported	Self-reported	Clinical records	Clinical records	Clinical records	Clinical records	Clinical records	Clinical records	Reported

Supplemental Table 1. Cohort-specific methods of data collection[a] (continued)

Cohort name (country)	Maternal height	Maternal pre-pregnancy weight	Maternal latest weight before delivery or gestational weight gain	Pre-eclampsia	Gestational hyperten-sion	Gestational diabetes	Mode of delivery	Gestational age at birth	Birth weight	Childhood weight and height
NINFEA (Italy)	Self-reported	Self-reported	Self-reported	Self-reported	Self-reported	Self-reported	Self-reported	Parental report	Parental report	Reported
PIAMA (The Netherlands)	Self-reported	Self-reported	Self-reported	Self-reported	Self-reported	Self-reported	Self-reported	Parental report	Parental report	Reported and measured (4 and 8y)
Piccolipiù (Italy)	Self-reported	Self-reported	Self-reported	Self-reported	Self-reported	Self-reported	Self-reported	Clinical records	Clinical records	Measured
PRIDE Study (The Netherlands)	Self-reported	Self-reported	Self-reported	Clinical records or Self-reported	Clinical records or Self-reported	Clinical records or Self-reported	Self-reported	Parental report	Parental report	NA
Project Viva (United States)	Self-reported	Self-reported	Clinical records	Clinical records	Clinical records	Clinical records	Clinical records	Parental report	Clinical records	Measured
REPRO_PL (Poland)	Measured	Self-reported	Measured	Clinical records	Clinical records	Clinical records	Clinical records	Clinical records	Clinical records	Measured
RHEA (Greece)	Measured	Self-reported	Measured	Self-reported	Measured or Self-reported	Self-reported	Self-reported	Clinical records	Clinical records	Clinical records or Measured
Slovak PCB study (Slovakia)	Self-reported	Self-reported	Self-reported	NA	NA	Self-reported	Clinical records	Clinical records	Clinical records	Measured
STEPS (Finland)	Self-reported	Self-reported	Self-reported	NA	NA	Clinical records	NA	National Longitudinal Census Files	National Longitudinal Census Files	Measured

Supplemental Table 1. Cohort-specific methods of data collection[a] (continued)

Cohort name (country)	Maternal height	Maternal pre-pregnancy weight	Maternal latest weight before delivery or gestational weight gain	Pre-eclampsia	Gestational hyperten-sion	Gestational diabetes	Mode of delivery	Gestational age at birth	Birth weight	Childhood weight and height
SWS (United Kingdom)	Measured	Measured	Measured	Clinical records	Clinical records	Clinical records	Clinical records	Measured	Clinical records	Measured
Hospital-based[b]										
Co.N.ER (Italy)	Self-reported	Self-reported	Self-reported	Self-reported	Self-reported	Self-reported	Self-reported	Parental report	Parental report	Reported
EDEN (France)	Measured	Self-reported	Clinical records	Clinical records	Clinical examination at 24 weeks of gestation and clinical records	Oral glucose tolerance test and clinical records	Clinical records	Clinical records	Clinical records	Clinical records or Measured
GASPII (Italy)	Self-reported	Self-reported	Self-reported	Self-reported	Self-reported	Self-reported	Clinical records	Measured	Measured	Measured
LUKAS (Finland)	Self-reported	Self-reported	Clinical records or Self-reported	NA	NA	NA	Reported by midwives	NA	Clinical records	Reported

[a] NA: not available (not collected or not provided) or not applicable (gestational age at birth (FCOU, GINIplus, LISAplus, LUKAS) and birth weight (GINIplus, LISAplus) due to selected samples).

[b] The hospital-based cohorts were used as validation sample.

Supplemental Table 2. Associations of general characteristics with total gestational weight gain[a]

	Total gestational weight gain, kg (95% CI)
Pre-pregnancy BMI (kg/m^2)[b]	-0.25 (-0.26, -0.24)
Underweight (<18.5 kg/m^2)	-0.16 (-0.29, -0.04)
Normal weight (18.5-24.9 kg/m^2)	Reference
Overweight (25.0-29.9 kg/m^2)	-0.79 (-0.85, -0.73)
Obesity grade 1 (30.0-34.9 kg/m^2)	-3.48 (-3.58, -3.37)
Obesity grade 2 (35.0-39.9 kg/m^2)	-5.73 (-5.92, -5.54)
Obesity grade 3 (≥40.0 kg/m^2)	-7.61 (-7.96, -5.54)
Maternal age (years)	-0.11 (-0.12, -0.11)
Parity	
Nulliparous	Reference
Multiparous	-1.07 (-1.12, -1.02)

[a] Values are regression coefficients from exploratory multilevel linear regression models that represent differences in total gestational weight gain for each of the characteristics listed. Models are not adjusted for covariates.
[b] Body mass index is calculated as weight in kilograms divided by height in meters squared.

Supplemental Table 3. Description of missing data

	Information available		Information missing	
Outcome	**n**	**%**	**n**	**%**
Main sample (n=196670)				
Used in models to construct ranges (main analysis)				
Pre-pregnancy BMI	196670	100.0	NA	NA
Total gestational weight gain	196670	100.0	NA	NA
Any adverse outcome [a]	196670	100.0	NA	NA
Separate outcomes (included in 'any adverse outcome')				
Gestational hypertensive disorders [b]	179577	91.3	17093	8.7
Gestational diabetes	189772	96.5	6898	3.5
Caesarean section	186884	95.0	9786	5.0
Preterm birth	188129	95.7	8541	4.3
Size-for-gestational-age at birth	190217	96.7	6453	3.3
All of the separate outcomes used to construct ranges (complete cases)[c]	169437[d]	86.2	27233[e]	13.8
Validation sample (Hospital-based) (n=3505)				
Any adverse outcome	3505	100.0	NA	NA
Gestational hypertensive disorders [b]	3082	87.9	423	12.1
Gestational diabetes	3084	88.0	421	12.0
Caesarean section	3490	99.6	15	0.4
Preterm birth	3089	88.1	416	11.9
Size-for-gestational-age at birth	3498	99.8	7	0.2
All of the separate outcomes (complete cases)	3059[d]	87.3	446[e]	12.7

[a] Any adverse outcome is defined as the presence of at least one of the following outcomes: pre-eclampsia, gestational hypertension, gestational diabetes, caesarean section, preterm birth and small or large size-for-gestational age.
[b] Includes both pre-eclampsia and gestational hypertension.
[c] Sensitivity analysis with complete cases is shown in **Supplemental Table 10**.
[d] Reflects the number of participants with information on all outcomes included in 'any adverse outcome'.
[e] Reflects the number of participants with missing information on one or more of the outcomes included in 'any adverse outcome'.

Supplemental Table 4. Characteristics of the hospital-based population, used as a validation sample.[a]

	Total group
	n=3505
Pre-pregnancy body mass index, median (Q1, Q3)[b]	22.0 (20.1, 24.8)
Pre-pregnancy body mass index[b]	
Underweight (<18.5 kg/m^2)	277 (7.9)
Normal weight (18.5-24.9 kg/m^2)	2400 (68.4)
Overweight (25.0-29.9 kg/m^2)	577 (16.5)
Obesity grade 1 (30.0-34.9 kg/m^2)	188 (5.4)
Obesity grade 2 (35.0-39.9 kg/m^2)	53 (1.5)
Obesity grade 3 (≥40.0 kg/m^2)	10 (0.3)
Total gestational weight gain (kg)	
Median (Q1, Q3)	13.0 (10.7, 16.0)
P2.5, P97.5	4.0, 24.0
Maternal age (years), median (Q1, Q3)	31.0 (27.7, 34.7)
Education[c]	
Low, n (%)	721 (20.6)
Medium, n (%)	1254 (35.9)
High, n (%)	1521 (43.5)
Country	
France, n (%)	1794 (51.2)
Italy, n (%)	1296 (37.0)
Finland, n (%)	415 (11.8)
Gestational hypertension, n (%)[d]	102 (3.4)
Pre-eclampsia, n (%)[e]	61 (2.0)
Gestational diabetes, n (%)[f]	162 (5.3)
Caesarean section, n (%)	653 (18.7)
Preterm birth, n (%)[g]	140 (4.5)
Small size-for-gestational-age, n (%)[h]	349 (10.0)
Large size-for-gestational-age, n (%)[i]	348 (10.0)
Childhood underweight, n (%)[j]	21 (0.8)
Childhood overweight, n (%)[k]	563 (20.5)
Any adverse outcome, n (%)[l]	1423 (40.6)

[a] Values are median (Q1, Q3), median (P2.5, P97.5) or n (valid %). [b] Body mass index is calculated as weight in kilograms divided by height in meters squared. [c] Education level was based on cohort-specific criteria. Each cohort used their own country-specific criteria to define low, medium and high educational level. These 3 categories were subsequently used in the meta-analysis. [d] Gestational hypertension is defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg after 20 weeks of gestation in previously normotensive women. [e] Pre-eclampsia is defined as gestational hypertension plus proteinuria. [f] Gestational diabetes is defined as either a random glucose level >11.0 mmol/L, a fasting glucose level ≥7.0 mmol/L or a fasting glucose level between 6.1 and 6.9 mmol/L with a subsequent abnormal glucose tolerance test (glucose level >7.8 mmol/L after glucose intake). [g] Preterm birth is defined as gestational age at birth <37 weeks. [h] Small size-for-gestational-age at birth is defined as sex- and gestational age adjusted birth weight <10th percentile. [i] Large size-for-gestational-age at birth is defined as sex- and gestational age adjusted birth >90th percentile. [j] Childhood underweight at the highest age available for each child (median (Q1, Q3): 84.9 (61.9, 95.9) months) is defined as sex- and age adjusted standard deviation scores (SDS) <-2 SDS for children of 2-5 years of age, and <-2 SDS for children of >5 years. [k] Childhood overweight at the highest age available for each child (median (Q1, Q3): 84.9 (61.9, 95.9) months) is defined as sex- and age adjusted standard deviation scores (SDS) >2.0 SDS for children of 2-5 years of age, and >1 SDS for children of >5 years. [l] Any adverse outcome includes pre-eclampsia, gestational hypertension, gestational diabetes, caesarean section, preterm birth, small size-for-gestational-age, and large size-for-gestational-age.

Supplemental Table 5. Description of exposures and general characteristics per cohort[a,b]

	Participants, n	Maternal pre-pregnancy BMI[c]	Total gestational weight gain (kg)		Maternal age (years)	Education, n(%)[d]		
Cohort name (country)		**median (Q1, Q3)**	**median (Q1, Q3)**	**P2.5, P97.5**	**median (Q1, Q3)**	**Low**	**Medium**	**High**
Main analysis								
ALSPAC (United Kingdom)	10156	22.2 (20.5, 24.5)	12.5 (9.5, 15.5)	4.0, 22.3	28.0 (25.0, 32.0)	6100 (63.1)	2262 (23.4)	1305 (13.5)
DNBC (Denmark)	60963	22.7 (20.7, 25.5)	15.0 (12.0, 18.0)	4.0, 28.0	30.2 (27.4, 33.3)	5461 (9.0)	23332 (38.4)	31959 (52.6)
FCOU (Ukraine)	3261	21.6 (19.9, 24.0)	12.0 (9.2, 15.0)	3.0, 21.5	23.0 (20.0, 27.0)	163 (5.1)	2177 (68.1)	857 (26.8)
GECKO Drenthe(The Netherlands)	2044	23.7 (21.5, 26.8)	13.0 (10.0, 17.0)	4.0, 25.0	31.0 (28.0, 34.0)	1257 (62)	771 (38.0)	0 (0.0)
Gen3G (Canada)	844	23.3 (20.9, 27.3)	13.7 (10.7, 17.0)	3.2, 24.1	28.0 (25.0, 31.0)	NA	NA	NA
GENESIS (Greece)	2173	21.9 (20.2, 24.0)	13.0 (10.0, 17.0)	3.0, 28.7	30.3 (27.2, 33.3)	95 (4.5)	1096 (52.0)	918 (43.5)
GENERATION R (The Netherlands)	6808	22.6 (20.8, 25.4)	12.0 (9.0, 16.0)	1.0, 25.0	30.4 (26.1, 33.6)	698 (10.6)	3055 (46.2)	2855 (43.2)
GENERATION XXI (Portugal)	7220	22.9 (21.0, 25.8)	13.0 (10.0, 17.0)	2.0, 26.0	29.0 (25.0, 33.0)	2329 (32.3)	3157 (43.8)	1717 (23.8)
GINIplus (Germany)	2262	22.1 (20.5, 24.2)	13.0 (10.0, 15.7)	5.0, 25.0	31.0 (29.0, 34.0)	264 (11.7)	963 (42.7)	1030 (45.6)
HUMIS (Norway)	1045	23.4 (21.3, 26.1)	14.0 (11.0, 18.0)	5.0, 27.0	30.0 (27.0, 33.0)	109 (12.0)	177 (19.5)	622 (68.5)
INMA (Spain)	1933	22.5 (20.7, 25.1)	13.5 (10.5, 16.6)	4.1, 24.5	30.0 (28.0, 33.0)	504 (26.1)	790 (41.0)	635 (32.9)
KOALA (The Netherlands)	2621	22.7 (20.9, 25.3)	14.0 (11.0, 17.0)	4.0, 25.0	32.0 (30.0, 34.0)	258 (10.4)	988 (39.7)	1242 (49.9)
Krakow Cohort (Poland)	498	21.0 (19.5, 22.7)	15.0 (12.0, 18.0)	7.0, 27.6	28.0 (26.0, 30.0)	48 (9.6)	188 (37.8)	262 (52.6)
LISAplus (Germany)	2837	21.7 (20.2, 24.0)	14.0 (11.5, 17.0)	6.0, 25.5	31.0 (28.0, 34.0)	278 (9.9)	1081 (38.6)	1443 (51.5)
MoBa (Norway)	73462	23.1 (21.1, 25.9)	15.0 (11.0, 18.0)	3.2, 27.0	30.0 (27.0, 33.0)	21573 (29.9)	31440 (43.6)	19027 (26.4)
NINFEA (Italy)[e]	2134	21.3 (19.8, 23.9)	12.0 (10.0, 15.0)	3.0, 22.0	33.0 (30.0, 36.0)	84 (3.9)	729 (34.2)	1317 (61.8)
PIAMA (The Netherlands)	3388	22.2 (20.6, 24.3)	13.0 (10.0, 16.0)	5.0, 25.0	30.0 (28.0, 33.0)	757 (22.4)	1399 (41.3)	1228 (36.3)
Piccolipiù (Italy)	3173	21.7 (19.9, 24.2)	13.0 (10.0, 15.0)	5.3, 22.0	34.0 (30.0, 37.0)	377 (11.9)	1372 (43.4)	1414 (44.7)
PRIDE Study (The Netherlands)	1081	22.5 (20.6, 24.7)	14.0 (11.0, 17.0)	4.0, 25.0	30.0 (28.0, 33.0)	20 (1.9)	196 (18.5)	843 (79.6)
Project Viva (United States)	2021	23.5 (21.0, 27.1)	15.5 (12.3, 19.1)	4.1, 27.3	32.3 (29.1, 35.4)	699 (34.8)	717 (35.7)	595 (29.6)

REPRO_PL (Poland)	1204	21.5 (19.8, 23.7)	12.0 (9.0, 15.0)	4.0, 22.5	28.0 (25.0, 31.0)	150 (12.5)	368 (30.6)	685 (56.9)
RHEA (Greece)	699	23.3 (21.2, 25.9)	13.0 (10.0, 17.0)	4.0, 26.0	30.0 (27.0, 33.0)	102 (14.6)	361 (51.7)	235 (33.7)
Slovak PCB study (Slovakia)	983	21.2 (19.5, 24.0)	13.0 (10.0, 17.0)	4.9, 24.0	25.0 (22.0, 29.0)	423 (43.1)	472 (48.1)	87 (8.9)
STEPS (Finland)	1406	23.0 (21.1, 26.0)	13.9 (10.8, 17.4)	2.5, 25.4	30.7 (28.0, 33.6)	127 (9.3)	388 (28.3)	855 (62.4)
SWS (United Kingdom)	2454	24.2 (21.9, 27.4)	11.9 (8.3, 15.7)	0.1, 25.3	30.2 (27.2, 32.7)	316 (12.9)	1445 (59.0)	688 (28.1)
Hospital-based[f]								
Co.N.ER (Italy)	625	21.1 (19.7, 23.2)	13.0 (10.0, 16.0)	7.0, 22.4	33.8 (30.9 ,36.5)	109 (17.5)	279 (44.7)	236 (37.8)
EDEN (France)	1794	22.1 (20.1, 25.2)	13.0 (11.0, 16.3)	3.0, 24.0	29.5 (26.2 ,32.6)	502 (28.1)	324 (18.1)	960 (53.8)
GASPII (Italy)	671	21.3 (19.8, 23.6)	13.0 (10.5, 16.0)	6.0, 23.3	33.0 (30.0, 36.0)	92 (13.7)	338 (50.4)	241 (35.9)
LUKAS (Finland)	415	24.0 (21.9, 27.2)	13.8 (11.0, 17.8)	4.2, 25.3	30.9 (27.4, 34.6)	18 (4.3)	313 (75.4)	84 (20.2)

[a] Values are median (Q1, Q3), median (P2.5, P97.5) or n (valid %).
[b] NA: not available (not collected or not provided).
[c] Body mass index is calculated as weight in kilograms divided by height in meters squared.
[d] Education level was based on cohort-specific criteria. Each cohort used their own country-specific criteria to define low, medium and high educational level. These 3 categories were subsequently used in the meta-analysis.
[e] Subset of participants with 4-years follow-up completed.
[f] The hospital-based cohorts were used as validation sample.

Supplemental Table 6. Description of outcomes per cohort[a,b]

Cohort name (country)	Participants, n	Gestational hypertension, n (%)[d]	Pre-eclampsia, n (%)[e]	Gestational diabetes, n (%)[f]	Caesarean section, n (%)	Preterm birth, n (%)[g]	Small size-for-gestational-age, n (%)[h]	Large size-for-gestational-age, n (%)[i]	Childhood underweight, n (%)[j]	Childhood overweight, n (%)[k]	Any adverse outcome, n (%)[l]
Main analysis											
ALSPAC (United Kingdom)	10156	1482 (14.9)	206 (2.4)	76 (0.7)	1068 (17.1)	463 (4.6)	1001 (10.0)	1001 (10.0)	116 (1.6)	1874 (25.5)	4156 (40.9)
DNBC (Denmark)	60963	2239 (4.0)	2230 (4.0)	416 (0.7)	9290 (15.3)	2625 (4.3)	6064 (10.0)	6063 (10.0)	728 (2.3)	4930 (15.3)	22997 (37.7)
FCOU (Ukraine)	3261	284 (9.2)	186 (6.3)	9 (0.3)	273 (8.5)	NA	338 (10.5)	319 (10.0)	47 (2.6)	208 (11.6)	1197 (36.7)
GECKO Drenthe(The Netherlands)	2044	142 (8.0)	38 (2.3)	78 (3.8)	271 (15.0)	80 (4.0)	197 (10.0)	197 (10.0)	NA	348 (21.4)	780 (38.2)
Gen3G (Canada)	844	42 (5.0)	9 (1.1)	71 (8.6)	144 (17.1)	39 (4.6)	84 (10.0)	84 (10.0)	NA	NA	360 (42.7)
GENESIS (Greece)	2173	NA	NA	34 (1.6)	NA	206 (9.5)	209 (10.0)	208 (10.0)	11 (0.6)	289 (15.6)	579 (26.6)
GENERATION R (The Netherlands)	6808	0 (0.0)	140 (2.2)	72 (1.1)	726 (11.9)	349 (5.1)	675 (10.0)	675 (10.0)	42 (0.8)	1425 (25.6)	2202 (32.3)
GENERATION XXI (Portugal)	7220	132 (1.9)	112 (1.6)	474 (6.6)	2526 (35.4)	526 (7.3)	719 (10.0)	718 (10.0)	24 (0.4)	2001 (35.5)	3917 (54.3)
GINIplus (Germany)	2262	NA	NA	58 (2.6)	445 (19.7)	NA	NA	NA	45 (2.0)	362 (16)	493 (21.8)
HUMIS (Norway)	1045	40 (4.2)	85 (8.5)	6 (0.6)	195 (18.7)	85 (8.2)	103 (9.9)	103 (9.9)	21 (2.3)	101 (10.9)	444 (42.5)
INMA (Spain)	1933	NA	NA	195 (11.9)	334 (17.6)	76 (3.9)	192 (10.0)	192 (10.0)	5 (0.3)	406 (27.8)	801 (41.4)
KOALA (The Netherlands)	2621	82 (3.2)	30 (1.2)	21 (0.8)	307 (11.7)	81 (3.1)	260 (10.0)	260 (10.0)	63 (3.2)	204 (10.5)	887 (33.8)
Krakow Cohort (Poland)	498	21 (4.2)	1 (0.2)	21 (4.2)	96 (19.3)	18 (3.6)	49 (9.9)	49 (9.9)	8 (1.9)	94 (22.5)	216 (43.4)
LISAplus (Germany)	2837	NA	NA	56 (3.7)	481 (17.2)	NA	NA	NA	50 (2.2)	332 (14.8)	525 (18.5)
MoBa (Norway)	73462	1448 (2.0)	2666 (3.7)	587 (0.8)	9831 (13.4)	2919 (4.0)	7300 (10.0)	7304 (10.0)	1234 (2.4)	7531 (14.8)	25826 (35.2)
NINFEA (Italy)[c]	2134	105 (5.3)	47 (2.4)	153 (7.5)	555 (29.2)	81 (3.8)	210 (10.0)	209 (10.0)	64 (3.8)	156 (9.3)	1030 (48.3)
PIAMA (The Netherlands)	3388	163 (8.0)	52 (2.7)	18 (0.9)	284 (8.5)	162 (4.8)	336 (10.0)	336 (10.0)	26 (1.1)	391 (17.2)	1122 (33.1)
Piccolipiù (Italy)	3173	114 (3.6)	35 (1.1)	283 (9.0)	854 (27.0)	94 (3.0)	316 (10.0)	314 (9.9)	24 (3.6)	40 (6.0)	1572 (49.5)

PRIDE Study (The Netherlands)	1081	85 (8.7)	33 (3.6)	15 (1.5)	106 (10.5)	44 (4.2)	104 (10.0)	104 (10.0)	NA	NA	398 (36.8)
Project Viva (United States)	2021	134 (6.9)	63 (3.4)	113 (5.6)	476 (23.6)	108 (5.3)	202 (10.0)	202 (10.0)	8 (0.6)	345 (25.6)	954 (47.2)
REPRO_PL (Poland)	1204	51 (4.2)	2 (0.2)	45 (3.7)	404 (34.9)	41 (3.4)	120 (10.0)	118 (9.8)	6 (2.3)	35 (13.4)	636 (52.8)
RHEA (Greece)	699	28 (4.6)	4 (0.7)	57 (9.3)	347 (49.9)	83 (12.0)	68 (9.9)	66 (9.6)	2 (0.3)	79 (12.4)	455 (65.1)
Slovak PCB study (Slovakia)	983	NA	NA	9 (0.9)	14 (1.4)	22 (2.2)	97 (9.9)	99 (10.1)	9 (2.0)	169 (37.7)	230 (23.4)
STEPS (Finland)	1406	NA	NA	54 (6.0)	NA	54 (3.8)	140 (10.0)	140 (10.0)	NA	19 (4.6)	357 (25.4)
SWS (United Kingdom)	2454	91 (3.8)	57 (2.4)	25 (1.0)	540 (26.2)	94 (3.8)	243 (10.0)	244 (10.0)	9 (0.4)	379 (18.2)	1027 (41.9)
Hospital-based[m]											
Co.N.ER (Italy)	625	21 (3.5)	16 (2.7)	16 (2.6)	121 (19.8)	27 (4.3)	62 (9.9)	62 (9.9)	13 (2.5)	121 (23.4)	259 (41.4)
EDEN (France)	1794	51 (2.9)	37 (2.1)	118 (6.6)	271 (15.1)	86 (4.8)	180 (10.1)	179 (10.0)	7 (0.5)	143 (11.1)	716 (39.9)
GASPII (Italy)	671	30 (4.5)	8 (1.3)	28 (4.2)	209 (31.1)	27 (4.0)	66 (9.9)	66 (9.9)	NA	NA	331 (49.3)
LUKAS (Finland)	415	NA	NA	NA	52 (12.5)	NA	41 (9.9)	41 (9.9)	1 (0.3)	114 (30.2)	117 (28.2)

[a] Values are median (Q1, Q3), median (P2.5, P97.5) or n (valid %).

[b] NA: not available (not collected or not provided) or not applicable (preterm birth (FCOU, GINIplus, LISAplus, LUKAS) and small and large size-for-gestational age (GINIplus, LISAplus) due to selected samples).

[c] Subset of participants with 4-years follow-up completed.

[d] Gestational hypertension is defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg after 20 weeks of gestation in previously normotensive women.

[e] Pre-eclampsia is defined as gestational hypertension plus proteinuria.

[f] Gestational diabetes is defined as either a random glucose level >11.0 mmol/L, a fasting glucose level ≥7.0 mmol/L or a fasting glucose level between 6.1 and 6.9 mmol/L with a subsequent abnormal glucose tolerance test (glucose level >7.8 mmol/L after glucose intake).

[g] Preterm birth is defined as gestational age at birth <37 weeks.

[h] Small size-for-gestational-age at birth is defined as sex- and gestational age adjusted birth weight <10th percentile.

[i] Large size-for-gestational-age at birth is defined as sex- and gestational age adjusted birth >90th percentile.

[j] Childhood underweight at the highest age available for each child (median (Q1, Q3): 84.9 (61.9, 95.9) months) is defined as sex- and age adjusted standard deviation scores (SDS) <-2.0 SDS for children of 2-5 years of age, and <-2.0 for children of >5 years.

[k] Childhood overweight at the highest age available for each child (median (Q1, Q3): 84.9 (61.9, 95.9) months) is defined as sex- and age adjusted standard deviation scores (SDS) >2.0 SDS for children of 2-5 years of age, and >1.0 SDS for children of >5 years.

[l] Any adverse outcome includes pre-eclampsia, gestational hypertension, gestational diabetes, caesarean section, preterm birth, small size-for-gestational-age, and large size-for-gestational-age.

[m] The hospital-based cohorts were used as validation sample.

Supplemental Table 7. Description of outcomes by gestational weight gain category[a]

	Pre-eclampsia			Gestational hypertension			Gestational diabetes			Caesarean section		
	Cases	**Total**	**%**	**Cases**	**Total**	**%**	**Cases**	**Total**	**%**	**Cases**	**Total**	**%**
Underweight (n=7809)												
<8	10	346	2.9	12	348	3.4	5	389	1.3	55	348	15.8
8 to 9.9	6	526	1.1	12	532	2.3	9	597	1.5	68	562	12.1
10 to 11.9	12	1011	1.2	17	1016	1.7	12	1117	1.1	144	1095	13.2
12 to 13.9	12	1235	1.0	22	1245	1.8	8	1365	0.6	174	1357	12.8
14 to 15.9	18	1181	1.5	19	1182	1.6	8	1269	0.6	134	1269	10.6
16 to 17.9	17	864	2.0	27	874	3.1	5	955	0.5	109	956	11.4
18 to 19.9	9	536	1.7	9	536	1.7	2	591	0.3	77	597	12.9
20 to 21.9	7	438	1.6	9	440	2.0	3	475	0.6	62	478	13.0
>=22	21	628	3.3	24	631	3.8	5	706	0.7	104	699	14.9
Normal weight (n=133788)												
Weight loss	2	100	2.0	2	100	2.0	3	107	2.8	18	106	17.0
0 to 1.9	6	309	1.9	6	309	1.9	9	341	2.6	52	325	16.0
2 to 3.9	6	499	1.2	17	510	3.3	22	560	3.9	67	524	12.8
4 to 5.9	21	1505	1.4	42	1526	2.8	43	1664	2.6	219	1542	14.2
6 to 7.9	84	3882	2.2	123	3921	3.1	112	4327	2.6	621	4049	15.3
8 to 9.9	151	8369	1.8	217	8435	2.6	170	9289	1.8	1172	8823	13.3
10 to 11.9	271	14885	1.8	405	15019	2.7	233	16444	1.4	2079	15933	13.0
12 to 13.9	386	20354	1.9	519	20487	2.5	224	22312	1.0	2910	21916	13.3
14 to 15.9	456	20396	2.2	567	20507	2.8	195	22221	0.9	2963	21973	13.5
16 to 17.9	358	15522	2.3	512	15676	3.3	132	16896	0.8	2326	16731	13.9
18 to 19.9	299	10954	2.7	356	11011	3.2	88	11862	0.7	1671	11794	14.2
20 to 21.9	319	8903	3.6	326	8910	3.7	76	9644	0.8	1432	9666	14.8
22 to 23.9	209	4962	4.2	155	4908	3.2	33	5330	0.6	851	5358	15.9
24 to 25.9	187	3473	5.4	137	3423	4	31	3745	0.8	631	3760	16.8
26 to 27.9	92	1476	6.2	59	1443	4.1	18	1624	1.1	289	1607	18.0
>=28.0	220	2339	9.4	140	2259	6.2	18	2590	0.7	524	2597	20.2

[a] Values are number of cases of each outcome, total number of participants, and absolute risks of adverse maternal and infant outcomes (%, calculated as (n (outcome) / n (gestational weight gain category within BMI group))*100) within in each gestational weight gain category.

Preterm birth			Small size-for-gestational-age			Large size-for-gestational age			Any adverse outcome		
Cases	Total	%	Cases	Total	%	Cases	Total	%	Cases	Total	%
54	381	14.2	125	390	32.1	3	268	1.1	203	404	50.2
55	568	9.7	147	585	25.1	12	450	2.7	252	619	40.7
78	1070	7.3	236	1087	21.7	26	877	3	434	1175	36.9
71	1299	5.5	275	1342	20.5	25	1092	2.3	506	1437	35.2
39	1217	3.2	195	1237	15.8	33	1075	3.1	387	1326	29.2
29	930	3.1	161	909	17.7	42	790	5.3	334	993	33.6
20	577	3.5	69	552	12.5	40	523	7.6	198	623	31.8
16	481	3.3	72	467	15.4	20	415	4.8	157	499	31.5
21	708	3.0	56	654	8.6	55	653	8.4	235	733	32.1
11	98	11.2	16	99	16.2	5	88	5.7	47	109	43.1
37	343	10.8	74	328	22.6	19	273	7.0	158	356	44.4
63	537	11.7	113	534	21.2	25	446	5.6	245	577	42.5
180	1604	11.2	305	1597	19.1	66	1358	4.9	691	1721	40.2
393	4142	9.5	811	4124	19.7	143	3456	4.1	1793	4463	40.2
630	9015	7.0	1488	8901	16.7	370	7783	4.8	3412	9658	35.3
888	16017	5.5	2253	15519	14.5	823	14089	5.8	5742	17081	33.6
916	21971	4.2	2549	20940	12.2	1354	19745	6.9	7424	23217	32.0
716	21973	3.3	2123	20493	10.4	1710	20080	8.5	7314	23073	31.7
502	16737	3.0	1445	15387	9.4	1507	15449	9.8	5648	17498	32.3
341	11817	2.9	925	10543	8.8	1362	10980	12.4	4161	12271	33.9
263	9729	2.7	665	8550	7.8	1208	9093	13.3	3511	10011	35.1
125	5369	2.3	333	4569	7.3	803	5039	15.9	2046	5526	37.0
112	3819	2.9	197	3188	6.2	625	3616	17.3	1548	3882	39.9
46	1619	2.8	104	1365	7.6	253	1514	16.7	691	1666	41.5
91	2631	3.5	126	2106	6.0	516	2496	20.7	1256	2679	46.9

Supplemental Table 7. Description of outcomes by gestational weight gain category (continued)[a]

	Pre-eclampsia			Gestational hypertension			Gestational diabetes			Caesarean section		
	Cases	Total	%	Cases	Total	%	Cases	Total	%	Cases	Total	%
Overweight (n=38828)												
Weight loss	11	192	5.7	3	184	1.6	9	206	4.4	34	195	17.4
0 to 1.9	15	489	3.1	28	502	5.6	31	530	5.8	82	510	16.1
2 to 3.9	16	598	2.7	17	599	2.8	24	651	3.7	98	623	15.7
4 to 5.9	31	1120	2.8	47	1136	4.1	47	1235	3.8	218	1206	18.1
6 to 7.9	63	1961	3.2	72	1970	3.7	82	2138	3.8	334	2071	16.1
8 to 9.9	97	2983	3.3	166	3052	5.4	87	3308	2.6	592	3235	18.3
10 to 11.9	135	4252	3.2	179	4296	4.2	132	4688	2.8	826	4608	17.9
12 to 13.9	186	4861	3.8	229	4904	4.7	118	5342	2.2	893	5313	16.8
14 to 15.9	202	4768	4.2	249	4815	5.2	86	5252	1.6	928	5177	17.9
16 to 17.9	188	3662	5.1	232	3706	6.3	71	4037	1.8	735	3983	18.5
18 to 19.9	147	2713	5.4	149	2715	5.5	42	2951	1.4	605	2943	20.6
20 to 21.9	172	2596	6.6	137	2561	5.3	34	2830	1.2	574	2835	20.2
22 to 23.9	83	1406	5.9	91	1414	6.4	22	1545	1.4	323	1555	20.8
24 to 25.9	101	1159	8.7	80	1138	7.0	13	1272	1.0	282	1272	22.2
26 to 27.9	55	545	10.1	33	523	6.3	6	592	1.0	148	600	24.7
>=28.0	135	983	13.7	64	912	7.0	14	1064	1.3	272	1084	25.1
Obesity grade 1 (n=11992)												
Weight loss	6	251	2.4	12	257	4.7	14	273	5.1	60	260	23.1
0 to 1.9	29	541	5.4	26	538	4.8	37	576	6.4	113	575	19.7
2 to 3.9	28	473	5.9	23	468	4.9	29	515	5.6	100	509	19.6
4 to 5.9	31	741	4.2	52	762	6.8	46	854	5.4	167	817	20.4
6 to 7.9	62	898	6.9	77	913	8.4	50	1034	4.8	232	1013	22.9
8 to 9.9	80	1166	6.9	95	1181	8.0	51	1313	3.9	292	1302	22.4
10 to 11.9	81	1264	6.4	100	1283	7.8	53	1426	3.7	317	1419	22.3
12 to 13.9	82	1246	6.6	107	1271	8.4	34	1419	2.4	321	1405	22.8
14 to 15.9	81	1136	7.1	83	1138	7.3	38	1267	3.0	301	1264	23.8
16 to 17.9	68	774	8.8	73	779	9.4	25	876	2.9	215	870	24.7
18 to 19.9	47	484	9.7	40	477	8.4	13	536	2.4	128	542	23.6
20 to 21.9	59	507	11.6	32	480	6.7	12	550	2.2	147	556	26.4
22 to 23.9	49	301	16.3	26	278	9.4	7	334	2.1	84	335	25.1
24 to 25.9	30	238	12.6	17	225	7.6	6	255	2.4	74	258	28.7
26 to 27.9	14	116	12.1	17	119	14.3	1	138	0.7	53	138	38.4
>=28.0	34	207	16.4	27	200	13.5	4	242	1.7	81	244	33.2

[a] Values are number of cases of each outcome, total number of participants, and absolute risks of adverse maternal and infant outcomes (%, calculated as (n (outcome) / n (gestational weight gain category within BMI group))*100) within in each gestational weight gain category.

Preterm birth			Small size-for-gestational-age			Large size-for-gestational age			Any adverse outcome		
Cases	Total	%	Cases	Total	%	Cases	Total	%	Cases	Total	%
21	199	10.6	28	188	14.9	18	178	10.1	88	213	41.3
42	534	7.9	73	501	14.6	35	463	7.6	236	546	43.2
49	638	7.7	80	603	13.3	40	563	7.1	249	667	37.3
71	1205	5.9	160	1134	14.1	96	1070	9.0	505	1271	39.7
148	2108	7.0	224	1970	11.4	167	1913	8.7	850	2206	38.5
191	3267	5.8	336	3004	11.2	313	2981	10.5	1383	3416	40.5
243	4638	5.2	418	4199	10.0	490	4271	11.5	1928	4847	39.8
217	5345	4.1	429	4768	9.0	627	4966	12.6	2148	5551	38.7
205	5249	3.9	384	4587	8.4	688	4891	14.1	2154	5400	39.9
133	4032	3.3	247	3413	7.2	629	3795	16.6	1787	4136	43.2
99	2973	3.3	189	2538	7.4	442	2791	15.8	1304	3045	42.8
110	2881	3.8	171	2320	7.4	545	2694	20.2	1325	2925	45.3
38	1563	2.4	83	1245	6.7	312	1474	21.2	760	1583	48.0
42	1288	3.3	73	1025	7.1	257	1209	21.3	636	1304	48.8
16	601	2.7	25	457	5.5	143	575	24.9	315	611	51.6
39	1093	3.6	43	786	5.5	297	1040	28.6	624	1107	56.4
26	266	9.8	29	242	12.0	26	239	10.9	128	275	46.5
46	588	7.8	59	523	11.3	64	528	12.1	280	596	47.0
36	513	7.0	56	471	11.9	46	461	10.0	234	533	43.9
54	834	6.5	70	750	9.3	96	776	12.4	377	872	43.2
66	1028	6.4	97	882	11.0	160	945	16.9	549	1068	51.4
81	1317	6.2	124	1142	10.9	183	1201	15.2	663	1359	48.8
64	1450	4.4	128	1241	10.3	213	1326	16.1	720	1487	48.4
83	1435	5.8	108	1181	9.1	254	1327	19.1	741	1463	50.6
51	1274	4.0	71	1031	6.9	237	1197	19.8	644	1301	49.5
38	890	4.3	50	699	7.2	191	840	22.7	472	905	52.2
22	550	4.0	29	416	7.0	134	521	25.7	290	561	51.7
33	566	5.8	31	424	7.3	142	535	26.5	319	575	55.5
16	334	4.8	17	252	6.7	77	312	24.7	197	341	57.8
13	261	5.0	11	198	5.6	61	248	24.6	144	262	55.0
3	137	2.2	6	90	6.7	48	132	36.4	101	143	70.6
11	249	4.4	14	183	7.7	63	232	27.2	160	251	63.7

Supplemental Table 7. Description of outcomes by gestational weight gain category (continued)[a]

	Pre-eclampsia			Gestational hypertension			Gestational diabetes			Caesarean section		
	Cases	**Total**	**%**	**Cases**	**Total**	**%**	**Cases**	**Total**	**%**	**Cases**	**Total**	**%**
Obesity grade 2 (n=3284)												
Weight loss	5	139	3.6	3	137	2.2	6	150	4.0	31	145	21.4
0.0 to 1.9	17	274	6.2	29	286	10.1	31	309	10	80	314	25.5
2.0 to 3.9	14	194	7.2	12	192	6.3	19	214	8.9	48	214	22.4
4.0 to 5.9	25	241	10.4	26	242	10.7	17	280	6.1	73	284	25.7
6.0 to 7.9	25	299	8.4	31	305	10.2	22	349	6.3	90	349	25.8
8.0 to 9.9	28	314	8.9	34	320	10.6	16	368	4.3	107	362	29.6
10.0 to 11.9	28	337	8.3	32	341	9.4	16	387	4.1	100	380	26.3
12.0 to 13.9	39	285	13.7	29	275	10.5	17	323	5.3	93	324	28.7
14.0 to 15.9	22	216	10.2	24	218	11	10	247	4.0	77	247	31.2
>=16.0	84	463	18.1	64	443	14.4	29	549	5.3	183	554	33.0
Obesity grade 3 (n=969)												
Weight loss	5	73	6.8	4	72	5.6	9	85	10.6	29	84	34.5
0.0 to 1.9	14	120	11.7	7	113	6.2	9	134	6.7	34	140	24.3
2.0 to 3.9	5	52	9.6	10	57	17.5	4	64	6.3	25	63	39.7
4.0 to 5.9	9	92	9.8	6	89	6.7	8	102	7.8	32	101	31.7
6.0 to 7.9	14	79	17.7	10	75	13.3	4	92	4.3	35	93	37.6
8.0 to 9.9	8	91	8.8	8	91	8.8	6	101	5.9	32	102	31.4
10.0 to 11.9	13	86	15.1	9	82	11.0	5	98	5.1	21	97	21.6
12.0 to 13.9	13	64	20.3	5	56	8.9	5	76	6.6	23	74	31.1
14.0 to 15.9	7	49	14.3	10	52	19.2	4	59	6.8	22	61	36.1
>=16.0	24	102	23.5	13	91	14.3	7	116	6.0	51	114	44.7

[a] Values are number of cases of each outcome, total number of participants, and absolute risks of adverse maternal and infant outcomes (%, calculated as (n (outcome) / n (gestational weight gain category within BMI group))*100) within in each gestational weight gain category.

Preterm birth			Small size-for-gestational-age			Large size-for-gestational age			Any adverse outcome		
Cases	Total	%	Cases	Total	%	Cases	Total	%	Cases	Total	%
8	149	5.4	14	127	11.0	21	134	15.7	65	151	43.0
22	317	6.9	26	270	9.6	46	290	15.9	175	322	54.3
9	216	4.2	17	182	9.3	32	197	16.2	105	221	47.5
20	287	7.0	13	237	5.5	51	275	18.5	168	297	56.6
15	353	4.2	22	288	7.6	62	328	18.9	189	363	52.1
26	374	7.0	21	290	7.2	80	349	22.9	220	380	57.9
22	390	5.6	31	320	9.7	71	360	19.7	212	398	53.3
14	328	4.3	23	255	9.0	73	305	23.9	195	334	58.4
11	247	4.5	20	186	10.8	60	226	26.5	152	251	60.6
30	556	5.4	37	400	9.3	153	516	29.7	384	567	67.7
10	85	11.8	7	73	9.6	11	77	14.3	52	87	59.8
11	142	7.7	12	121	9.9	19	128	14.8	73	144	50.7
7	66	10.6	10	48	20.8	18	56	32.1	46	67	68.7
3	108	2.8	7	86	8.1	21	100	21.0	56	108	51.9
6	93	6.5	10	72	13.9	21	83	25.3	64	96	66.7
5	106	4.7	6	83	7.2	20	97	20.6	60	107	56.1
6	100	6.0	10	77	13.0	23	90	25.6	56	101	55.4
8	75	10.7	7	55	12.7	20	68	29.4	48	77	62.3
4	62	6.5	5	40	12.5	21	56	37.5	44	64	68.8
9	117	7.7	6	73	8.2	43	110	39.1	93	118	78.8

Supplemental Table 8. Odds Ratios for the associations of gestational weight gain categories with any adverse outcome per maternal clinical body mass index group [a]

Underweight		Normal weight		Overweight	
Weight gain(kg)	**OR (95% CI)**	**Weight gain (kg)**	**OR (95% CI)**	**Weight gain (kg)**	**OR (95% CI)**
		Weight loss	1.5 (1.15,1.96)	**Weight loss**	0.99 (0.75,1.30)
		0 to 1.9	1.57 (1.31,1.88)	**0 to 1.9**	1.06 (0.89,1.26)
		2 to 3.9	1.42 (1.21,1.66)	**2 to 3.9**	0.81 (0.69,0.95)
		4 to 5.9	1.28 (1.16,1.40)	**4 to 5.9**	0.90 (0.80,1.01)
<8	2.01 (1.64,2.46)	**6 to 7.9**	1.28 (1.20,1.36)	**6 to 7.9**	0.84 (0.77,0.92)
8 to 9.9	1.33 (1.12,1.57)	**8 to 9.9**	1.04 (1.00,1.09)	**8 to 9.9**	0.91 (0.85,0.98)
10 to 11.9	1.12 (0.98,1.28)	**10 to 11.9**	0.96 (0.93,0.99)	**10 to 11.9**	0.89 (0.83,0.94)
12 to 13.9	1.04 (0.93,1.18)	**12 to 13.9**	0.88 (0.86,0.91)	**12 to 13.9**	0.85 (0.80,0.90)
14 to 15.9	0.74 (0.65,0.84)	**14 to 15.9**	0.87 (0.85,0.90)	**14 to 15.9**	0.90 (0.85,0.96)
16 to 17.9	0.95 (0.82,1.09)	**16 to 17.9**	0.91 (0.88,0.95)	**16 to 17.9**	1.07 (1.00,1.14)
18 to 19.9	0.88 (0.74,1.05)	**18 to 19.9**	1.00 (0.96,1.04)	**18 to 19.9**	1.05 (0.98,1.13)
20 to 21.9	0.84 (0.69,1.02)	**20 to 21.9**	1.06 (1.01,1.10)	**20 to 21.9**	1.18 (1.10,1.28)
>=22	0.88 (0.75,1.04)	**22 to 23.9**	1.17 (1.10,1.24)	**22 to 23.9**	1.33 (1.21,1.47)
		24 to 25.9	1.31 (1.23,1.40)	**24 to 25.9**	1.36 (1.22,1.52)
		26 to 27.9	1.42 (1.29,1.56)	**26 to 27.9**	1.56 (1.33,1.82)
		>= 28	1.77 (1.64,1.91)		1.87 (1.66,2.11)

[a]Values represent odds ratios (95% Confidence Intervals) reflecting the risk of any adverse outcome per gestational weight gain category for women with underweight, normal weight, overweight, obesity grade 1, obesity grade 2 and obesity grade 3, as compared to all other gestational weight gain categories in that specific clinical maternal BMI group. Any adverse outcome includes pre-eclampsia, gestational hypertension, gestational diabetes, caesarean section, preterm birth, small size-for-gestational-age, and large size-for-gestational-age.

Obesity grade 1		Obesity grade 2		Obesity grade 3	
Weight gain (kg)	**OR (95% CI)**	**Weight gain (kg)**	**OR (95% CI)**	**Weight gain (kg)**	**OR (95% CI)**
Weight loss	0.87 (0.68,1.11)	**Weight loss**	0.55 (0.39,0.78)	**Weight loss**	0.96 (0.60,1.53)
0 to 1.9	0.86 (0.73,1.02)	**0 to 1.9**	0.89 (0.70,1.13)	**0 to 1.9**	0.59 (0.41,0.85)
2 to 3.9	0.76 (0.64,0.91)	**2 to 3.9**	0.67 (0.51,0.88)	**2 to 3.9**	1.39 (0.81,2.39)
4 to 5.9	0.73 (0.64,0.84)	**4 to 5.9**	0.98 (0.77,1.25)	**4 to 5.9**	0.62 (0.41,0.94)
6 to 7.9	1.06 (0.94,1.21)	**6 to 7.9**	0.80 (0.65,1.00)	**6 to 7.9**	1.33 (0.85,2.08)
8 to 9.9	0.92 (0.82,1.03)	**8 to 9.9**	1.04 (0.84,1.30)	**8 to 9.9**	0.79 (0.53,1.19)
10 to 11.9	0.91 (0.82,1.02)	**10 to 11.9**	0.85 (0.68,1.05)	**10 to 11.9**	0.77 (0.50,1.16)
12 to 13.9	1.02 (0.91,1.13)	**12 to 13.9**	1.06 (0.84,1.34)	**12 to 13.9**	1.08 (0.67,1.76)
14 to 15.9	0.96 (0.86,1.08)	**14 to 15.9**	1.17 (0.90,1.53)	**14 to 15.9**	1.43 (0.82,2.47)
16 to 17.9	1.09 (0.95,1.25)	**>=16**	1.81 (1.49,2.19)	**>=16**	2.68 (1.68,4.27)
18 to 19.9	1.09 (0.92,1.30)				
20 to 21.9	1.25 (1.06,1.49)				
22 to 23.9	1.41 (1.13,1.75)				
24 to 25.9	1.24 (0.97,1.58)				
26 to 27.9	2.51 (1.74,3.61)				
>= 28	1.84 (1.42,2.38)				

Supplemental Table 9. Absolute Risk Reductions for the associations of gestational weight gain categories with any adverse outcome per maternal clinical body mass index group [a]

Underweight		Normal weight		Overweight	
Weight gain(kg)	**ARR (95% CI)**	**Weight gain (kg)**	**ARR (95% CI)**	**Weight gain (kg)**	**ARR (95% CI)**
		Weight loss	-0.09 (-0.18,0)	**Weight loss**	0.01 (-0.06,0.07)
		0 to 1.9	-0.10 (-0.15,-0.05)	**0 to 1.9**	-0.01 (-0.05,0.03)
		2 to 3.9	-0.08 (-0.12,-0.04)	**2 to 3.9**	0.05 (0.01,0.08)
		4 to 5.9	-0.06 (-0.08,-0.04)	**4 to 5.9**	0.02 (0.00,0.05)
<8	-0.16 (-0.21,-0.11)	**6 to 7.9**	-0.06 (-0.08,-0.05)	**6 to 7.9**	0.04 (0.02,0.06)
8 to 9.9	-0.07 (-0.11,-0.03)	**8 to 9.9**	-0.01 (-0.02,0.00)	**8 to 9.9**	0.02 (0.00,0.03)
10 to 11.9	-0.03 (-0.06,0,00)	**10 to 11.9**	0.01 (0.00,0.01)	**10 to 11.9**	0.02 (0.01,0.04)
12 to 13.9	-0.01 (-0.03,0.02)	**12 to 13.9**	0.03 (0.02,0.03)	**12 to 13.9**	0.04 (0.02,0.05)
14 to 15.9	0.07 (0.04,0.09)	**14 to 15.9**	0.03 (0.02,0.04)	**14 to 15.9**	0.02 (0.01,0.04)
16 to 17.9	0.01 (-0.02,0.04)	**16 to 17.9**	0.02 (0.01,0.03)	**16 to 17.9**	-0.01 (-0.03,0.00)
18 to 19.9	0.03 (-0.01,0.07)	**18 to 19.9**	0.00 (-0.01,0.01)	**18 to 19.9**	-0.01 (-0.03,0.01)
20 to 21.9	0.03 (-0.01,0.08)	**20 to 21.9**	-0.01 (-0.02,0.00)	**20 to 21.9**	-0.04 (-0.05,-0.02)
>=22	0.03 (-0.01,0.06)	**22 to 23.9**	-0.03 (-0.04,-0.02)	**22 to 23.9**	-0.06 (-0.09,-0.04)
		24 to 25.9	-0.06 (-0.07,-0.04)	**24 to 25.9**	-0.07 (-0.10,-0.04)
		26 to 27.9	-0.07 (-0.10,-0.05)	**26 to 27.9**	-0.10 (-0.14,-0.06)
		>= 28	-0.13 (-0.15,-0.11)	**>= 28**	-0.15 (-0.18,-0.12)

[a] Values represent absolute risk reductions (95% Confidence Intervals) reflecting the reduction of absolute risk of any adverse outcome per gestational weight gain category for women with underweight, normal weight, overweight, obesity grade 1, obesity grade 2 and obesity grade 3, as compared to all other gestational weight gain categories in that specific clinical maternal BMI group. Any adverse outcome includes pre-eclampsia, gestational hypertension, gestational diabetes, caesarean section, preterm birth, small size-for-gestational-age, and large size-for-gestational-age.

Obesity grade 1		Obesity grade 2		Obesity grade 3	
Weight gain (kg)	**ARR (95% CI)**	**Weight gain (kg)**	**ARR (95% CI)**	**Weight gain (kg)**	**ARR (95% CI)**
Weight loss	0.04 (-0.02,0.10)	**Weight loss**	0.14 (0.06,0.22)	**Weight loss**	0.01 (-0.09,0.12)
0 to 1.9	0.03 (-0.01,0.07)	**0 to 1.9**	0.03 (-0.03,0.08)	**0 to 1.9**	0.12 (0.03,0.21)
2 to 3.9	0.07 (0.02,0.11)	**2 to 3.9**	0.10 (0.03,0.17)	**2 to 3.9**	-0.08 (-0.20,0.03)
4 to 5.9	0.08 (0.04,0.11)	**4 to 5.9**	0.00 (-0.06,0.06)	**4 to 5.9**	0.10 (0.00,0.20)
6 to 7.9	-0.01 (-0.04,0.02)	**6 to 7.9**	0.05 (0.0,0.11)	**6 to 7.9**	-0.06 (-0.16,0.04)
8 to 9.9	0.02 (-0.01,0.04)	**8 to 9.9**	-0.01 (-0.07,0.04)	**8 to 9.9**	0.06 (-0.04,0.16)
10 to 11.9	0.02 (-0.01,0.05)	**10 to 11.9**	0.04 (-0.01,0.09)	**10 to 11.9**	0.06 (-0.04,0.17)
12 to 13.9	-0.01 (-0.03,0.02)	**12 to 13.9**	-0.02 (-0.07,0.04)	**12 to 13.9**	-0.01 (-0.13,0.10)
14 to 15.9	0.01 (-0.02,0.04)	**14 to 15.9**	-0.04 (-0.10,0.02)	**14 to 15.9**	-0.08 (-0.20,0.04)
16 to 17.9	-0.02 (-0.06,0.01)	**>=16**	-0.13 (-0.17,-0.09)	**>=16**	-0.20 (-0.28,-0.12)
18 to 19.9	-0.02 (-0.06,0.03)				
20 to 21.9	-0.06 (-0.10,-0.01)				
22 to 23.9	-0.08 (-0.13,-0.02)				
24 to 25.9	-0.05 (-0.11,0.01)				
26 to 27.9	-0.21 (-0.28,-0.13)				
>= 28	-0.14 (-0.20,-0.08)				

Supplemental Table 10. Classification of gestational weight gain by classifications from this study and IOM

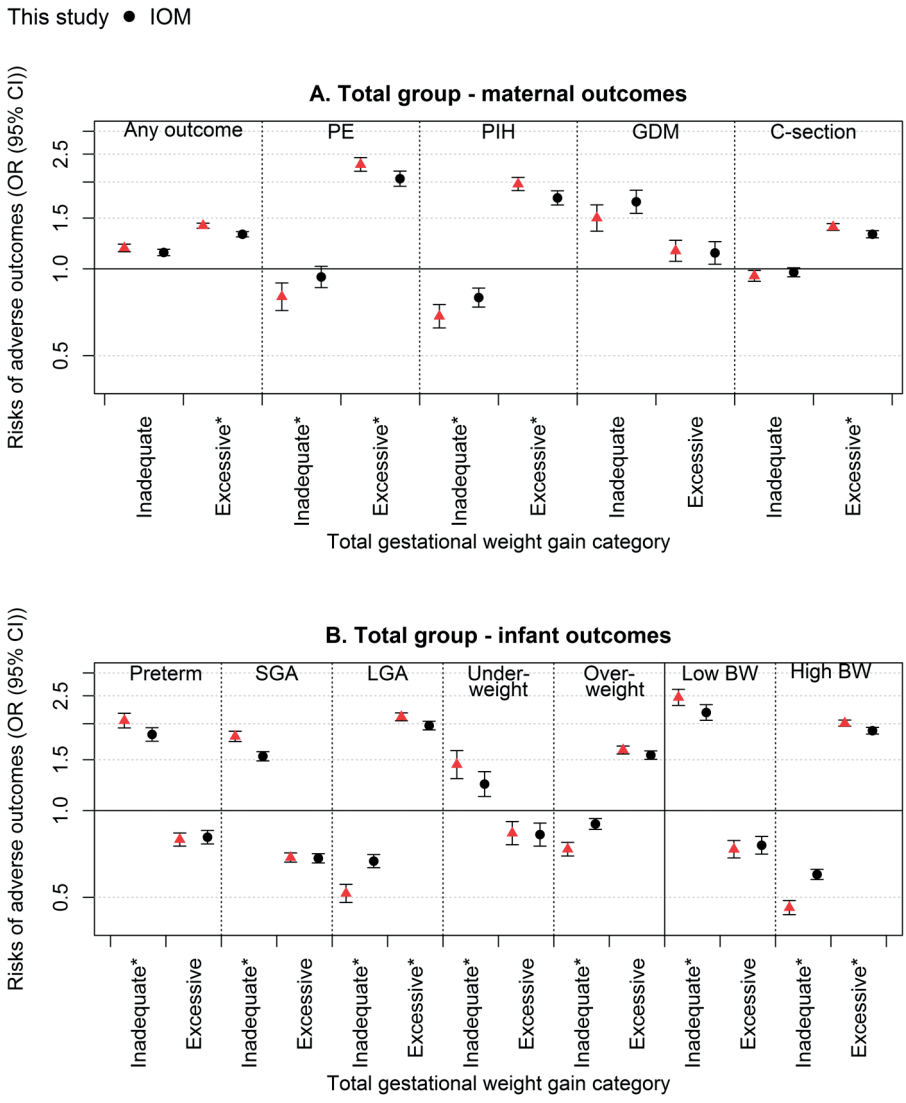
	This study		IOM	
	Range (kg)	**n (%)**	**Range (kg)**	**n (%)**
Underweight (n=7809)				
Inadequate	<14.0	3635 (46.5)	<12.5	2894 (37.1)
Adequate	14.0 – <16.0	1326 (17.0)	12.5 – 18.0	3416 (43.7)
Excessive	≥16.0	2848 (36.5)	>18.0	1499 (19.2)
Normal weight (n=133788)				
Inadequate	<10.0	16884 (12.6)	<11.5	32755 (24.5)
Adequate	10.0 – <18.0	80869 (60.5)	11.5 – 16.0	55188 (41.2)
Excessive	≥18.0	36035 (26.9)	>16.0	45845 (34.3)
Overweight (n=38828)				
Inadequate	<2.0	759 (2.0)	<7.0	3691 (9.5)
Adequate	2.0 – <16.0	233580 (60.1)	7.0 – 11.5	9309 (24.0)
Excessive	≥16.0	14711 (37.9)	>11.5	25828 (66.5)
Obesity grade 1 (n=11992)				
Inadequate	<2.0	871 (7.3)	<5.0	1798 (15.0)
Adequate	2.0 – <6.0	1405 (11.7)	5.0 – 9.0	2779 (23.2)
Excessive	≥6.0	9716 (81.0)	>9.0	7415 (61.8)
Obesity grade 2 (n=3284)				
Inadequate	NA	NA	<5.0	835 (25.4)
Adequate	Weight loss – <4.0[a]	694 (21.1)	5.0 – 9.0	865 (26.3)
Excessive	≥4.0	2590 (78.9)	>9.0	1584 (48.2)
Obesity grade 3 (n=969)				
Inadequate	Weight loss[b]	87 (9.0)	<5.0	350 (36.1)
Adequate	0 – <6.0	319 (32.9)	5.0 – 9.0	246 (25.4)
Excessive	≥6.0	563 (58.1)	>9.0	373 (38.5)
Total group (n=196670)				
Inadequate		22236 (11.3)		42323 (21.5)
Adequate		107971 (54.9)		71803 (36.5)
Excessive		66463 (33.8)		82544 (42.0)

Abbreviations: NA, not available; IOM, US Institute of Medicine (nowadays called National Academy of Medicine (NAM)).

[a] Median gestational weight gain in the weight loss category: -3.00 kg (range: -25.00 to -0.13) for obesity grade 2.

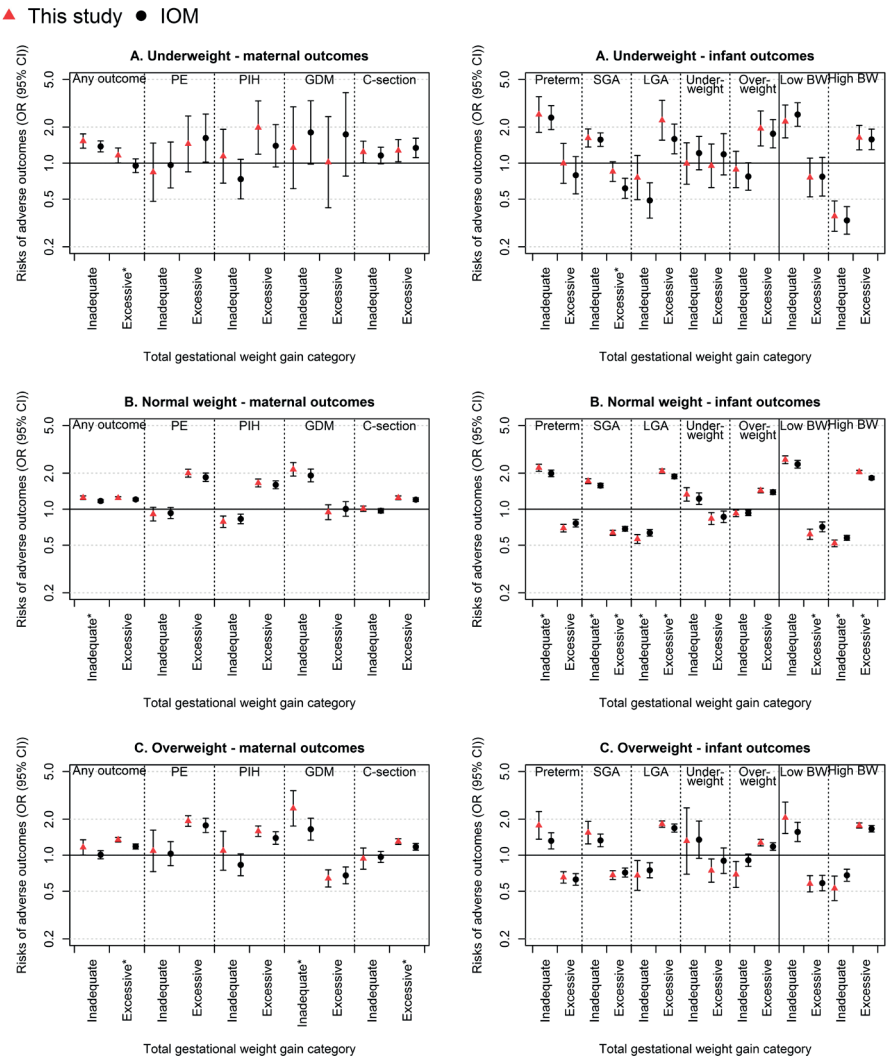
[b] Median gestational weight gain in the weight loss category: -3.00 kg (range: -13.58 to -0.40) for obesity grade 3.

Supplemental Figure 2. Associations of inadequate and excessive gestational weight gain by classifications from this study and IOM with adverse maternal and infant outcomes



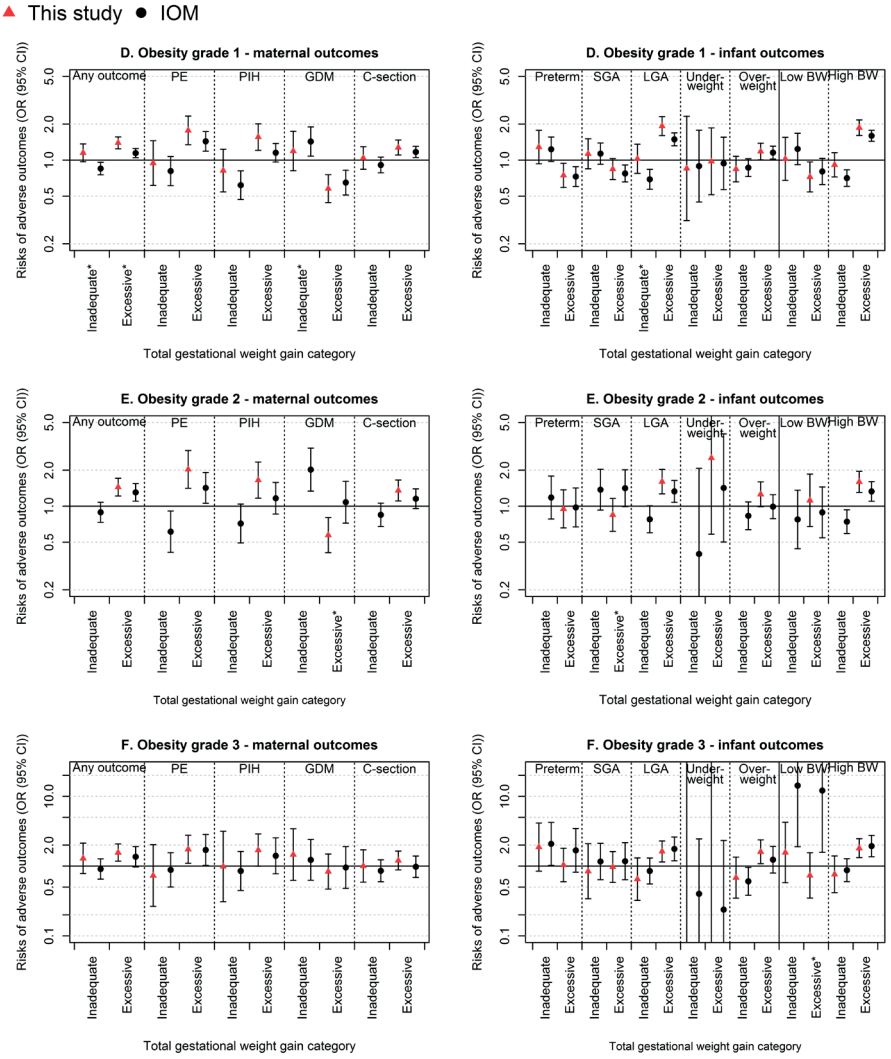
Abbreviations: IOM: US Institute of Medicine (nowadays called National Academy of Medicine (NAM)). Values represent odds ratios (OR) (95% Confidence Intervals (CI)) reflecting the risk of adverse maternal and infant outcomes in women with inadequate and excessive weight gain as compared to women with adequate weight gain, according to this study (red triangles) and IOM classifications (black dots). Inadequate and excessive gestational weight gain are defined as gestational weight gain below or above the ranges defined by this study and IOM, respectively (**Supplemental Table 10**). Any adverse outcome includes pre-eclampsia (PE), gestational hypertension (PIH), gestational diabetes (GDM), caesarean section, preterm birth, small size-for-gestational-age (SGA), and large size-for-gestational-age (LGA). Low and high birth and childhood under- and overweight were not included for the definition of the optimal weight gain ranges. * P-value for the difference between ORs for this study and IOM (calculated using Z= (logodds $_{\text{This study}}$ – logodds $_{\text{IOM}}$) /sqrt($SE_{\text{This study}}^2$+SE_{IOM}^2)).

Supplemental Figure 3. Associations of inadequate and excessive gestational weight gain by classifications from this study and IOM with adverse maternal and infant outcomes. by clinical body mass index group



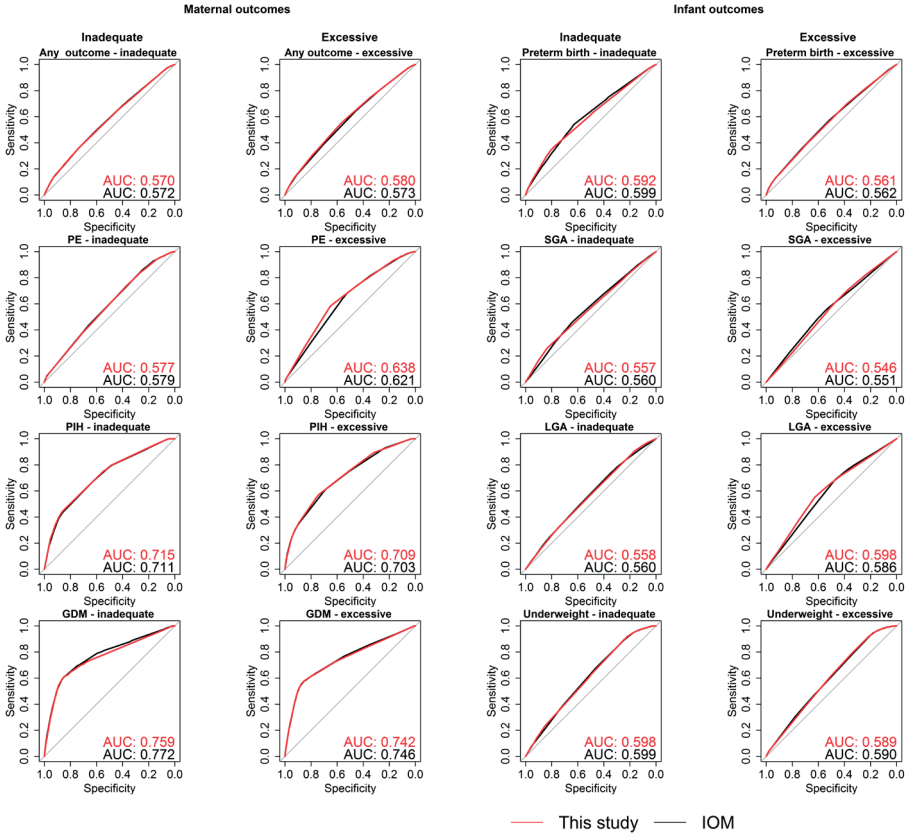
Values represent odds ratios (95% Confidence Intervals) reflecting the risk of adverse maternal or infant outcome in women with inadequate and excessive weight gain as compared to women with adequate weight gain, according to this study, red triangles) and IOM (US Institute of Medicine, nowadays called National Academy of Medicine (NAM)) classifications (black dots), per clinical BMI category. Inadequate and excessive gestational weight gain are defined as gestational weight gain below or above the ranges defined by this study and IOM, respectively (**Supplemental Table 10**). Any adverse outcome includes pre-eclampsia (PE), gestational hypertension (PIH), gestational diabetes (GDM), caesarean section, preterm birth, and small size-for-gestational-age (SGA), large size-for-gestational-age (LGA). Low and high birth weight and childhood under- and overweight were not included for the definition of the optimal weight gain ranges. * P-value for the difference between ORs for this study and IOM (calculated using Z= (logodds $_{\text{This study}}$ – logodds $_{\text{IOM}}$) /sqrt($SE_{\text{This study}}^{2}$+$SE_{\text{IOM}}^{2}$)).

Supplemental Figure 3. Associations of inadequate and excessive gestational weight gain by classifications from this study and IOM with adverse maternal and infant outcomes. by clinical body mass index group (continued)



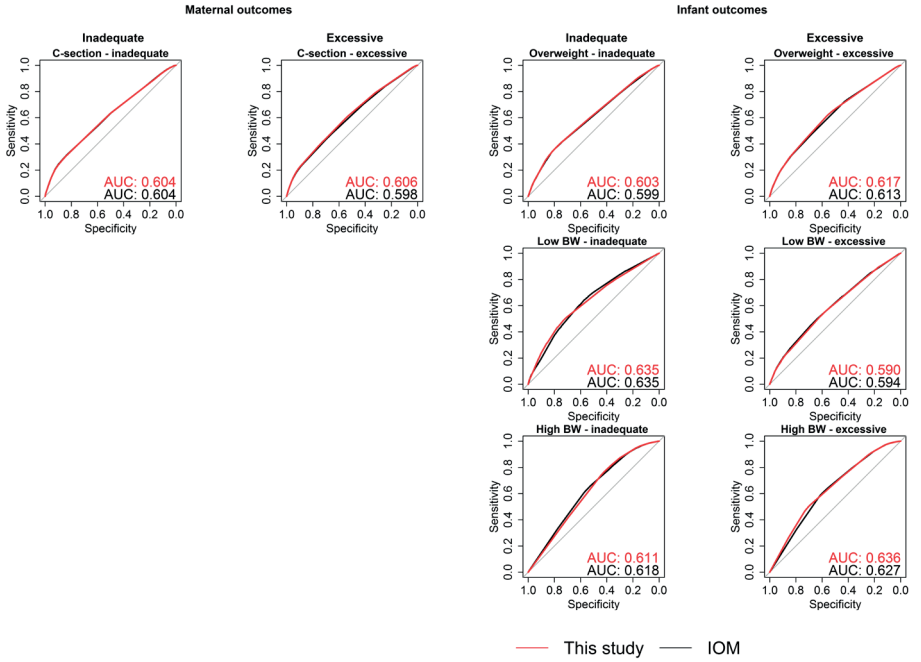
Values represent odds ratios (95% Confidence Intervals) reflecting the risk of adverse maternal or infant outcome in women with inadequate and excessive weight gain as compared to women with adequate weight gain, according to this study, red triangles) and IOM (US Institute of Medicine, nowadays called National Academy of Medicine (NAM)) classifications (black dots), per clinical BMI category. Inadequate and excessive gestational weight gain are defined as gestational weight gain below or above the ranges defined by this study and IOM, respectively (**Supplemental Table 10**). Any adverse outcome includes pre-eclampsia (PE), gestational hypertension (PIH), gestational diabetes (GDM), caesarean section, preterm birth, and small size-for-gestational-age (SGA), large size-for-gestational-age (LGA). Low and high birth weight and childhood under- and overweight were not included for the definition of the optimal weight gain ranges. * P-value for the difference between ORs for this study and IOM (calculated using Z= (logodds $_{\text{This study}}$ – logodds $_{\text{IOM}}$) /sqrt($SE_{\text{This study}}^{2}$+$SE_{\text{IOM}}^{2}$)).

Supplemental Figure 4. Receiver operator characteristic curves of inadequate and excessive gestational weight gain by classifications from this study and IOM for the detection of adverse maternal and infant outcomes



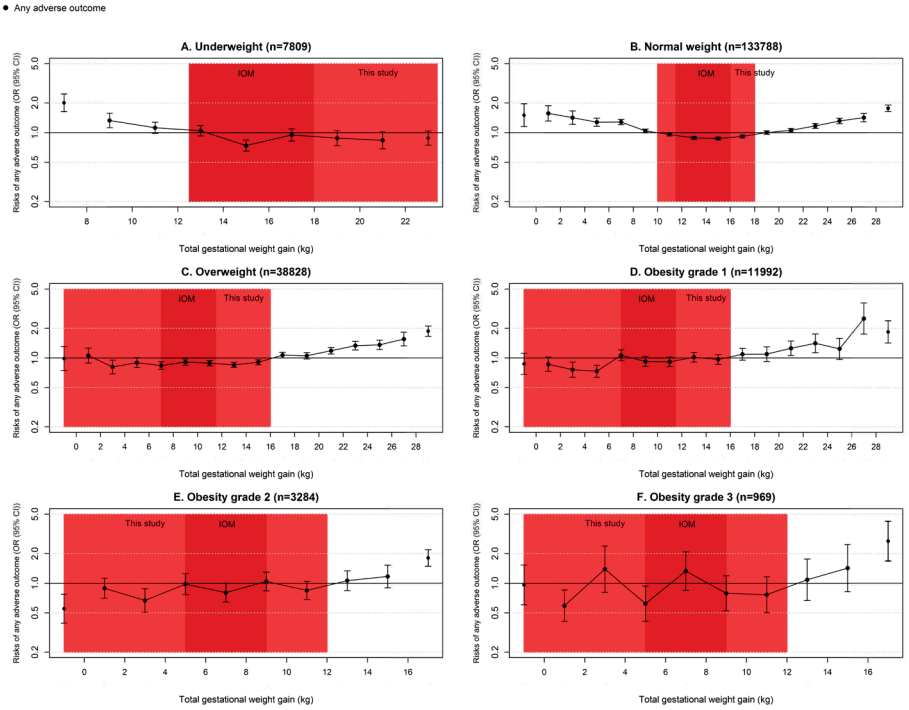
Abbreviations: AUC, area under the Receiver Operator Characteristic curve; IOM, US Institute of Medicine (nowadays called National Academy of Medicine (NAM)). Inadequate and excessive gestational weight gain are defined as gestational weight gain below or above the ranges defined by this study and IOM, respectively (**Supplemental Table 10**). Any adverse outcome includes pre-eclampsia (PE), gestational hypertension (PIH), gestational diabetes (GDM), caesarean section, preterm birth, small size-for-gestational-age (SGA), and large size-for-gestational-age (LGA). Low and high birth weight and childhood under- and overweight were not included for the definition of the optimal weight gain ranges.

Supplemental Figure 4. Receiver operator characteristic curves of inadequate and excessive gestational weight gain by classifications from this study and IOM for the detection of adverse maternal and infant outcomes (continued)



Abbreviations: AUC, area under the Receiver Operator Characteristic curve; IOM, US Institute of Medicine (nowadays called National Academy of Medicine (NAM)). Inadequate and excessive gestational weight gain are defined as gestational weight gain below or above the ranges defined by this study and IOM, respectively (**Supplemental Table 10**). Any adverse outcome includes pre-eclampsia (PE), gestational hypertension (PIH), gestational diabetes (GDM), caesarean section, preterm birth, small size-for-gestational-age (SGA), and large size-for-gestational-age (LGA). Low and high birth weight and childhood under- and overweight were not included for the definition of the optimal weight gain ranges.

Supplemental Figure 5. Associations of gestational weight gain categories with any adverse outcome per maternal clinical body mass index group, ranges from this study based on protective associations only



The symbols represent odds ratios (95% Confidence Intervals) reflecting the risk of any adverse outcome per gestational weight gain category for women with (A) underweight, (B) normal weight, (C) overweight, (D) obesity grade 1, (E) obesity grade 2 and (F) obesity grade 3, as compared to all other gestational weight gain categories in that specific clinical maternal body mass index group. The symbols represent the mean for all participants in each gestational weight gain category. The percentages below each of the figures represent the number of participants in that gestational weight gain category as a percentage of all participants within that BMI category. Participants in the extreme categories of gestational weight gain had values beyond the most extreme labeled tick marks. Any adverse outcome includes pre-eclampsia, gestational hypertension, gestational diabetes, caesarean section, preterm birth, small size-for-gestational-age, and large size-for-gestational-age. This figure is equal to **Figure 3**, but shows ranges from this study when optimal weight gain would be defined as all associations below 1, regardless of statistical significance. The red area represents the optimal weight gain range according to this study, the grey area represents the weight gain ranges as recommended by the US Institute of Medicine (IOM, nowadays called National Academy of Medicine (NAM)).

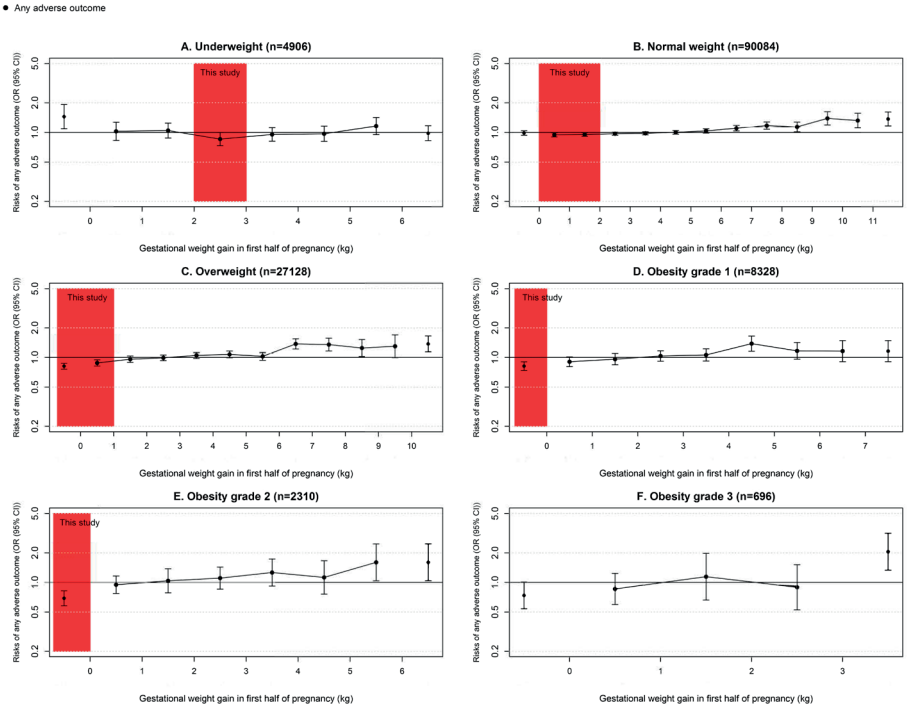
Supplemental Table 11. Optimal total gestational weight gain ranges, sensitivity analyses[a]

Model	Underweight	Normal weight	Overweight	Obesity grade 1	Obesity grade 2	Obesity grade 3
Main model (any adverse outcome)[b]	**14.0-<16.0**	**10.0 - <18.0**	**2.0 - <16.0**	**2.0 - <6.0**	**Weight loss- <4.0**	**0.0 - <6.0**
Account for length of gestation						
Main model additionally adjusted for gestational age at birth	14.0 - <16.0	8.0 - <16.0	2.0 - <16.0	Weight loss – <6.0	Weight loss - <4.0	0.0 - <2.0
Study sample with term births only	14.0 - <16.0	10.0 - <18.0	2.0 - <16.0	0.0 – <6.0	Weight loss -<4.0	0.0 - <2.0
Account for maternal age and parity						
Main model additionally adjusted for maternal age and parity	14.0 - <16.0	10.0 - <18.0	2.0 - <16.0	2.0 - <6.0	Weight loss - <4.0	0.0 - <6.0
Definition of 'any adverse outcome'						
Gestational diabetes excluded from definition	14.0 - <16.0	10.0 - <18.0	2.0 - <16.0	Weight loss -<6.0	Weight loss – <8.0	0.0 - <6.0
Pre-eclampsia excluded from definition	≥14.0	12.0 - <18.0	2.0 - <16.0	2.0 - <6.0	Weight loss - <4.0	0.0 - <6.0
Gestational diabetes and pre-eclampsia excluded from definition	≥14.0	12.0 - <18.0	2.0 - <16.0	0.0 – <6.0	Weight loss – <4.0	0.0 - <6.0
Preterm birth excluded from definition	14.0 - <16.0	10.0 - <18.0	2.0 - <16.0	0.0 – 6.0	Weight loss – <4.0	0.0 - <2.0
Gestational diabetes, pre-eclampsia and preterm birth excluded from definition	14.0 - <16.0	10.0 - <18.0	2.0 - <16.0	Weight loss – <6.0	Weight loss – <4.0	0.0 - <2.0
Caesarean section excluded from definition	≥14.0	12.0 - <18.0	6.0 - <16.0	2.0 - <6.0	Weight loss – <4.0	0.0 - <6.0
Childhood underweight and overweight included in the definition	14.0 - <16.0	10.0 - <18.0	2.0 - <16.0	0.0 - <6.0	Weight loss – <4.0	0.0 - <6.0
Selection study population						
Complete cases only	14.0 - <16.0	10.0 - <18.0	2.0 - <16.0	0.0 - <6.0	Weight loss – <4.0	0.0 - <2.0

[a] Values represent optimal gestational weight gain ranges (kg) based on each of the sensitivity analyses.

[b] Main model includes pre-eclampsia, gestational hypertension, gestational diabetes, caesarean section, preterm birth, small size-for-gestational-age, and large size-for-gestational-age.

Supplemental Figure 6. Associations of categories of gestational weight gain in first half of pregnancy with any adverse outcome per maternal clinical body mass index group



The symbols represent odds ratios (95% Confidence Intervals) reflecting the risk of any adverse outcome per category of gestational weight gain in first half of pregnancy for women with (A) underweight, (B) normal weight, (C) overweight, (D) obesity grade 1, (E) obesity grade 2 and (F) obesity grade 3, as compared to all other gestational weight gain categories in that specific clinical maternal body mass index group. The percentages below each of the figures represent the number of participants in that gestational weight gain category as a percentage of all participants within that BMI category. The symbols represent the mean for all participants in each gestational weight gain category. Participants in the extreme categories of gestational weight gain had values beyond the most extreme labeled tick marks. Because of the relatively low levels of gestational weight gain in first half of pregnancy, we categorized weight gain within this time interval into categories of 1 kg each. Any adverse outcome includes pre-eclampsia, gestational hypertension, gestational diabetes, caesarean section, preterm birth, small size-for-gestational-age, and large size-for-gestational-age. The red area represents the optimal weight gain range according to the current analysis. No optimal weight gain range was observed for obesity grade 3 due to low numbers.

Supplemental Table 12. Classification of gestational weight gain in first half of pregnancy by classification from this study

	This study	
	Range (kg)	**n (%)**
Underweight (n=4906)		
Inadequate	<2.0	1315 (26.8)
Adequate	2.0 – <3.0	941 (19.2)
Excessive	≥3.0	2650 (54.0)
Normal weight (n=90084)		
Inadequate	Weight loss[a]	7272 (8.1)
Adequate	0.0 – <2.0	21481(23.8)
Excessive	≥2.0	61331 (68.1)
Overweight (n=27128)		
Inadequate	NA	NA
Adequate	<1.0[a]	7662 (28.2)
Excessive	≥1.0	19466 (71.8)
Obesity grade 1 (n=8328)		
Inadequate	NA	NA
Adequate	Weight loss[a]	2053 (24.7)
Excessive	≥0.0	6275 (75.3)
Obesity grade 2 (n=2310)		
Inadequate	NA	NA
Adequate	Weight loss[a]	784 (33.9)
Excessive	≥0.0	1526 (66.1)
Obesity grade 3	NA	NA
Total group (n=132756)		
Inadequate		8587 (6.5)
Adequate		32921 (24.8)
Excessive		91248 (68.7)

Abbreviations: NA, not available.

[a] Results need to be interpreted with caution, as the effect and safety of weight loss during pregnancy are not known.

Supplemental Table 13. Classification table for ranges of this study for weight gain in first half of pregnancy vs. total gestational weight gain [a, b]

	Total pregnancy			
First half	**Inadequate**	**Adequate**	**Excessive**	**Total**
Inadequate	3634 (25.1)	4462 (5.8)	791 (1.7)	8587
Adequate	5309 (36.6)	21296 (29.5)	6316 (13.7)	32921
Excessive	5545 (38.3)	46683 (64.7)	39020 (84.6)	91248
Total	14488	72141	46127	132756

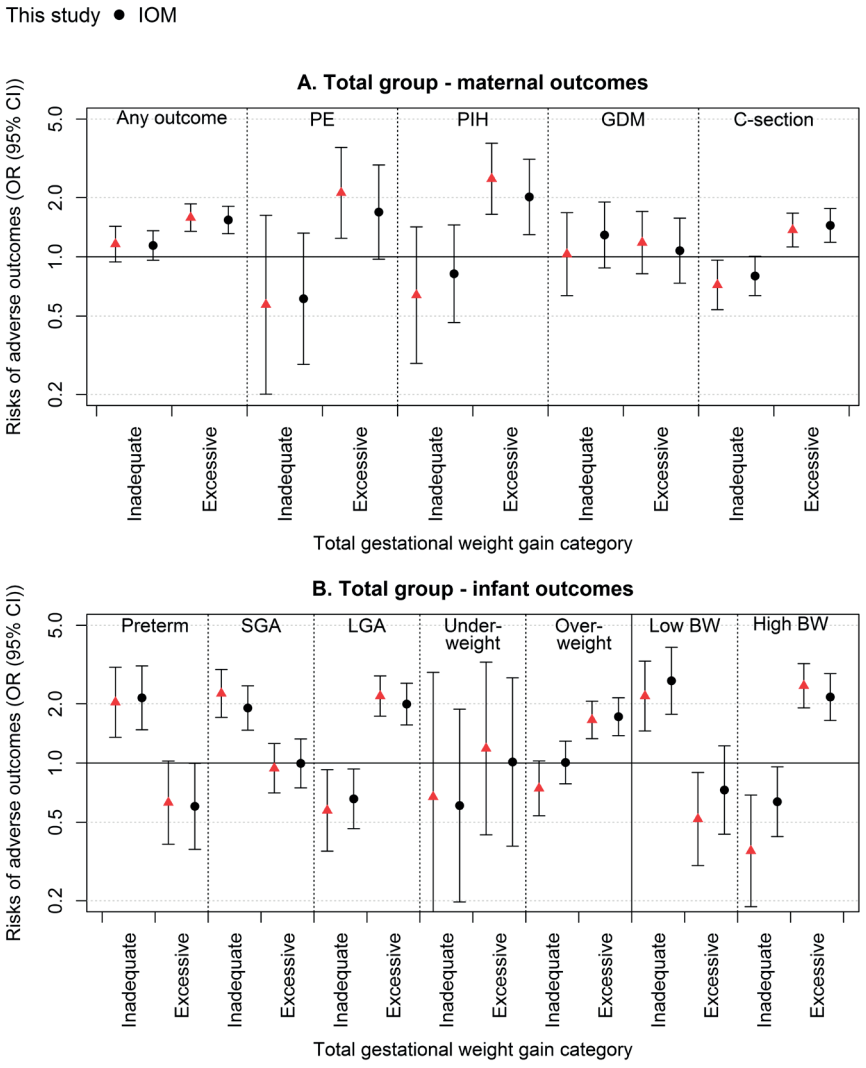
[a] Values are n (% within groups of total gestational weight gain (columns)).
[b] As no inadequate weight gain categories were observed in first half of pregnancy for most BMI categories, classification for inadequate gestational weight gain needs to be interpreted carefully.

Supplemental Table 14. Classification of gestational weight gain by classifications from this study and IOM, hospital-based population (used as validation sample)

	This study		IOM	
	Range (kg)	**n (%)**	**Range (kg)**	**n (%)**
Underweight (n=277)				
Inadequate	<14.0	134 (48.4)	<12.5	99 (35.7)
Adequate	14.0 – <16.0	58 (20.9)	12.5 – 18.0	136 (49.1)
Excessive	≥16.0	85 (30.7)	>18.0	42 (15.2)
Normal weight (n=2400)				
Inadequate	<10.0	303 (12.6)	<11.5	664 (27.7)
Adequate	10.0 – <18.0	1656 (69.0)	11.5 – 16.0	1142 (47.6)
Excessive	≥18.0	441 (18.4)	>18.0	594 (24.8)
Overweight (n=577)				
Inadequate	<2.0	7 (1.2)	<7.0	57 (9.9)
Adequate	2.0 – <16.0	404 (70.0)	7.0 – 11.5	171 (26.6)
Excessive	≥16.0	166 (28.8)	>11.5	349 (60.5)
Obesity grade 1 (n=188)				
Inadequate	<2.0	6 (3.2)	<5.0	23 (12.2)
Adequate	2.0 – <6.0	25 (13.3)	5.0 – 9.0	65 (34.6)
Excessive	≥6.0	157 (83.5)	>9.0	100 (53.2)
Obesity grade 2 (n=53)				
Inadequate	NA	NA	<5.0	15 (28.3)
Adequate	Weight loss – <4.0	13 (24.5)	5.0 – 9.0	17 (32.1)
Excessive	≥4.0	40 (75.5)	>9.0	21 (39.6)
Obesity grade 3 (n=10)				
Inadequate	Weight loss	1 (10.0)	<5.0	6 (60.0)
Adequate	0 – <6.0	6 (60.0)	5.0 – 9.0	2 (20.0)
Excessive	≥6.0	3 (30.0)	>9.0	2 (20.0)
Total group (n=3505)				
Inadequate		451 (12.9)		864 (24.7)
Adequate		2162 (61.7)		1533 (43.7)
Excessive		892 (25.4)		738 (31.6)

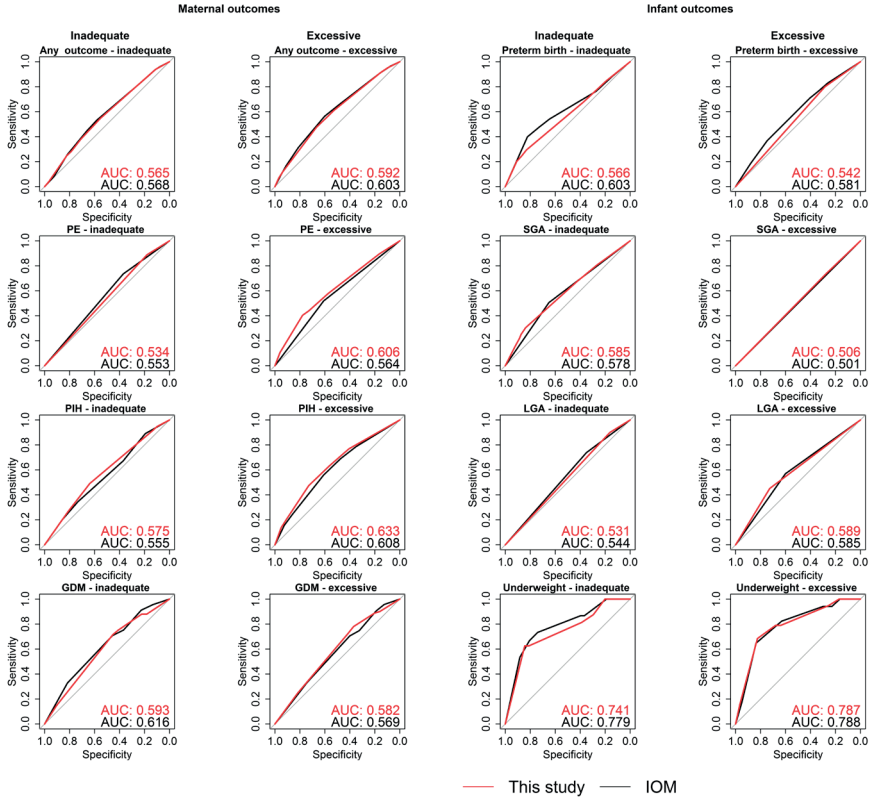
Abbreviations: NA, not available; US Institute of Medicine (nowadays called National Academy of Medicine (NAM)).

Supplemental Figure 7. Associations of inadequate and excessive gestational weight gain by classifications from this study and IOM with adverse maternal and infant outcomes, hospital-based population (used as validation sample)



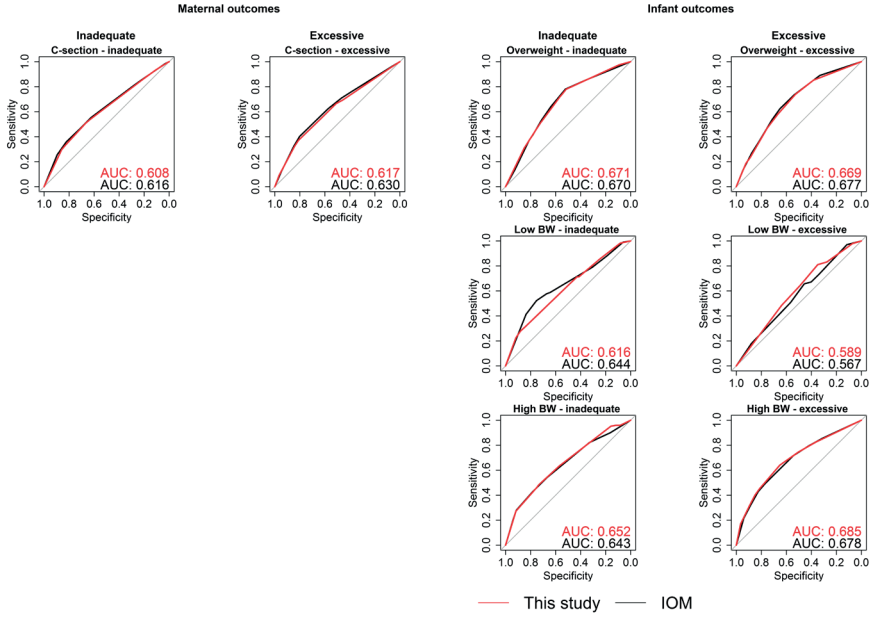
Abbreviations: IOM, US Institute of Medicine (nowadays called National Academy of Medicine (NAM)). Values represent odds ratios (OR) (95% Confidence Intervals (CI)) reflecting the risk of adverse maternal and infant outcomes in women with inadequate and excessive weight gain as compared to women with adequate weight gain, according to this study (red triangles) and IOM classifications (black dots). Inadequate and excessive gestational weight gain are defined as gestational weight gain below or above the ranges from this study and IOM, respectively (**Supplemental Table 14**). Any adverse outcome includes pre-eclampsia (PE), gestational hypertension (PIH), gestational diabetes (GDM), caesarean section, preterm birth, small size-for-gestational-age (SGA), and large size-for-gestational-age (LGA). Low and high birth and childhood under- and overweight were not included for the definition of the optimal weight gain ranges. * P-value for the difference between ORs for this study and IOM (calculated using Z= (logodds $_{\text{This study}}$ – logodds $_{\text{IOM}}$) / sqrt($SE_{\text{This study}}^{2}$+$SE_{\text{IOM}}^{2}$)).

Supplemental Figure 8. Receiver operator characteristic curves of inadequate and excessive gestational weight gain by classifications from this study and IOM for the detection of adverse maternal and infant outcomes, hospital-based population (used as validation sample)

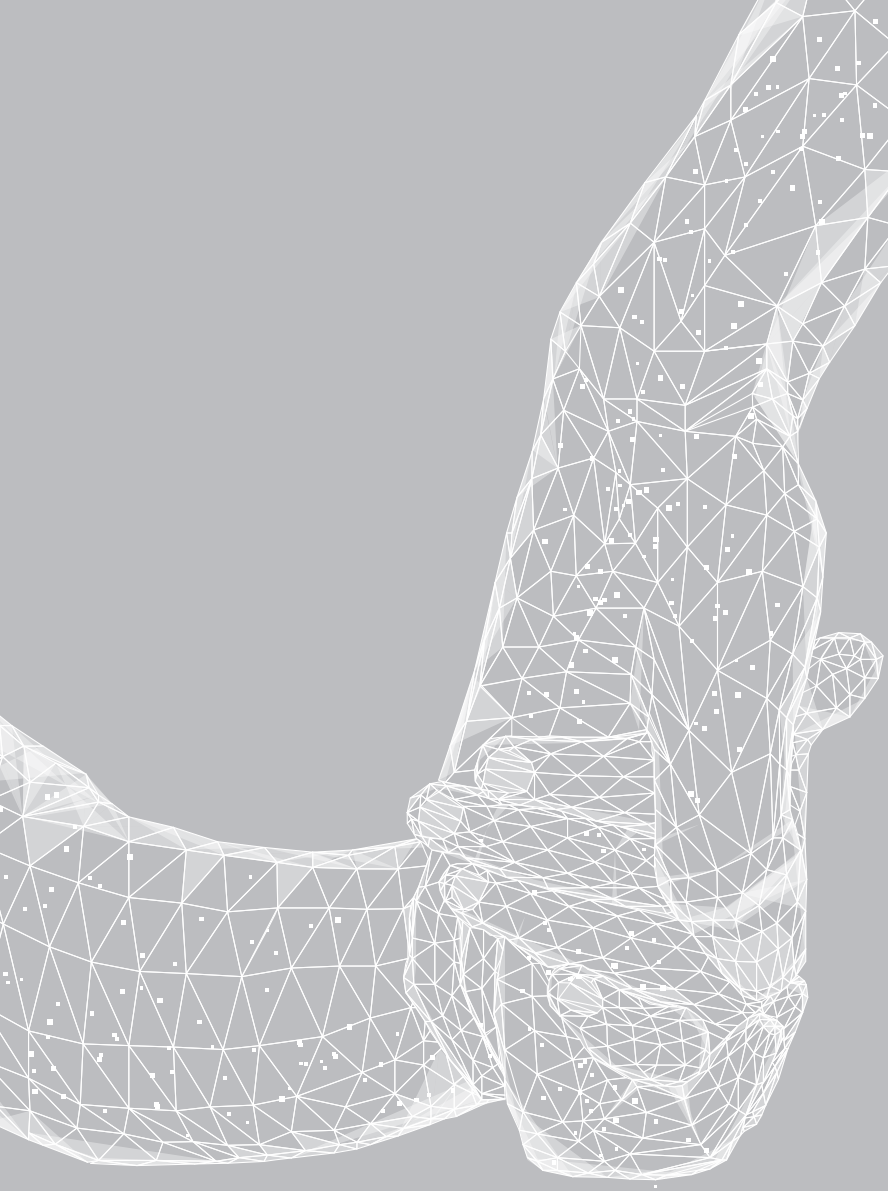


Abbreviations: AUC, area under the Receiver Operator Characteristic curve; IOM, US Institute of Medicine (nowadays called National Academy of Medicine (NAM)). Inadequate and excessive gestational weight gain are defined as gestational weight gain below or above the ranges from this study and IOM, respectively (**Supplemental Table 14**). Any adverse outcome includes pre-eclampsia (PE), gestational hypertension (PIH), gestational diabetes (GDM), caesarean section, preterm birth, small size-for-gestational-age (SGA), and large size-for-gestational-age (LGA). Low and high birth weight and childhood under- and overweight were not included for the definition of the optimal weight gain ranges.

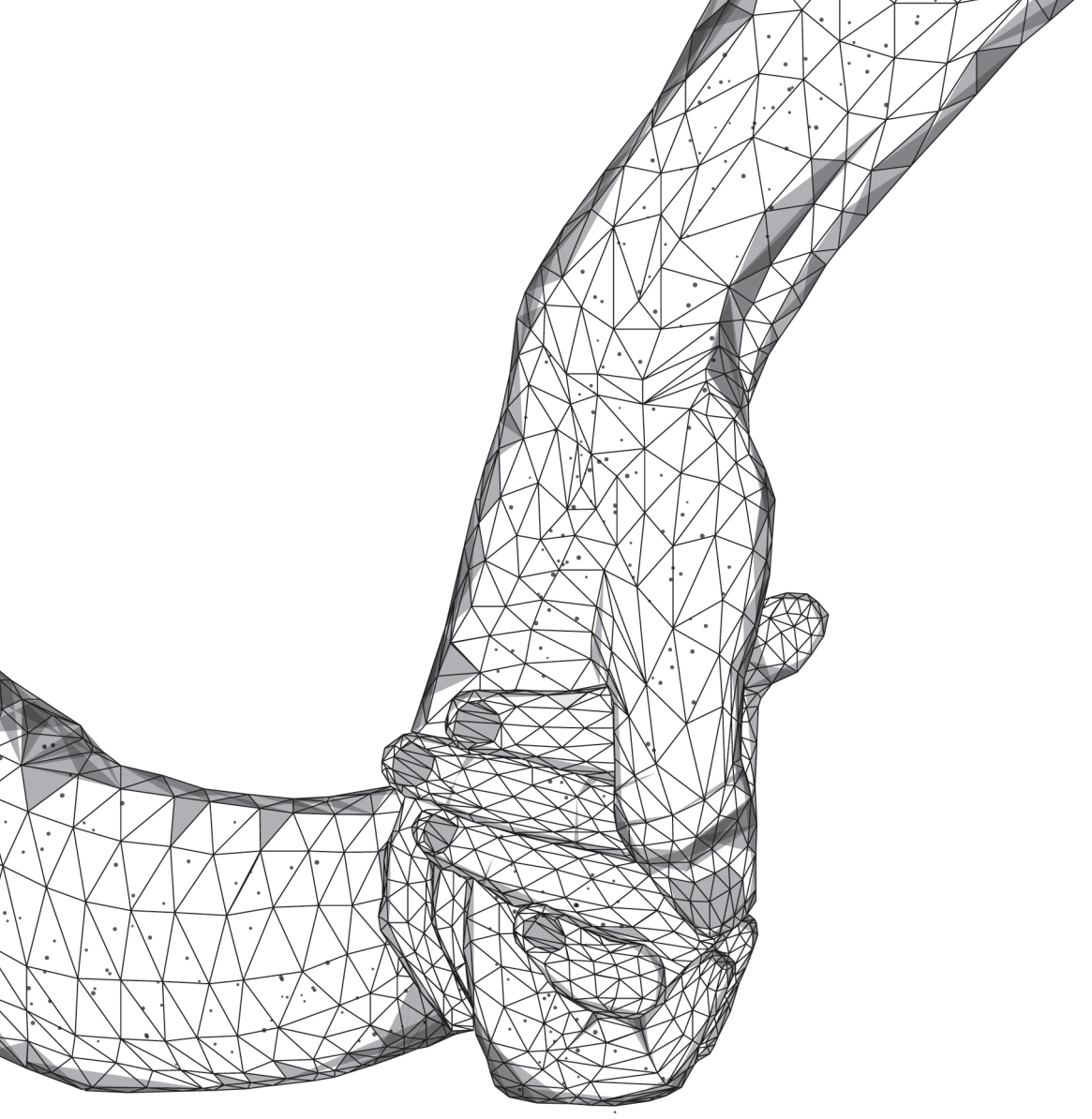
Supplemental Figure 8. Receiver operator characteristic curves of inadequate and excessive gestational weight gain by classifications from this study and IOM for the detection of adverse maternal and infant outcomes, hospital-based population (used as validation sample) (continued)



Abbreviations: AUC, area under the Receiver Operator Characteristic curve; IOM, US Institute of Medicine (nowadays called National Academy of Medicine (NAM)). Inadequate and excessive gestational weight gain are defined as gestational weight gain below or above the ranges from this study and IOM, respectively (**Supplemental Table 14**). Any adverse outcome includes pre-eclampsia (PE), gestational hypertension (PIH), gestational diabetes (GDM), caesarean section, preterm birth, small size-for-gestational-age (SGA), and large size-for-gestational-age (LGA). Low and high birth weight and childhood under- and overweight were not included for the definition of the optimal weight gain ranges.



4 Maternal and childhood metabolism



4.1 Maternal glucose concentrations and childhood cardio-metabolic outcomes

Wahab RJ, **Voerman E**, Jansen PW, Oei EHG, Steegers EAP, Jaddoe VWV, Gaillard R. Maternal glucose concentrations in early pregnancy and cardiometabolic risk factors in childhood.

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ABSTRACT

Objective: This study aimed to examine the associations of maternal early-pregnancy glucose and insulin concentrations with offspring cardiometabolic risk factors and fat distribution.
Methods: In a population-based prospective cohort study among 3,737 mothers and their children, random maternal glucose and insulin concentrations were measured at a median gestational age of 13.2 (95% range 10.5-17.1) weeks. Childhood fat, blood pressure, and blood concentrations of lipids, glucose, and insulin at the age of 10 years were measured.
Results: Higher maternal early-pregnancy glucose and insulin concentrations were associated with a higher risk of childhood overweight, and higher maternal early-pregnancy insulin concentrations were associated with an increased childhood risk of clustering of cardiometabolic risk factors (all $P < 0.05$). These associations were explained by maternal prepregnancy BMI. Independent of maternal prepregnancy BMI, one SD score (SDS) higher maternal early-pregnancy glucose and insulin concentrations were associated with higher childhood glucose (0.08 SDS, 95% CI: 0.04-0.11) and insulin concentrations (0.07 SDS, 95% CI: 0.03-0.10), but not with childhood blood pressure, lipids, and fat measures.
Conclusions: These results suggest that maternal early-pregnancy random glucose and insulin concentrations are associated with childhood glucose and insulin concentrations but not with other childhood cardiometabolic risk factors.

INTRODUCTION

Gestational diabetes is associated with increased risks of offspring obesity, type 2 diabetes, and metabolic syndrome (1-5). Increasing evidence has suggested that these risks might not be confined to women diagnosed with gestational diabetes but that they may already exist in offspring exposed to maternal glucose concentrations below diagnostic thresholds (6, 7). Previous studies have reported associations of maternal glucose concentrations in mid- and late pregnancy with offspring cardiometabolic risk factors (6, 7). However, as fetal cardiovascular and metabolic development already starts in the first trimester, early pregnancy may already be a critical period for the adverse influence of a suboptimal maternal glucose metabolism on the development of the fetal cardiometabolic system. Increases of maternal glucose and insulin concentrations from early pregnancy onward may directly affect placental development and increase nutrient transfer to the developing fetus. This may subsequently lead to increased fetal growth as well as adaptations in adipogenesis and pancreatic and vascular development. These adaptations may increase the susceptibility to cardiometabolic disease in later life (4, 8-12). Altered childhood body fat development may especially be involved in the associations of maternal glycemia with offspring cardiometabolic risk factors (9). A few studies have shown an association of maternal fasting glucose concentrations in pregnancy with increased childhood sum of skinfolds and waist circumference (6, 7, 13). However, it is not clear whether this includes overall fat or more specifically visceral fat accumulation, which is known to be more strongly related with cardiometabolic disease (14, 15). We hypothesized that higher maternal early-pregnancy glucose concentrations are associated with an unfavorable offspring cardiometabolic risk profile and suboptimal body fat distribution.

Therefore, in a population-based prospective cohort from early pregnancy onward among 3,737 mothers and their children, we assessed the associations of maternal early-pregnancy glucose and insulin concentrations across the full range with cardiometabolic risk factors and detailed measurements of general and abdominal fat in childhood. We additionally explored whether these associations are independent of maternal lifestyle factors and birth, infant, or childhood characteristics.

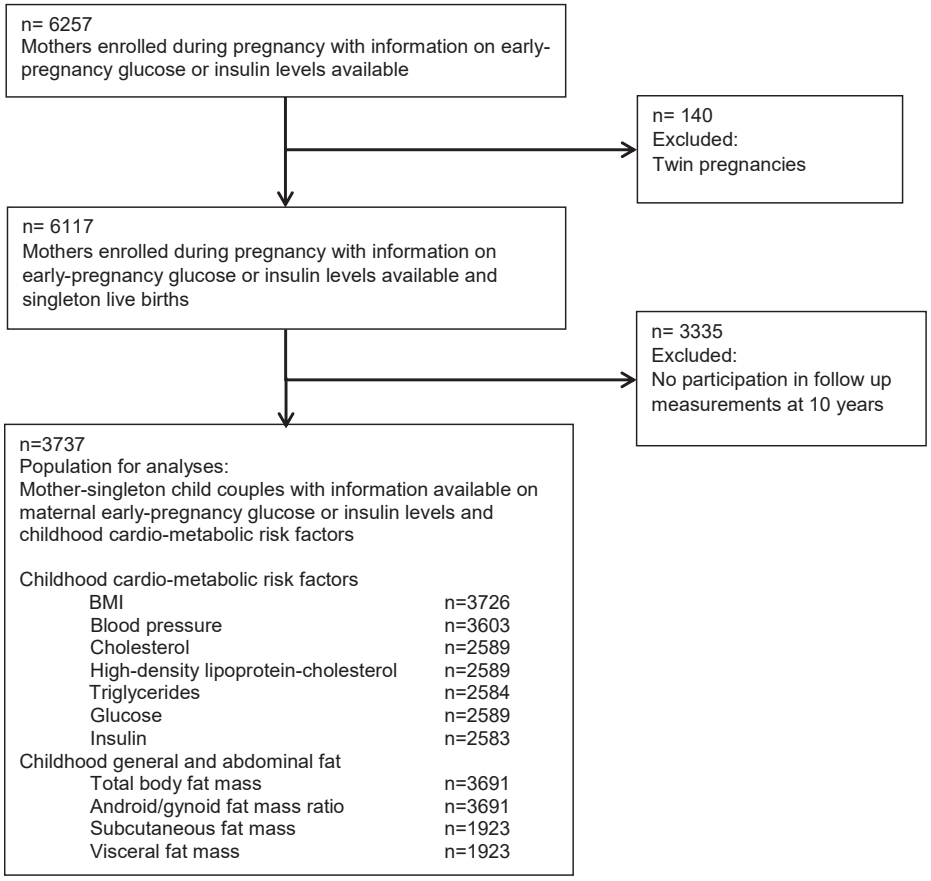
METHODS

Study design and participants

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onward in Rotterdam, The Netherlands (16). Approval for the study was obtained from the Medical Ethical Committee of Erasmus University Medical Center, Rotterdam. Written consent was obtained from the parents of all participants. In total,

8,879 pregnant women were enrolled between 2001 and 2005. Of these, 6,117 mothers had early-pregnancy information on glucose and insulin concentrations available and had singleton live-born children. Cardiometabolic follow-up measurements at the age of 10 years were available for 3,737 of their children (**Figure 1**). Main reasons for missing data were participants lost to follow-up and no consent or failure of venous punctures (16).

Figure 1. Flow chart of the study participants



Maternal early-pregnancy glucose and insulin concentrations

Nonfasting blood samples were collected at enrollment in the study before 18 weeks of gestation (median: 13.2 weeks; 95% range: 10.5-17.1). Glucose concentration (millimoles per liter) is an enzymatic quantity and was measured with c702 module on the Cobas 8000 analyzer (Roche, Almere, the Netherlands). Insulin concentration (picomoles per liter) was measured with electrochemiluminescence immunoassay on the Cobas e411 analyzer (Roche).

Childhood cardiometabolic risk factors and general and abdominal fat measurements

At the age of 10 years, we measured height and weight without shoes and heavy clothing and calculated BMI (kilograms per meter squared). Childhood BMI standard deviation scores (SDS) adjusted for sex and age were constructed based on Dutch reference growth charts (Growth Analyzer 4.0; Dutch Growth Research Foundation, Rotterdam, Netherlands) (17). We defined childhood overweight and underweight by categorizing childhood weight status according to the International Obesity Task Force cutoffs (18). Overweight and obesity were combined into one category, and children with underweight were excluded only in this variable (n = 266). We observed similar results when children with underweight were included in the analyses (results not shown). Systolic and diastolic blood pressures (millimeters of mercury) were measured at the right brachial artery, four times with 1-minute intervals, using the validated automatic sphygmanometer Datascope Accutorr Plus (Paramus, New Jersey) (19). Mean systolic and diastolic blood pressure values were calculated using the last three blood pressure measurements. We obtained nonfasting venous blood samples and measured total cholesterol (millimoles per liter), high-density lipoprotein (HDL) cholesterol (millimoles per liter), triglycerides (millimoles per liter), glucose (millimoles per liter), and insulin (picomoles per liter) concentrations.

We measured total, android, and gynoid body fat mass by dual-energy x-ray absorptiometry (Lunar iDXA; GE Healthcare, Madison, Wisconsin) and calculated android/gynoid fat mass ratio (20). Abdominal subcutaneous and visceral fat measures were obtained from magnetic resonance imaging (MRI) scans using a 3.0-T MRI (Discovery MR750w; GE Healthcare, Milwaukee, Wisconsin) as described previously (16, 21). Childhood body fat mass is strongly influenced by height of the child (22). To enable assessment of the associations of maternal glucose metabolism with childhood adiposity measures independent of childhood size, we constructed childhood fat mass measures independent of height of the child. Using log-log regressions, we estimated the optimal adjustment for childhood height needed to construct height-independent fat mass measures (details in **Supplemental Methods 1**) (22-24). We calculated total fat mass and subcutaneous fat mass indices (total and subcutaneous fat mass/height^4) and visceral fat mass index (visceral fat mass/height^3).

Clustering of cardiometabolic risk factors was defined as having three or more of the following components: visceral fat mass index ≥ 75th percentile, systolic or diastolic blood pressure ≥ 75th percentile, triglycerides ≥ 75th percentile, or HDL cholesterol ≤ 25th percentile; and insulin ≥ 75th percentile (25). Because waist circumference was not available, we used visceral fat mass index as a proxy for waist circumference.

Covariates

Information on maternal educational level, ethnicity, parity, weight just before pregnancy, maximum weight during pregnancy, smoking, and total daily energy intake (in kilojoules) dur-

ing pregnancy was obtained through questionnaires (16). Maternal height was measured at intake without shoes and BMI was calculated (16). We obtained information about diagnosis of gestational diabetes and child's sex, gestational age at birth, and birth weight from medical records (16). Preterm birth was defined as a gestational age at birth < 37 weeks. We created gestational age- and sex-adjusted SDS of birth weight using North-European reference growth charts (26). We defined small for gestational age and large for gestational age at birth as the lowest and the highest 10 percentiles of gestational-age–adjusted birth weight, respectively. We obtained information on breastfeeding in infancy by questionnaire (16).

Statistical analysis

First, we performed a nonresponse analysis to compare children with and without follow-up measurements at the age of 10 years. Second, we assessed the associations of maternal early-pregnancy glucose and insulin concentrations across the full range with the risks of childhood overweight and clustering of cardiometabolic risk factors using multiple logistic regression models. Third, we used multiple linear regression models to assess the associations of maternal early-pregnancy glucose and insulin concentrations with childhood BMI, blood pressure, lipids, and glucose and insulin concentrations across the full range separately and with detailed childhood general and abdominal fat measurements. We used three different models for the analyses. The first was the basic model, which was adjusted for gestational age at enrollment and child's age and sex at follow-up measurements. The second was the confounder model, which was the basic model additionally adjusted for confounding covariates and was considered as the main model. Based on literature, maternal ethnicity, educational level, parity, smoking, and daily total caloric intake were considered as potential confounders. Only maternal ethnicity and educational level were selected in the model based on their association with exposures and outcomes and change in effect estimates of > 10% in our study sample. The third model was the maternal BMI model, which was the confounder model additionally adjusted for maternal prepregnancy BMI. Because previous studies have suggested that associations between gestational diabetes and childhood BMI are largely explained by maternal prepregnancy BMI, we constructed this separate maternal prepregnancy BMI model (12). Correlation coefficients for correlation between maternal glucose and insulin concentrations and prepregnancy BMI were 0.16 and 0.20 for maternal glucose and insulin concentrations, respectively. For associations that persisted after adjustment for maternal prepregnancy BMI, we further explored whether these associations were mediated by gestational weight gain, birth weight, infant breastfeeding, or childhood BMI by adding these variables separately to the maternal BMI model. We tested for interactions of maternal glucose and insulin with maternal BMI, maternal ethnicity, and child's sex, but none was significant and no further stratified analyses were performed (27-29). We performed the following sensitivity analyses: (1) we excluded women with a diagnosis of gestational diabetes (n = 34) because we were interested in the associations of maternal glucose and

insulin concentrations within a nondiabetic population; (2) we repeated the analyses excluding children born preterm, small for gestational age at birth, or large for gestational age at birth to explore whether these adverse birth outcomes explained potential associations.

Not normally distributed exposure and outcome measures were log transformed. To enable comparison of effect estimates, we constructed SDS of exposures and outcomes. To reduce selection bias because of missing data, multiple imputations of covariates (pooled results of five imputed data sets) were performed (30). We applied Bonferroni correction to take multiple testing into account. As outcomes were strongly correlated, we divided the αof 0.05 by four categories (fat measures, blood pressure, lipid concentrations, and glucose/insulin concentrations), resulting in $P < 0.013$. All analyses were performed using SPSS Statistics version 24.0 for Windows (IBM Corp., Armonk, New York).

RESULTS

Characteristics of study participants

Table 1 shows the population characteristics. In early pregnancy, the mean maternal glucose concentration was 4.4 mmol/L (SD 0.9) and the median insulin concentration was 114.0 pmol/L (95% range: 24.1-491.8). Nonresponse analyses showed that mothers of children included in the analyses compared with mothers lost to follow-up were, on average, older, more frequently European, and more highly educated and that they had a higher prepregnancy weight and had children with a higher birth weight. No differences in early-pregnancy glucose and insulin concentrations were present (**Supplemental Table 1**).

Table 1. Characteristics for the study population

	Total group (n = 3,737)
Maternal characteristics	
Age at enrolment, mean (SD), years	30.7 (4.7)
Height, mean (SD), cm	168.2 (7.4)
Prepregnancy weight, median (95%), kg	65.0 (50.3; 90.0)
Prepregnancy BMI, median (95%), kg/m^2	22.6 (18.8; 31.9)
Ethnicity, n (%)	
Dutch	2193 (58.7)
European	299 (8.0)
Cape Verdean	153 (4.1)
Dutch Antillean	66 (1.8)
Moroccan	169 (4.5)
Surinamese	272 (7.3)
Turkish	218 (5.8)

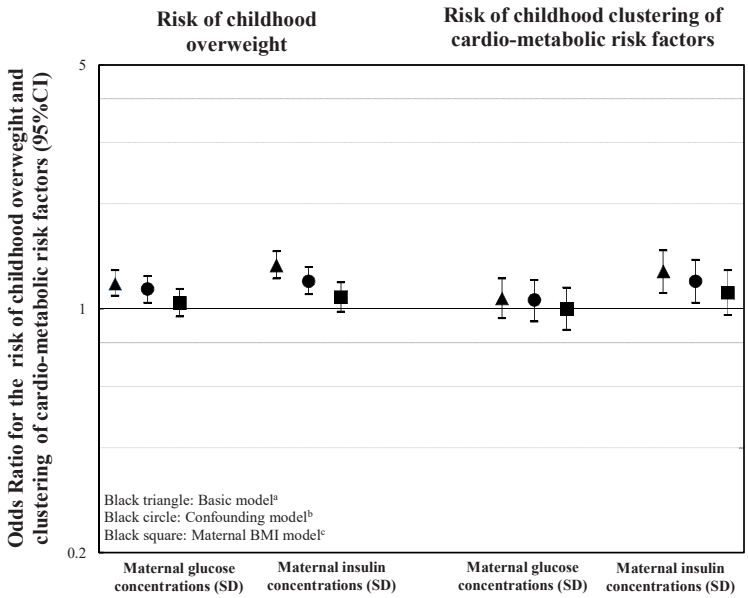
Table 1. Characteristics for the study population (continued)

	Total group (n = 3,737)
Education, n high (%)	1855 (49.6)
Parity, No. nulliparous (%)	2230 (59.7)
Smoking during pregnancy, n yes (%)	853 (22.8)
Gestational weight gain, mean (SD), kg	15.1 (5.7)
Daily energy intake, mean (SD), kJ	8581 (2294)
Gestational age at intake, median (95%), weeks	13.2 (10.5; 17.1)
Glucose concentration, mean (SD), mmol/l	4.4 (0.9)
Insulin concentration, median (95%), pmol/l	114.0 (24.1; 491.8)
Gestational diabetes, n (%)	34 (0.9)
Infant characteristics	
Sex, n female (%)	1894 (50.7)
Gestational age at birth, median (95%), weeks	40.3 (37.14; 42.14)
Birth weight, mean (SD), grams	3437 (550)
Small for gestational age, n (%)	373 (10)
Large for gestational age, n (%)	373 (10)
Preterm birth, n (%)	155 (4)
Ever breastfeeding, n yes (%)	2878 (77)
Childhood characteristics	
Age, mean (SD), years	9.8 (0.4)
Height, mean (SD), cm	141.6 (6.7)
Weight, median (95%), kg	33.8 (26.4; 49.7)
BMI, median (95%), kg/m^2	16.9 (14.4; 23.3)
Fat	
Total fat mass, median (95%)	8417 (4905; 19116)
Android/gynoid fat mass ratio, median (95%)	0.24 (0.16; 0.44)
Subcutaneous fat mass, median (95%), g	1294 (642; 4271)
Visceral fat mass, median (95%), g	369 (187; 853)
Blood pressure	
Systolic, mean (SD), mmHg	103.1 (7.9)
Diastolic, mean (SD), mmHg	58.5 (6.4)
Lipid concentrations	
Total cholesterol, mean (SD), mmol/l	4.31 (0.66)
High-density lipoprotein-cholesterol, mean (SD), mmol/l	1.48 (0.34)
Triglycerides, median (95%), mmol/l	0.98 (0.47; 2.28)
Glucose, mean (SD), mmol/l	5.20 (0.94)
Insulin, median (95%), pmol/l	174.60 (45.87; 512.40)
Overweight/obese, n (%)	643 (17.2)
Clustering of cardio-metabolic risk factors, n (%)	261 (7)

Childhood cardiometabolic risk factors

Figure 2 shows that, in the confounder model, 1-SDS higher maternal early-pregnancy glucose and insulin concentrations were associated with an increased risk of childhood overweight (odds ratio [OR] 1.14, 95% CI: 1.04-1.24 and OR 1.20, 95% CI: 1.10-1.32 per SDS increase in maternal glucose and insulin concentrations, respectively). A 1-SDS higher maternal early-pregnancy insulin concentration, but not glucose concentration, was associated with clustering of cardiometabolic risk factors in childhood (OR 1.20, 95% CI: 1.04-1.38 per SDS increase in maternal insulin concentration). All of these associations attenuated to nonsignificance after adjustment for maternal prepregnancy BMI.

Figure 2. Associations of maternal early-pregnancy glucose and insulin concentrations and childhood risks of overweight and clustering of cardio-metabolic risk factors



Values represent odds ratios (95% confidence interval) from logistic regression models that reflect the risks of childhood overweight for SDS change in maternal glucose and insulin concentrations.

[a]Basic model includes gestational age at enrolment, child's age and sex at follow up measurements

[b]Confounding model includes the basic model additionally adjusted for ethnicity, maternal educational level

[c]Maternal BMI model includes the confounder model additionally adjusted for maternal prepregnancy BMI

Table 2 shows the associations of maternal glucose and insulin concentrations with each of the childhood cardiometabolic risk factors separately. In the confounder model, a 1-SDS higher maternal glucose concentration was associated with lower HDL cholesterol (–0.04 SDS, 95% CI: –0.08 to –0.01 per SDS increase in glucose concentration). A 1-SDS higher maternal insulin concentration was associated with higher childhood BMI (0.05 SDS, 95% CI: 0.02 to 0.08 per SDS increase in insulin concentration) and systolic blood pressure (0.04 SDS, 95% CI: 0.01 to 0.07 per SDS increase in insulin concentration). These associations attenuated to nonsignificance after adjustment for maternal prepregnancy BMI. A 1-SDS higher maternal early-pregnancy glucose concentration was associated with higher glucose concentration in childhood (0.08 SDS, 95% CI: 0.04-0.11 per SDS increase in maternal glucose concentration), whereas a 1-SDS higher maternal early-pregnancy insulin concentration was associated with higher childhood insulin concentration (0.07 SDS, 95% CI: 0.03-0.10 per SDS increase in maternal insulin concentration). The association of maternal glucose concentration with childhood glucose concentration was not affected by additional adjustment for maternal prepregnancy BMI, whereas the association of maternal early-pregnancy insulin concentration with childhood insulin concentration only slightly attenuated after adjustment for maternal prepregnancy BMI. Further adjustment for gestational weight gain, birth weight, infant breastfeeding, and childhood BMI did not materially affect the associations (**Supplemental Table 2**).

Childhood general and abdominal fat

Table 3 shows that in the confounder model, a 1-SDS higher maternal early-pregnancy insulin concentration, but not glucose concentration, was associated with higher childhood total fat mass index (0.06 SDS, 95% CI: 0.03-0.09 per SDS increase in insulin concentration), android/gynoid fat mass ratio (0.05 SDS, 95% CI: 0.02-0.08 per SDS increase in insulin concentration), and subcutaneous fat mass index (0.07 SDS, 95% CI: 0.03-0.11 per SDS increase in insulin concentration). All of these associations of maternal insulin concentration with childhood total fat mass index, android/gynoid fat mass ratio, and abdominal subcutaneous fat mass index attenuated to nonsignificance after adjustment for maternal prepregnancy BMI. No associations of maternal glucose or insulin concentrations with childhood visceral fat mass index were present.

Sensitivity analyses

No differences in findings were present when mothers with gestational diabetes were excluded from the analyses (data not shown). We observed largely similar results when children with adverse birth outcomes were excluded from the analyses (**Supplemental Tables 3-6**).

Table 2. Associations of maternal early-pregnancy glucose and insulin concentrations with childhood cardio-metabolic risk factors

Model	BMI (SDS) (n=3726)	Systolic blood Pressure (SDS) (n=3603)	Diastolic blood Pressure (SDS) (n=3603)	Total Cholesterol Concentrations (SDS) (n=2589)	HDL-cholesterol Concentrations (SDS) (n=2589)	Triglyceride Concentrations (SDS) (n=2584)	Glucose Concentrations (SDS) (n=2589)	Insulin Concentrations (SDS) (n=2583)
Maternal glucose concentrations (SDS)								
Basic model[a]	0.04 (0.00; 0.07)	0.03 (0.00; 0.06)	0.04 (0.01; 0.07)*	-0.01 (-0.05; 0.03)	-0.05 (-0.08; -0.01)*	-0.02 (-0.06; 0.02)	0.08 (0.04; 0.11)*	0.04 (0.00; 0.08)
Confounding model[b]	0.02 (-0.01; 0.06)	0.02 (-0.01; 0.06)	0.03 (0.00; 0.07)	-0.01 (-0.05; 0.03)	-0.04 (-0.08; -0.01)*	-0.03 (-0.06; 0.01)	0.08 (0.04; 0.11)*	0.04 (0.00; 0.07)
Maternal BMI model[c]	N.A.	N.A.	0.02 (-0.01; 0.06)	N.A.	-0.03 (-0.07; 0.01)	N.A.	0.08 (0.04; 0.12)*	0.03 (-0.01; 0.06)
Maternal insulin concentrations (SDS)								
Basic model[a]	0.08 (0.05; 0.12)*	0.06 (0.03; 0.09)*	0.05 (0.01; 0.08)*	0.00 (-0.04; 0.04)	-0.06 (-0.10; -0.02)*	0.01 (-0.03; 0.05)	0.02 (-0.02; 0.06)	0.08 (0.04; 0.12)*
Confounding model[b]	0.05 (0.02; 0.08)*	0.04 (0.01; 0.07)*	0.03 (-0.01; 0.06)	-0.01 (-0.04; 0.03)	-0.05 (-0.09; -0.01)	0.00 (-0.04; 0.04)	0.02 (-0.02; 0.06)	0.07 (0.03; 0.10)*
Maternal BMI model[c]	-0.01 (-0.05; 0.02)	0.01 (-0.02; 0.05)	N.A.	N.A.	N.A.	N.A.	N.A.	0.05 (0.02; 0.09)*

Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal glucose and insulin concentrations. Estimates are based on multiple imputed data.

SDS: standard deviation score, HDL-cholesterol: High-density lipoprotein-cholesterol, N.A.: not applicable

[a]Basic model includes gestational age at enrolment, child's age and sex at follow up measurements

[b]Confounding model includes the basic model additionally adjusted for ethnicity, maternal educational level

[c]Maternal BMI model includes the confounder model additionally adjusted for maternal prepregnancy BMI

*p-value<0.013 (Bonferroni corrected p-value for multiple testing)

Table 3. Associations of maternal early-pregnancy glucose and insulin concentrations with childhood general and abdominal fat

Model	Total fat mass Index (SDS) (n=3684)	Android/gynoid fat mass ratio (SDS) (n=3691)	Subcutaneous fat mass index (SDS) (n=1919)[d]	Visceral fat mass index (SDS) (n=1919)[d]
Maternal glucose concentrations (SDS)				
Basic model[a]	0.05 (0.02; 0.08)*	0.04 (0.00; 0.07)	0.04 (-0.01; 0.08)	-0.01 (-0.05; 0.04)
Confounding model[b]	0.03 (0.00; 0.06)	0.02 (-0.01; 0.05)	0.03 (-0.02; 0.07)	-0.01 (-0.06; 0.03)
Maternal BMI model[c]	N.A.	N.A.	N.A.	N.A.
Maternal insulin concentrations (SDS)				
Basic model[a]	0.11 (0.08; 0.14)*	0.09 (0.06; 0.12)*	0.11 (0.06; 0.15)*	0.03 (-0.01; 0.08)
Confounding model[b]	0.06 (0.03; 0.09)*	0.05 (0.02; 0.08)*	0.07 (0.02; 0.11)*	0.02 (-0.02; 0.07)
Maternal BMI model[c]	0.01 (-0.02; 0.04)	0.01 (-0.02; 0.04)	0.02 (-0.02; 0.06)	N.A.

Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal glucose and insulin concentrations. Estimates are based on multiple imputed data.
SDS: standard deviation score, N.A.: not applicable
[a]Basic model includes gestational age at enrolment, child's age and sex at follow up measurements
[b]Confounding model includes the basic model additionally adjusted for ethnicity, maternal educational level
[c]Maternal BMI model includes the confounder model additionally adjusted for maternal prepregnancy BMI
[d]Magnetic resonance imaging follow up measurements were performed in a subgroup of children
*p-value<0.013 (Bonferroni corrected p-value for multiple testing)

DISCUSSION

In this prospective cohort study, we observed that higher maternal early-pregnancy glucose and insulin concentrations were associated with higher childhood glucose and insulin concentrations at the age of 10 years. The associations of maternal early-pregnancy glucose and insulin concentrations with other childhood cardiometabolic risk factors and detailed measurements of general and abdominal fat were explained by maternal prepregnancy BMI.

Interpretation of main findings

A high number of pregnancies are complicated by gestational diabetes. Next to an increased risk of maternal complications, intrauterine exposure to gestational diabetes is associated with adverse cardiometabolic outcomes in the offspring (4). Previous studies have already reported associations between higher late-pregnancy maternal glucose concentrations already below the clinical threshold of gestational diabetes with offspring cardiometabolic risk factors (6, 31, 32). A study among 970 Chinese mother-child pairs reported that third-trimester maternal fasting glucose concentrations were associated with a higher risk for obesity, higher systolic blood pressure, and abnormal glucose tolerance at the age of 7 years, independent of maternal prepregnancy BMI (6). A cohort study in the United Kingdom including 2,563 women and their offspring showed that, independent of maternal prepreg-

nancy BMI, glycosuria in midpregnancy was associated with higher offspring BMI and fasting insulin concentrations but not with blood pressure and lipid concentrations (31). It is likely that women who develop gestational diabetes or hyperglycemia later in pregnancy already have a suboptimal glucose metabolism in early pregnancy, a critical period for placental and fetal cardiometabolic development (9, 33). Suboptimal maternal glucose and insulin concentrations in early pregnancy may adversely affect placental development, predisposing to alterations in fetal nutrient supply, growth, and development (34). In addition, suboptimal maternal early-pregnancy glucose concentrations may have direct adverse influences on fetal cardiometabolic development (9).

In the current study, we observed that higher maternal glucose and insulin concentrations in early pregnancy were associated with higher childhood risks of overweight and clustering of cardiometabolic risk factors. However, these associations attenuated after adjustment for maternal prepregnancy BMI. These findings suggest that maternal prepregnancy BMI, a known risk factor for insulin resistance in pregnancy and cardiometabolic risk factors in childhood, explains the associations of maternal early-pregnancy glucose and insulin concentrations with childhood overweight and cardiometabolic risk factors (9). When we further explored the associations of maternal early-pregnancy glucose and insulin concentrations with individual cardiometabolic risk factors, we observed that higher maternal glucose and insulin concentrations were associated with higher offspring glucose and insulin concentrations, respectively. These associations were independent of maternal prepregnancy BMI, gestational weight gain, birth weight, infant breastfeeding, and childhood BMI. Findings were also similar when we excluded children with adverse birth outcomes from the analyses. Thus, these factors do not seem to explain the associations of maternal glucose and insulin concentrations with childhood glucose metabolism. This suggests that at least part of the association may be due to an intrauterine effect of maternal glucose and insulin concentrations on offspring glucose metabolism. Similar to previous studies performed later in pregnancy using fasting glucose samples, we did not find an association of maternal early-pregnancy glucose and insulin concentrations with childhood BMI, blood pressure, and lipid concentrations, independent of maternal prepregnancy BMI (31). Thus, our results suggest that maternal glucose and insulin concentrations, as soon as early pregnancy, are related to higher childhood glucose and insulin concentrations, irrespective of maternal, birth, and childhood characteristics, but not to other cardiometabolic outcomes. Whether maternal factors other than impaired glucose metabolism as a consequence of higher maternal BMI, such as altered maternal hormone status, play a role in the association of maternal prepregnancy BMI with childhood BMI, blood pressure, and lipids should be further studied.

Animal and mechanistic studies proposed that offspring fat accumulation and adverse fat distribution might be involved in the associations of maternal hyperglycemia with offspring cardiometabolic risk factors. Observational studies have confirmed this hypothesis and reported associations of maternal fasting glucose concentrations in pregnancy with adverse

offspring body fat composition, measured by sum of skinfolds and waist circumference (6, 7, 31, 35, 36). However, these measures are suboptimal, as waist circumference does not distinguish subcutaneous from visceral fat, whereas visceral abdominal fat is much more closely related to risk of cardiometabolic disease in later life (14). In the present study, we observed that higher maternal early-pregnancy insulin concentrations but not glucose concentrations were associated with childhood total body fat mass, android/gynoid fat mass ratio, and subcutaneous abdominal fat mass. In line with the associations of maternal glucose and insulin concentrations with childhood BMI, blood pressure, and lipids, all associations of maternal glucose and insulin concentrations with detailed measurements of childhood general and abdominal fat in the present study were fully explained by maternal prepregnancy BMI. Contrary to our hypothesis, no specific associations with childhood visceral fat mass were present. It might be that associations with childhood visceral fat are more apparent among higher risk populations or at older ages. Further studies are needed to explore the detailed role of a suboptimal offspring body fat distribution in response to impaired maternal glucose metabolism during pregnancy within different populations and using advanced imaging techniques. Based on our results, it seems that maternal early-pregnancy glucose and insulin concentrations are associated with childhood subcutaneous fat accumulation, but these associations are explained by maternal prepregnancy BMI.

Within this study, we only observed independent associations of maternal early-pregnancy glucose and insulin concentrations with childhood glucose and insulin concentrations. These associations provide insight into potential underlying mechanisms, and they may be explained through several pathways. First, shared genetic factors are expected to have a contribution in the association between maternal glucose and insulin concentrations with offspring glucose and insulin concentrations (37). Second, higher maternal early-pregnancy glucose concentrations lead to fetal hyperinsulinemia, whereas higher maternal early-pregnancy insulin concentrations are involved in protein, lipolysis, and early placental development. Together, this could cause alternations in fetal nutrient supply, affecting fetal pancreatic beta-cell development and increasing fetal insulin secretion. These irreversible alterations may subsequently lead to increased glucose and insulin concentrations in childhood (9, 38, 39). Furthermore, higher maternal glucose concentrations may also be involved in gene expression through DNA methylation, leading to altered insulin secretion in the offspring (40). Further studies are needed to disentangle the complex mechanisms underlying the association of maternal glucose and insulin concentrations with childhood glucose metabolism.

The observed effect estimates for the associations of maternal early-pregnancy glucose and insulin concentrations with childhood glucose and insulin concentrations were relatively small but they may be important on a population level. Previous studies have shown that childhood glucose and insulin concentrations tend to track into adulthood. A study among 1,766 children showed that children with higher fasting glucose concentrations at the age of 10 years had a higher risk of developing type 2 diabetes in adolescence (6). Similarly,

a study among 1,723 children reported that children with higher fasting glucose concentrations within the normal range had a higher risk of prediabetes and type 2 diabetes in adulthood (7). A study among 4,857 American Indian children without diabetes showed that children with higher glucose concentrations after a glucose tolerance test had a higher risk of premature death, but this effect was not independent of concurrent childhood BMI (41). Together, these findings suggest that even subclinical differences in childhood glucose and insulin concentrations may be related to the development of type 2 diabetes in later life (42). Maternal prepregnancy BMI seems to explain the associations of maternal glucose and insulin concentrations with other childhood cardiometabolic risk factors and childhood body fat development. This suggests that preventive strategies, aimed at improving offspring cardiometabolic health, might be more effective when focusing on optimizing maternal prepregnancy BMI than on optimizing maternal glucose concentrations from early pregnancy onward.

Methodological considerations

Strengths of this study are the prospective design, large sample size, and the use of detailed fat measures obtained through MRI. Although only 61% of children from mothers with information on glucose and insulin concentrations in pregnancy participated in follow-up measurements, we do not expect that nonresponse affected our effect estimates, as maternal insulin and glucose concentrations did not differ between these groups. The generalizability of our results may be affected by a selection toward a relatively healthy, high-educated study population. We obtained nonfasting glucose and insulin concentrations, sampled on nonfixed times throughout the day. This may have led to nondifferential misclassification, causing an underestimation of our associations. Although we simultaneously measured insulin concentrations to substantiate our findings, random glucose concentrations cannot directly assess insulin resistance. However, random glucose concentrations are useful for identifying women at risk for gestational diabetes and they are used in clinical practice as a screening method in early pregnancy (43, 44). In addition, we measured maternal glucose and insulin concentrations once during early pregnancy. Impaired glucose tolerance in early pregnancy has been suggested to persist throughout pregnancy (33). Further studies are needed with multiple, more detailed maternal glucose measurements, including fasting glucose concentrations and detailed postprandial glucose measurements throughout pregnancy. These studies also need to use more advanced statistical methods to provide further insight into critical periods for potential adverse effects of impaired maternal glucose metabolism on offspring glucose metabolism. We did not have information available on clinical diagnosis of type 2 diabetes in the offspring. However, we expect the percentage of childhood type 2 diabetes according to clinical diagnosis within our cohort to be low, as the average age of the children in our cohort is 9.8 years, whereas the onset of type 2 diabetes mostly occurs at later childhood ages (45). Further studies are needed to assess whether maternal early-pregnancy

glucose and insulin concentrations are also associated with the risk of type 2 diabetes in the offspring during adolescence. Finally, although we had detailed information on maternal and childhood sociodemographic and lifestyle factors available, because of the observational study design, residual confounding by, for example, childhood dietary factors and physical activity may have influenced our results.

Conclusions

Maternal early-pregnancy random glucose and insulin concentrations were associated with higher childhood glucose and insulin concentrations, independent of maternal and childhood characteristics. When taking maternal prepregnancy BMI into account, no associations of maternal glucose and insulin concentrations with other childhood cardiometabolic risk factors were present.

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SUPPLEMENTAL MATERIAL

Supplemental Methods 1. **Log-log regression analyses**

For our fat measures, we created index variables, which were made independent of height. We did this by dividing our fat measurements by the optimal adjustment for height. The optimal adjustment was determined using log-log regression analyses (1). Total fat mass, subcutaneous fat mass, visceral fat mass and height were log-transformed using natural logs. We performed linear regression analyses with log-fat measures as the dependent variable and log- height as the independent variable. The regression slope corresponds with the power by which height should be raised. This resulted in the following index values of the fat measures: total fat mass divided by height^4, subcutaneous fat mass divided by height^4 and visceral fat mass divided by height^3.

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Supplemental Table 1. Non-response analysis for loss to follow-up at the age of 10 years (n=6117)

	Follow up at 10 years (n=3737)	No follow up at 10 years (n=2380)	P-value*
Maternal characteristics			
Age at enrolment, mean (SD), years	30.7 (4.7)	28.3(5.2)	<0.01
Height, mean (SD), cm	168.2 (7.4)	166.5 (7.3)	<0.01
Pre-pregnancy weight, median (95%), kg	65.0 (50.3; 90.0)	63 (50.0; 92.0)	<0.01
Pre-pregnancy BMI, median (95%), kg/m^2	22.6 (18.8; 31.9)	22.6 (18.6; 32.8)	0.64
Gestational weight gain, mean (SD), kg	15.1 (5.7)	15.1 (6.3)	0.82
Gestational age at intake, median (95%), weeks	13.2 (10.5; 17.1)	13.4 (10.4; 17.4)	<0.01
Parity, n nulliparous (%)	2230 (59.7)	1244 (52.3)	<0.01
Ethnicity, n (%)			<0.01
Dutch/European	2492 (66.7)	1088 (49.7)	
Other	1191 (31.9)	1103 (46.3)	
Education level, n high (%)	1855 (49.6)	695 (33.0)	<0.01
Smoking during pregnancy, n yes (%)	853 (22.8)	673 (29.2)	<0.01
Folic acid supplement use, n yes (%)	2363 (63.2)	1126 (47.3)	<0.01
Glucose, mean (SD), mmol/l	4.40 (0.86)	4.38 (0.82)	0.39
Insulin, median (95%), pmol/l	114.0 (24.05)	115.35	0.06
Gestational diabetes, n (%)	34 (0.9)	28 (1.2)	0.27
Daily calorie intake, mean (SD), kcal	2050 (548)	2008 (588)	0.02
Birth characteristics			
Gender, n female (%)	1894 (50.7)	1123 (47.2)	0.01
Birth weight, mean (SD), grams	3437 (550)	3386 (583)	<0.01
Gestational age at birth, median (95%), weeks	40.3 (37.1; 42.1)	40.0 (36.4; 42.0)	<0.01

*Differences in subject characteristics between the groups were evaluated using unpaired t-tests for the normally distributed continuous variables, Mann-Whitney U tests for the not-normally distributed continuous variables and chi-square tests for proportions.

Supplemental Table 2. Associations of maternal early-pregnancy glucose and insulin concentrations with childhood glucose and insulin concentrations after adjustment for maternal and childhood characteristics

Model	Glucose Concentrations (SDS) (n=2589)	Insulin Concentrations (SDS) (n=2583)
Maternal glucose concentrations (SDS)		
Gestational weight gain model[a]	0.07 (0.03; 0.12)*	0.03 (-0.02; 0.07)
Birth weight model[b]	0.08 (0.04; 0.12)*	0.03 (-0.01; 0.07)
Infant model[c]	0.07 (0.03; 0.11)*	0.03 (-0.01; 0.06)
Child BMI model[d]	0.07 (0.03; 0.11)*	0.03 (-0.01; 0.07)
Maternal insulin concentrations (SDS)		
Gestational weight gain model[a]	0.02 (-0.02; 0.07)	0.05 (0.01; 0.10)*
Birth weight model[b]	0.03 (-0.01; 0.07)	0.06 (0.02; 0.10)*
Infant model[c]	0.02 (-0.02; 0.07)	0.06 (0.02; 0.10)*
Child BMI model[d]	0.02 (-0.02; 0.07)	0.06 (0.02; 0.09)*

[a]Gestational weight gain model includes the maternal BMI model additionally adjusted for gestational weight gain
[b]Birth weight model includes the maternal BMI model additionally adjusted for gestational-age-adjusted birth weight
[c]Infant model includes maternal BMI model additionally adjusted for breastfeeding in infancy
[d]Child BMI model, the maternal BMI model additionally adjusted for child's BMI during follow up measurement at 10 years
*p-value<0.013 (Bonferroni corrected p-value for multiple testing)

Supplemental Table 3. Associations of maternal early-pregnancy glucose and insulin concentrations with childhood cardio-metabolic risk factors after exclusion of children born premature

Model	BMI (SDS) (n=3571)	Systolic blood Pressure (SDS) (n=3454)	Diastolic blood Pressure (SDS) (n=3454)	Total Cholesterol Concentrations (SDS) (n=2485)	HDL-cholesterol Concentrations (SDS) (n=2485)	Triglyceride Concentrations (SDS) (n=2480)	Glucose Concentrations (SDS) (n=2485)	Insulin Concentrations (SDS) (n=2480)
Maternal glucose concentrations (SDS)								
Basic model[a]	0.04 (0.00; 0.07)	0.03 (0.00; 0.06)	0.04 (0.01; 0.07)	-0.01 (-0.03; 0.02)	-0.05 (-0.08; -0.01)*	-0.01 (-0.03; 0.01)	0.08 (0.04; 0.12)*	0.04 (0.00; 0.08)
Confounding model[b]	0.02 (-0.01; 0.06)	0.03 (-0.01; 0.06)	0.03 (0.00; 0.06)	-0.01 (-0.03; 0.02)	-0.04 (-0.08; -0.01)*	-0.02 (-0.03; 0.00)	0.08 (0.04; 0.12)*	0.04 (0.00; 0.07)
Maternal BMI model[c]	N.A.	N.A.	0.02 (-0.01; 0.05)	N.A.	-0.03 (-0.07; 0.01)	N.A.	0.08 (0.04; 0.12)*	0.03 (-0.01; 0.06)
Maternal insulin concentrations (SDS)								
Basic model[a]	0.09 (0.05; 0.12)*	0.06 (0.03; 0.09)*	0.05 (0.01; 0.08)*	0.00 (-0.03; 0.02)	-0.02 (-0.03; 0.00)	0.00 (-0.02; 0.02)	0.04 (0.00; 0.08)	0.08 (0.04; 0.11)*
Confounding model[b]	0.05 (0.02; 0.09)*	0.04 (0.01; 0.08)	0.03 (-0.01; 0.06)	-0.01 (-0.03; 0.02)	-0.01 (-0.03; 0.00)	0.00 (-0.02; 0.02)	0.04 (0.00; 0.08)	0.06 (0.02; 0.10)*
Maternal BMI model[c]	-0.01 (-0.04; 0.03)	0.02 (-0.02; 0.05)	N.A.	N.A.	N.A.	N.A.	N.A.	0.05 (0.01; 0.09)*

Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal glucose and insulin concentrations. Estimates are based on multiple imputed data.

SDS: standard deviation score, HDL-cholesterol: High-density lipoprotein-cholesterol, N.A.: not applicable

[a]Basic model includes gestational age at enrolment, child's age and sex at follow up measurements

[b]Confounding model includes the basic model additionally adjusted for ethnicity, maternal educational level

[c]Maternal BMI model includes the confounder model additionally adjusted for maternal prepregnancy BMI

*p-value<0.013 (Bonferroni corrected p-value for multiple testing)

Supplemental Table 4. Associations of maternal early-pregnancy glucose and insulin concentrations with childhood cardio-metabolic risk factors after exclusion of children small or large for gestational age at birth

Model	BMI (SDS) (n=2977)	Systolic blood Pressure (SDS) (n=2884)	Diastolic blood Pressure (SDS) (n=2884)	Total Cholesterol Concentrations (SDS) (n=2068)	HDL-cholesterol Concentrations (SDS) (n=2069)	Triglyceride Concentrations (SDS) (n=2064)	Glucose Concentrations (SDS) (n=2070)	Insulin Concentrations (SDS) (n=2064)
Maternal glucose concentrations (SDS)								
Basic model[a]	0.04 (0.00; 0.07)	0.03 (-0.01; 0.07)	0.04 (0.00; 0.07)	-0.01 (-0.03; 0.02)	-0.05 (-0.08; -0.01)*	0.00 (-0.03; 0.02)	0.06 (0.01; 0.10)*	0.04 (0.00; 0.08)
Confounding model[b]	0.02 (-0.02; 0.06)	0.02 (-0.01; 0.06)	0.03 (-0.01; 0.07)	-0.01 (-0.03; 0.02)	-0.04 (-0.08; -0.01)*	-0.01 (-0.03; 0.01)	0.06 (0.01; 0.10)*	0.04 (-0.01; 0.08)
Maternal BMI model[c]	N.A.	N.A.	0.02 (-0.02; 0.06)	N.A.	-0.01 (-0.02; 0.01)	N.A.	0.06 (0.01; 0.10)*	0.02 (-0.02; 0.07)
Maternal insulin concentrations (SDS)								
Basic model[a]	0.09 (0.05; 0.12)*	0.07 (0.03; 0.10)*	0.05 (0.01; 0.08)*	0.00 (-0.03; 0.03)	-0.02 (-0.03; 0.00)	0.01 (-0.01; 0.03)	0.01 (-0.03; 0.06)	0.08 (0.04; 0.12)*
Confounding model[b]	0.05 (0.01; 0.09)*	0.05 (0.01; 0.08)*	0.03 (-0.01; 0.06)	0.00 (-0.03; 0.03)	-0.02 (-0.03; 0.00)	0.00 (-0.02; 0.03)	0.02 (-0.03; 0.06)	0.07 (0.03; 0.11)*
Maternal BMI model[c]	-0.01 (-0.05; 0.03)	0.02 (-0.01; 0.06)	N.A.	N.A.	-0.01 (-0.02; 0.01)	N.A.	N.A.	0.05 (0.01; 0.10)

Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal glucose and insulin concentrations. Estimates are based on multiple imputed data.

SDS: standard deviation score, HDL-cholesterol: High-density lipoprotein-cholesterol, N.A.: not applicable

[a]Basic model includes gestational age at enrolment, child's age and sex at follow up measurements

[b]Confounding model includes the basic model additionally adjusted for ethnicity, maternal educational level

[c]Maternal BMI model includes the confounder model additionally adjusted for maternal prepregnancy BMI

*p-value<0.013 (Bonferroni corrected p-value for multiple testing)

Supplemental Table 5. Associations of maternal early-pregnancy glucose and insulin concentrations with childhood general and abdominal fat after exclusion of children born premature

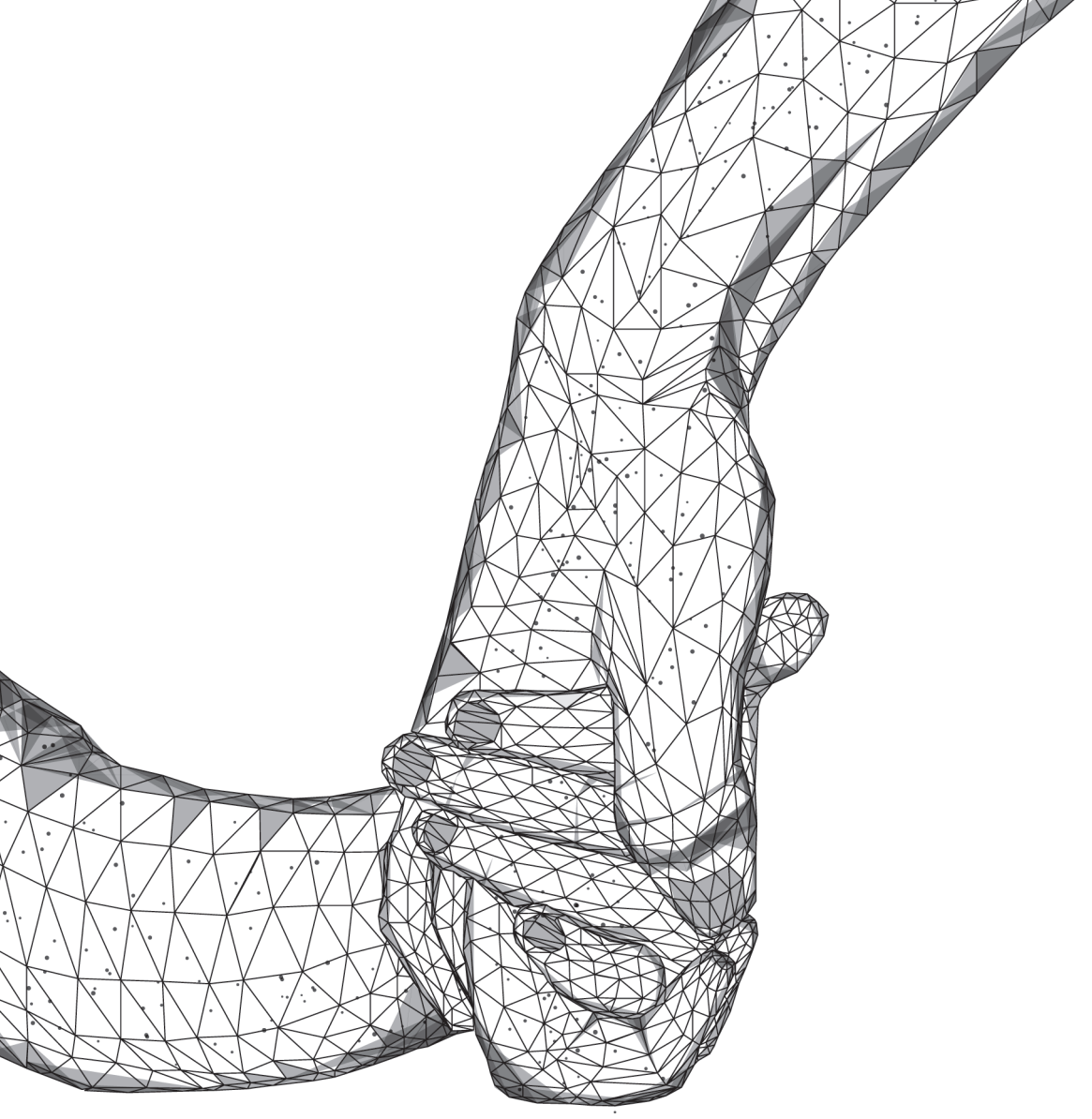
Model	Total fat mass Index (SDS) (n=3540)	Android/gynoid fat mass ratio (SDS) (n=3540)	Subcutaneous fat mass index (SDS) (n=1846)[d]	Visceral fat mass index (SDS) (n=1846)[d]
Maternal glucose concentrations (SDS)				
Basic model[a]	0.05 (0.02; 0.08)*	0.04 (0.01; 0.07)	0.03 (-0.01; 0.08)	-0.01 (-0.06; 0.03)
Confounding model[b]	0.03 (0.00; 0.06)	0.02 (-0.01; 0.06)	0.02 (-0.02; 0.06)	-0.02 (-0.06; 0.03)
Maternal BMI model[c]	N.A.	N.A.	N.A.	N.A.
Maternal insulin concentrations (SDS)				
Basic model[a]	0.11 (0.08; 0.14)*	0.09 (0.06; 0.12)*	0.10 (0.06; 0.15)*	0.03 (-0.02; 0.07)
Confounding model[b]	0.07 (0.04; 0.10)*	0.05 (0.02; 0.08)*	0.07 (0.03; 0.11)*	0.02 (-0.03; 0.06)
Maternal BMI model[c]	0.02 (-0.01; 0.05)	0.01 (-0.02; 0.05)	0.02 (-0.02; 0.06)	N.A.

Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal glucose and insulin concentrations. Estimates are based on multiple imputed data.
N.A.: not applicable, SDS: standard deviation score
[a]Basic model includes gestational age at enrolment, child's age and sex at follow up measurements
[b]Confounding model includes the basic model additionally adjusted for ethnicity, maternal educational level
[c]Maternal BMI model includes the confounder model additionally adjusted for maternal prepregnancy BMI
[d]Magnetic resonance imaging follow up measurements were performed in a subgroup of children
*p-value<0.013 (Bonferroni corrected p-value for multiple testing)

Supplemental Table 6. Associations of maternal early-pregnancy glucose and insulin concentrations with childhood general and abdominal fat after exclusion of children small or large for gestational age at birth

Model	Total fat mass Index (SDS) (n=2951)	Android/gynoid fat mass ratio (SDS) (n=2951)	Subcutaneous fat mass index (SDS) (n=1544)[d]	Visceral fat mass index (SDS) (n=1544)[d]
Maternal glucose concentrations (SDS)				
Basic model[a]	0.04 (0.01; 0.08)	0.04 (0.00; 0.07)	0.04 (-0.01; 0.09)	-0.01 (-0.06; 0.04)
Confounding model[b]	0.03 (-0.01; 0.06)	0.02 (-0.01; 0.05)	0.03 (-0.02; 0.07)	-0.02 (-0.07; 0.03)
Maternal BMI model[c]	N.A.	N.A.	N.A.	-0.06 (-0.11; -0.01)
Maternal insulin concentrations (SDS)				
Basic model[a]	0.11 (0.08; 0.14)*	0.10 (0.06; 0.13)*	0.11 (0.06; 0.15)*	0.03 (-0.02; 0.08)
Confounding model[b]	0.06 (0.03; 0.09)*	0.06 (0.03; 0.10)*	0.07 (0.03; 0.12)*	0.02 (-0.03; 0.06)
Maternal BMI model[c]	0.01 (-0.02; 0.04)	0.02 (-0.01; 0.06)	0.02 (-0.03; 0.06)	N.A.

Values represent regression coefficients (95% confidence interval) from linear regression models that reflect differences in childhood outcomes in SDS per SDS change in maternal glucose and insulin concentrations. Estimates are based on multiple imputed data.
N.A.: not applicable, SDS: standard deviation score
[a]Basic model includes gestational age at enrolment, child's age and sex at follow up measurements
[b]Confounding model includes the basic model additionally adjusted for ethnicity, maternal educational level
[c]Maternal BMI model includes the confounder model additionally adjusted for maternal prepregnancy BMI
[d]Magnetic resonance imaging follow up measurements were performed in a subgroup of children
*p-value<0.013 (Bonferroni corrected p-value for multiple testing)



4.2 Critical periods and growth patterns from fetal life onwards associated with childhood insulin levels

Voerman E, Jaddoe VWV, Franco OH, Steegers EAP, Gaillard R. Critical periods and growth patterns from fetal life onwards associated with childhood insulin levels.

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ABSTRACT

Aims/hypothesis: We aimed to identify critical periods and specific longitudinal growth patterns from fetal life onwards associated with childhood insulin and C-peptide levels.

Methods: In a prospective population-based cohort study of 4328 children, we repeatedly measured (femur) length and (estimated fetal) weight from the second trimester of fetal life until 6 years of age. BMI was calculated from 6 months onwards. Insulin and C-peptide levels were measured at 6 years of age.

Results: Preterm birth and small or large size for gestational age at birth were not associated with childhood insulin levels. Conditional growth modelling showed that, independent of growth in other time intervals, weight growth in each time interval from birth onwards, length growth from 6 months onwards and BMI growth from 12 months onwards were positively associated with childhood insulin levels. The strongest associations were present for weight and BMI growth between 48 and 72 months of age. Repeated measurement analyses showed that, compared with children in the lowest quartile of childhood insulin, those in the highest quartile had a higher length from birth onwards and a higher weight and BMI from 24 months onwards. These differences increased with age. No associations were observed for fetal growth characteristics. Similar results were observed for C-peptide levels.

Conclusions/interpretation: Our results suggest that rapid length, weight and BMI growth from birth onwards, but not during fetal life, is associated with higher insulin levels in childhood.

INTRODUCTION

A large body of evidence suggests that adverse exposures in early life influence the risk of type 2 diabetes in later life (1). Multiple previous studies have shown that adults born with either a low or high birthweight are at increased risk of type 2 diabetes (2, 3). Birthweight is often used as a proxy for fetal growth. However, since birthweight is the result of different fetal growth patterns and is the starting point of infant growth, birthweight is not a causal factor per se in the development of type 2 diabetes. Studies assessing the associations of directly measured fetal growth characteristics with measures of glucose or insulin metabolism in later life are scarce and focused on measures of growth during specific trimesters only (4, 5). As the development of the endocrine pancreas starts as early as the first trimester (6), fetal life might be a critical period for glucose and insulin metabolism, and the development of type 2 diabetes in later life.

More research has been performed on childhood growth patterns related to the risk of type 2 diabetes in later life (7-11). These studies showed that participants who developed type 2 diabetes in adulthood were small at birth and thin in infancy. During childhood, they gained weight rapidly, leading to an average or above average BMI at the age of 11 years. Furthermore, weight gain during the first 6 months of life was more strongly related to the risk of insulin resistance in adulthood compared with weight gain later in infancy (9).

Thus far, there have been no studies to explore the associations of directly measured fetal growth characteristics or the combined associations of repeatedly measured fetal and childhood growth characteristics with insulin metabolism in later life. It is also not clear which time period of growth is most important for later insulin metabolism. Therefore, we aimed to identify critical periods and specific growth patterns from fetal life onwards that are important for the development of suboptimal insulin metabolism in childhood. Among 4328 participants of a population-based prospective cohort study from early pregnancy onwards, we assessed the associations of directly measured fetal and childhood growth characteristics with insulin and C-peptide levels at 6 years of age.

METHODS

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards performed in Rotterdam, the Netherlands (12, 13). The study was approved by the Medical Ethical Committee of the Erasmus Medical Center, University Medical Center, Rotterdam (MEC 198.782/2001/31). Written informed consent was obtained from all mothers. The response rate at birth was 61%. In total, 9901 children were enrolled in the study. As growth represents a change in size between two time points,

we included children who had measurements of fetal or childhood growth characteristics available at, at least, two different time points (n=9639). Of these children, 9395 were singleton and live-born, 6522 participated in the follow-up measurements at 6 years of age and 4328 had information on insulin or C-peptide levels available (**Supplemental Figure 1**). Missing data on insulin and C-peptide levels were mainly due to lack of consent for venous punctures or non-successful venous punctures (13).

Fetal and childhood growth characteristics

We performed fetal ultrasound examinations in each trimester of pregnancy. As described previously, gestational age was established using data from the first fetal ultrasound examination (14). Second and third trimester fetal head circumference (HC), abdominal circumference (AC) and femur length (FL) were measured to the nearest millimetre using standardised ultrasound procedures. We calculated estimated fetal weight (EFW) using the formula of Hadlock et al (15): $\log_{10}$ EFW=1.5662–0.0108 (HC)+0.0468 (AC)+0.171 (FL)+0.00034 $(HC)^2$–0.003685 (AC× FL). Information on child's sex, gestational age, weight and length at birth was obtained from medical records. Preterm birth was defined as a gestational age at birth <37 weeks. Low birthweight was defined as a birthweight ≤2500 g and high birthweight was defined as a birthweight ≥4000 g. Small and large size for gestational age at birth were defined as the lowest and the highest ten percentiles of gestational age and sex-adjusted birthweight in the complete cohort.

Well-trained staff in community health centres obtained childhood growth characteristics (length and weight) according to standard schedules and procedures at 6, 12, 24, 36 and 48 months of age. Growth characteristics at 72 months were obtained in a dedicated research centre following standardised protocols. We calculated BMI (kg/m^2) at each time point from 6 months onwards. Standard deviation scores (SDS) were constructed for all growth characteristics using reference charts from the complete cohort for fetal measurements (14), northern European growth charts for birth measurements (16) and Dutch growth reference charts for childhood growth characteristics (Growth Analyzer 3.0; Dutch Growth Research Foundation, Rotterdam, the Netherlands) (17).

Childhood insulin and C-peptide levels

At 6 years of age, 30 min fasting venous blood samples were obtained in a well-equipped and dedicated research centre in the Erasmus Medical Center Sophia Children's hospital, Rotterdam, the Netherlands (12). All blood samples were stored for a maximum of 4 h at 4°C. Blood samples were transported twice daily to the laboratory facility of the regional laboratory in Rotterdam, the Netherlands (STAR-MDC), where they were processed and stored within 4 h of venous puncture (13). Insulin (pmol/l) and C-peptide levels (nmol/l) were obtained enzymatically using a Cobas 8000 Analyzer (Roche, Almere, the Netherlands). Quality control samples demonstrated intra- and inter-assay coefficients of variation of 1.39% and 2.40%,

respectively. As C-peptide is secreted in equal amounts but has a longer half-life compared with insulin, we included C-peptide levels as an additional, more stable, outcome measure.

Covariates

We assessed maternal age, pre-pregnancy weight, parity, ethnicity, educational level and folic acid use by questionnaire at enrolment in the study. Maternal height was measured and pre-pregnancy BMI was calculated. Smoking during pregnancy was repeatedly assessed by questionnaire throughout pregnancy. We obtained information on gestational hypertensive disorders (gestational hypertension and preeclampsia) and gestational diabetes from medical records (18). Information on breastfeeding and the timing of introduction to solid foods was assessed by questionnaire during infancy, and the average time watching television was assessed by questionnaire at 6 years of age.

Statistical analysis

First, we assessed the associations of gestational age at birth (preterm, term), birthweight (low, normal, high) and size for gestational age at birth (small, appropriate, large) with childhood insulin and C-peptide levels using multiple linear regression models. Second, to identify specific critical periods of growth associated with childhood insulin and C-peptide levels, we used conditional growth modelling (19-21). We constructed length, weight and BMI growth variables, which are statistically independent from each other, using the standardised residuals obtained from the linear regression models of length, weight and BMI regressed on all prior corresponding growth measurements (**Supplemental Methods 1**). This enables inclusion of the growth measurements at all time points simultaneously in one model, and thus to estimate the independent and mutually adjusted influence of growth during each time interval on childhood insulin and C-peptide levels (19-21). Third, in order to examine the associations of longitudinal fetal and childhood growth patterns with childhood insulin and C-peptide levels, we used unbalanced repeated measurement models. These models allow for incomplete outcome data and take the correlation between repeated measurements of the same participant into account by modelling the correlated errors of these measurements. For these analyses, we constructed quartiles of childhood insulin and C-peptide levels. These categories were included in the models as intercept and as interaction terms with (gestational) age to study the (gestational) age-independent effects (difference constant over time) as well as (gestational) age-dependent effects (difference non-constant over time). The actual models are described in more detail in **Supplemental Methods 2**.

All models were first adjusted for the child's sex and age at insulin and C-peptide measurement only (basic models), and were subsequently adjusted for maternal and childhood sociodemographic and lifestyle related characteristics (adjusted models). The models for birth outcomes were also adjusted for childhood BMI at insulin and C-peptide measurement. We included covariates in the models based on their associations with the outcomes of inter-

est in previous studies, a significant association with the determinants and outcomes, or a change in effect estimates of >10%. The associations between all growth characteristics and childhood insulin and C-peptide levels were linear. We tested for interactions between birthweight and BMI at the age of insulin and C-peptide measurement, birthweight and child's sex, and BMI at the age of insulin and C-peptide measurement and child's sex in the models described above, but no significant interactions were present (p values for interaction >0.05). In order to obtain normal distributions, we square root transformed insulin and C-peptide levels. SDS were constructed for insulin and C-peptide levels, defined as (observed value minus mean value of the reference population)/SD, to enable comparison of effect estimates. We used multiple imputation for missing values of covariates (<32%) and for the growth characteristics (<46%) for conditional growth modelling only by generating five independent datasets using the Markov Chain Monte Carlo (MCMC) method. Pooled effect estimates were presented. We performed a sensitivity analysis in children with growth characteristics available at all time points, which did not materially change the main findings (results not shown). The repeated measurement analyses were performed using the Statistical Analysis System version 9.3 (SAS Institute, Cary, NC, USA). All other analyses were performed using the Statistical Package of Social Sciences version 21.0 for Windows (IBM, Armonk, NY, USA).

RESULTS

Study population

Table 1 shows the characteristics of the study population. At 6 years of age, the median insulin and C-peptide levels were 112 pmol/l (95% range 17, 395) and 0.95 nmol/l (95% range 0.30, 2.14), respectively. **Supplemental Table 1** shows the growth characteristics at each time point. Nonresponse analysis showed that the mothers of children not included in the analyses were more likely to have a lower level of education and to smoke more often compared with the mothers of children included in the analyses (**Supplemental Table 2**). Furthermore, children not included in the analysis were less often from European descent. No or minor differences in childhood growth characteristics were observed between the groups.

Critical periods of early growth

We did not observe associations of gestational age at birth, birthweight or size for gestational age at birth with childhood insulin levels in the basic or the adjusted models (**Table 2**). After additional adjustment for childhood current BMI, tendencies to inverse associations of birthweight and size for gestational age at birth with childhood insulin levels were present. The results were similar for childhood C-peptide levels (**Supplemental Table 3**).

Figure 1 shows the associations of growth during specific time intervals, conditional on prior growth measurements, with childhood insulin levels (adjusted models). No associations

Table 1. Characteristics of the study population

	Total group
	N=4,328
Maternal characteristics	
Age at intake (years), mean (SD)	30.7 (5.1)
Height (cm), mean (SD)	167.7 (7.5)
Pre-pregnancy weight (kg), median (95% range)	64.0 (49.0, 98.0)
Pre-pregnancy BMI (kg/m^2), median (95% range)	22.7 (18.1, 34.5)
Highest education completed, *n* (%)	
Primary	343 (8.7)
Secondary	1692 (43.1)
Higher	1887 (48.1)
Parity, nulliparous (%)	2299 (55.1)
Ethnicity, European (%)	2605 (61.9)
Folic acid use, yes (%)	2239 (75.3)
Smoking during pregnancy, yes (%)	601 (16.0)
Gestational diabetes, yes (%)	42 (1.0)
Pre-eclampsia, yes (%)	65 (1.8)
Gestational hypertension, yes (%)	155 (4.2)
Child characteristics	
Sex, male (%)	2235 (51.6)
Gestational age at birth (weeks), median (95% range)	40.1 (35.8, 42.3)
Birthweight (g), median (95% range)	3450 (2261, 4474)
Ever breastfed, yes (%)	3150 (92.5)
Introduction of solid foods before 6 months, yes (%)	2328 (89.9)
Ethnicity, European (%)	2730 (64.8)
Television watching >2 h/day, *n* (%)	672 (19.9)
Age at 6-year follow-up examination (years), median (95% range)	6.0 (5.7, 8.0)
BMI (kg/m^2), median (95% range)	15.9 (13.7, 21.2)
Insulin at 6 years (pmol/l), median (95% range)	112 (17, 395)
C-peptide at 6 years (nmol/l), median (95% range)	0.95 (0.30, 2.14)

were observed for growth during specific periods in fetal life. Independent of growth in other time intervals, weight growth in each time interval from birth onwards, length growth in each time interval from 6 months onwards and BMI growth in each time interval from 12 months onwards were positively associated with childhood insulin levels ($p < 0.05$). The strongest associations were present for weight and BMI growth between 48 and 72 months. The results obtained from the basic model were consistent (**Supplemental Figure 2**). Similar results were also observed for childhood C-peptide levels (**Supplemental Figures 2 and 3**).

Table 2. Associations of birth outcomes with childhood insulin levels (*n*=4286)

Birth outcome	*n*	Insulin (SDS [95% CI])		
		Basic model	**Adjusted model**	**BMI-adjusted model**
Gestational age at birth				
Preterm (<37 weeks)	213	0 (-0.14, 0.14)	-0.03 (-0.18, 0.13)	-0.07 (-0.22, 0.08)
Term (≥37 weeks)	4051	Reference	Reference	Reference
p value for trend	4264	0.957	0.743	0.358
Birthweight				
Low (≤2500 g)	187	-0.01 (-0.16, 0.14)	0.06 (-0.12, 0.23)	0.12 (-0.06, 0.29)
Normal (2500–3999 g)	3474	Reference	Reference	Reference
High (≥4000 g)	625	-0.03 (-0.12, 0.05)	-0.07 (-0.16, 0.02)	-0.13 (-0.22, -0.04)*
p value for trend	4286	0.529	0.098	0.002
Size for gestational age				
SGA (≤10th percentile)	363	-0.01 (-0.11, 0.10)	0.01 (-0.10, 0.12)	0.08 (-0.03, 0.19)
AGA (10–90th percentile)	3447	Reference	Reference	Reference
LGA (≥90th percentile)	444	-0.04 (-0.14, 0.06)	-0.06 (-0.16, 0.04)	-0.12 (-0.22, -0.02)*
p value for trend	4254	0.635	0.276	0.005

Values are regression coefficients that reflect the differences in insulin SDS between the groups of the different birth outcomes

The basic models are adjusted for child's sex and age at insulin and C-peptide measurement. The adjusted models are further adjusted for maternal pre-pregnancy BMI, maternal age, parity, smoking during pregnancy, folic acid use, maternal education level, gestational diabetes, gestational hypertensive disorders, ethnicity of the child, gestational age at birth (for birthweight) and birthweight (for gestational age at birth). The BMI-adjusted models are the adjusted models with additional adjustment for childhood BMI at insulin and C-peptide measurement

p values for trend were obtained by entering the categorical variables to the models as continuous variables

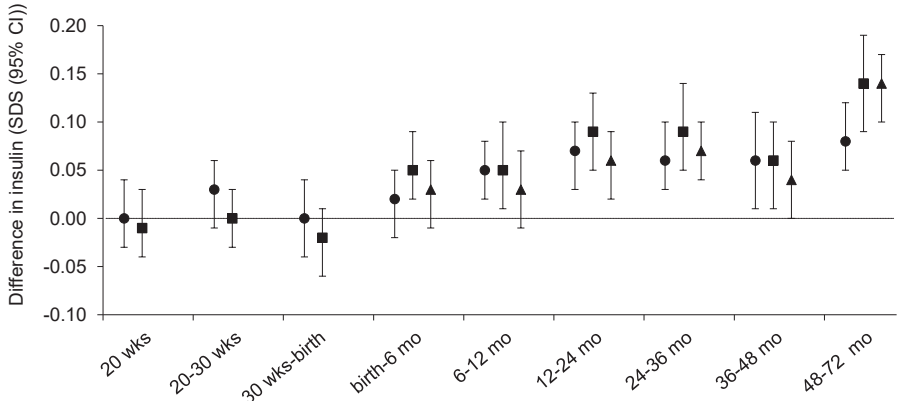
*$p<0.05$

AGA, appropriate for gestational age; LGA, large for gestational age; SGA, small for gestational age

Fetal and childhood growth patterns

Figure 2a–c shows the growth patterns from fetal life onwards for children in the higher childhood insulin quartiles compared with children in the lowest childhood insulin quartile. The overall growth patterns for fetal weight and length did not differ between the insulin quartiles (p values for trend >0.05). During childhood, length, weight and BMI gain were faster for children in the higher insulin quartiles (p values for trend <0.05). The largest differences were observed for children in the highest insulin quartile. Compared with children in the lowest insulin quartile, children in the highest insulin quartile were taller from birth onwards. Furthermore, they had a higher weight and BMI from 24 months onwards. These differences increased with age. **Supplemental Figure 4a-c** shows the growth patterns associated with childhood C-peptide levels, which were similar to those for insulin levels. The regression coefficients for (gestational) age-independent (intercept) and (gestational) age-dependent effects (interaction between the insulin or C-peptide quartiles and (gestational) age) are given in **Supplemental Tables 4 and 5**.

Figure 1. Associations of fetal and childhood growth, conditional on prior measurements, with childhood insulin levels (*n*=4293)



Values are regression coefficients representing differences in childhood insulin SDS per standardised residual change in growth characteristic in each time interval. The standardised residuals were obtained from models in which the growth measures of interest were regressed on the prior corresponding growth measures. For models presented for length and weight, the initial measure of size (starting point) was at 20 weeks of gestation (FL and EFW), and for BMI at 6 months of age. The models are adjusted for child's sex, age at insulin and C-peptide measurement, maternal pre-pregnancy BMI, maternal age, parity, smoking during pregnancy, folic acid use, maternal education level, gestational diabetes, gestational hypertensive disorders, ethnicity of the child, gestational age at birth, breastfeeding, timing of introduction of solid foods and time watching television. Circles, length; squares, weight; triangles, BMI; wks, weeks; mo, months.

DISCUSSION

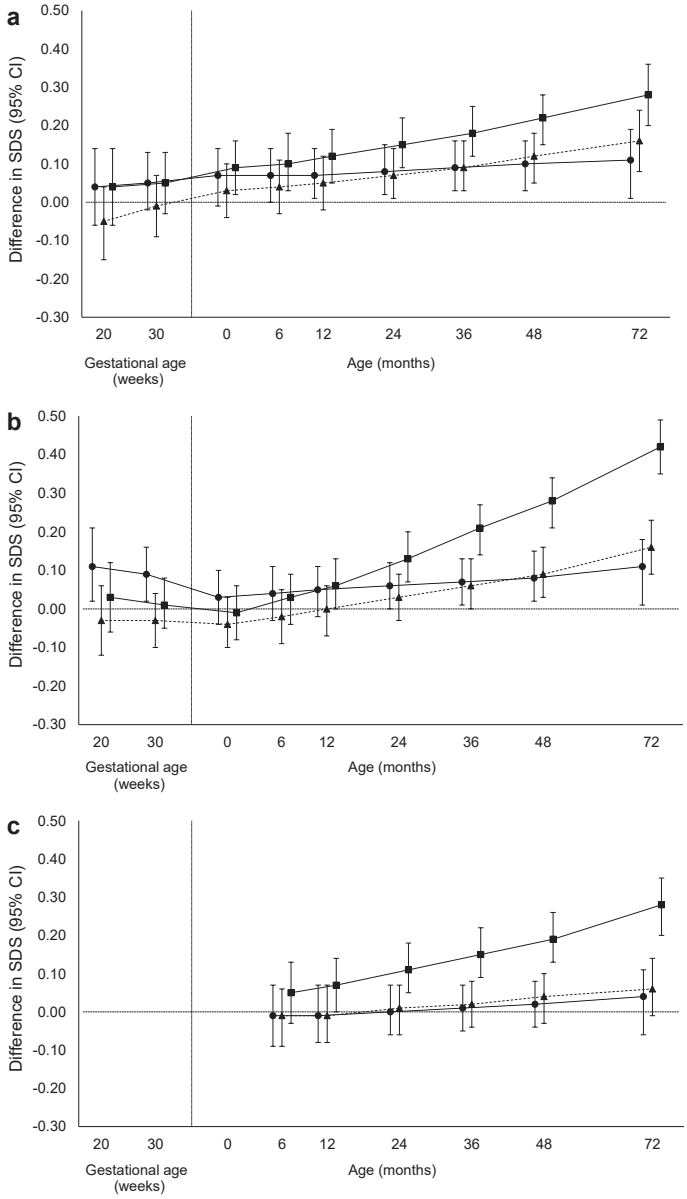
In this prospective population-based cohort study, rapid length, weight and BMI growth from birth onwards was associated with higher childhood insulin levels. Although the strongest associations were present for weight and BMI growth between 48 and 72 months, we also observed independent associations of postnatal weight and BMI growth with childhood insulin levels at earlier time intervals. No associations were observed for fetal growth characteristics.

Strengths and limitations

In this prospective population-based study, we had repeatedly measured growth characteristics available from fetal life onwards, enabling us to study the combined associations of fetal and childhood growth characteristics with childhood insulin and C-peptide levels. Of all eligible children at baseline, 54% were not included in the analyses. We consider it unlikely that this led to selection bias, since no or only minor differences were observed between the growth characteristics of children included in the analyses and children not included in the analyses.

The fasting time before venous puncture was limited. Due to the design of the study and the young age of the children, we were not able to collect blood samples after a longer fasting period (12). This may have led to some non-differential misclassification and un-

Figure 2. Fetal and childhood growth patterns according to insulin quartile (*n*=4293)



Results are based on repeated linear regression models and reflect the differences in SDS of (**a**) length (based on 39,022 measurements), (**b**) weight (based on 42,630 measurements) and (**c**) BMI (based on 23,277 measurements) growth in children with insulin levels in the second, third and fourth quartiles (insulin levels 62.7–112.4, 112.5–186.9 and 187.0–569.7 pmol/l, respectively), compared with those with insulin levels in the first quartile (2.7–62.6 pmol/l). The reference value is an SDS of 0. The models were adjusted for child's sex, maternal pre-pregnancy BMI, maternal age, parity, smoking during pregnancy, folic acid use, maternal education level, gestational diabetes, gestational hypertensive disorders, ethnicity of the child, gestational age at birth, breastfeeding, timing of introduction of solid foods and time watching television. Circles/dotted line, second insulin quartile; triangles/dashed line, third insulin quartile; squares/solid line, fourth insulin quartile.

derestimation of the observed effect estimates. This may especially affect childhood insulin levels, which are less stable and have a shorter half-life compared with C-peptide levels. In addition, information on glucose levels was not available and therefore we were unable to estimate insulin sensitivity. However, as blood glucose levels are less variable than insulin levels, especially in children, and most of the variability in insulin sensitivity is due to insulin levels, we consider insulin levels to be a useful proxy of insulin sensitivity (22, 23). Further studies are needed to assess the associations of fetal and childhood growth with detailed and fasting measures of offspring glucose and insulin metabolism.

As insulin is known to stimulate growth and fat deposition, it might be possible that the associations observed were due to reverse causation (24). However, as information on insulin and C-peptide levels was only available at 6 years of age, we were not able to assess this possibility in our study.

The prospective design of the study from early pregnancy onwards enabled us to take into account numerous potential maternal and childhood confounders. However, some residual confounding might be present in the reported effect estimates as, for example, detailed dietary information was not available.

Interpretation of main findings

An accumulating body of evidence suggests that adverse exposures in early life influence the risk of type 2 diabetes in later life. Multiple studies have reported associations of birthweight with measures of glucose and insulin metabolism in children as well as adults, some of them depending on adjustment for current body size (2-4, 9, 25). We did not observe associations of gestational age at birth, birthweight or size for gestational age at birth with childhood insulin or C-peptide levels. After adjustment for childhood current BMI, tendencies to inverse associations for birthweight and size for gestational age at birth were present. However, it has been argued that adjusting early size for later size reflects the change in size between these two time points (26). This suggests that the change between the growth characteristics at birth and in childhood is related to childhood insulin and C-peptide levels rather than birthweight per se.

Thus far, critical periods from fetal life onwards associated with the development of type 2 diabetes remain unclear, as previous studies mainly used birthweight as a proxy of fetal growth. Among 123 Danish adolescents, fetal growth velocity during the third trimester was not associated with any measure of insulin or glucose metabolism (4). In our own study cohort, first trimester crown to rump length was not associated with childhood insulin levels (5). In childhood, results from the Helsinki birth cohort suggest that growth during the first 6 months is critical for the development of insulin resistance in adulthood (9). In a study of 1124 children from England and Wales, both a lower birthweight and a higher childhood ponderal index were associated with higher fasting and postload insulin levels at 10–11 years, with stronger effect estimates for the childhood ponderal index (25). Furthermore, a

recent study among 3301 European children with a mean age of 8.5 years showed that rapid BMI growth between 9 months and 6 years was related to a higher risk of insulin resistance (27). In our current study, growth during fetal life was not associated with childhood insulin or C-peptide levels, independent of growth in other time intervals. Weight growth from birth onwards, length growth from 6 months onwards and BMI growth from 12 months onwards were independently and positively associated with childhood insulin and C-peptide levels. The strongest associations were present for weight and BMI growth between 48 and 72 months. These results suggest that growth during childhood, especially in weight and BMI, is important for the development of a suboptimal insulin metabolism in childhood, irrespective of growth during fetal life.

Observations from previous studies suggest that a growth pattern characterised by a low birthweight, followed by a rapid childhood weight gain, is associated with increased risk of insulin resistance or type 2 diabetes in later life (9-11). We observed no associations of fetal growth patterns with childhood insulin and C-peptide levels. During childhood, children with higher insulin and C-peptide levels had a growth pattern characterised by a high weight and BMI, which increased throughout childhood. This suggests that children with relatively high childhood insulin or C-peptide levels grow faster during childhood but might not have grown differently during fetal life.

Thus, our results suggest that rapid growth throughout childhood is important for the development of suboptimal insulin metabolism in childhood, rather than fetal growth. It has been proposed that adverse exposures during fetal life lead to reduced muscle mass, reduced muscle sensitivity to insulin, and changes in the structure and function of the beta cells, which may subsequently lead to insulin resistance and beta cell dysfunction in later life (28-30). Since only childhood growth characteristics were related to childhood insulin and C-peptide levels in our study, the mechanisms underlying these associations may involve differences in the body composition of the child, for example an increased body fat mass resulting from rapid postnatal growth or genetic influences, rather than developmental adaptations during fetal life (21, 31-33). However, it is possible that associations of fetal growth with insulin or glucose metabolism become apparent later in life. Furthermore, the widely described associations of low birthweight with increased risk of type 2 diabetes may be explained by beta cell dysfunction, which we were unable to assess in this study (34). If our findings are confirmed by other studies with directly measured fetal growth data, detailed childhood growth data and more detailed measures of childhood glucose and insulin metabolism, including measures of beta cell function, our results underline the importance of strategies aimed at preventing rapid weight gain throughout childhood to improve insulin metabolism in later life.

Conclusions

Our results suggest that rapid length, weight and BMI gain from birth onwards, but not growth during fetal life, is associated with the development of suboptimal insulin metabolism in childhood. Further research is needed to replicate our findings and to explore the underlying mechanisms.

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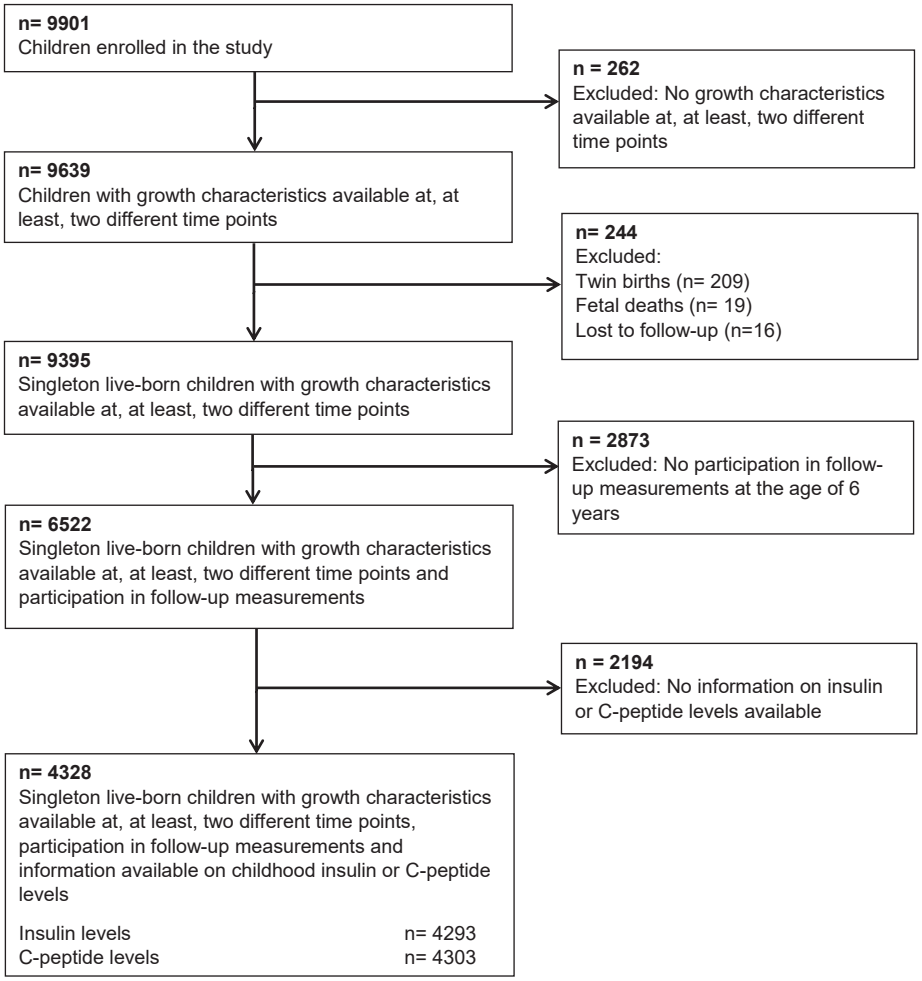
SUPPLEMENTAL MATERIAL

Supplemental Figure 1. Flow chart of the study participants



Supplemental Methods 1. Conditional growth modelling

We used conditional growth modelling in order to identify critical periods of growth associated with childhood insulin and C-peptide levels (1-3). We first calculated the predicted value of the growth measurement at the time point of interest, using a linear regression model including all prior corresponding growth measurements. The standardised residuals of these models indicate to what degree a growth measure of interest differs from that predicted by all previous growth measures, and represent the excess growth during the time interval prior to the growth measurement of interest. As these standardised residuals are not correlated, we subsequently included the standardised residuals plus the initial measure of size (femur length and estimated fetal weight at 20 weeks of gestation for models of length and weight, and BMI at the age of 6 months for models of BMI) simultaneously in one linear regression model, estimating the effect of growth during each time interval on childhood insulin or C-peptide levels. The regression coefficients of these models represent the difference in childhood insulin or C-peptide levels per standardised residual change in growth in each time interval, adjusted for growth during the other time intervals. Conditional growth modelling enables us to examine the associations of growth during specific time-periods, independent of growth in other time intervals and to compare the strength of the effect estimates between time intervals. This method thus allows us to identify periods of growth which are most important for later childhood insulin and C-peptide levels (1-3).

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Supplemental Methods 2. Unbalanced repeated measurement models

We analysed the fetal and childhood growth patterns among children in the 2nd, 3rd and 4th insulin and C-peptide quartiles, as compared to children in the 1st quartiles using unbalanced repeated measurement regression models. These models allow for incomplete outcome data and take the correlation between repeated measurements of the same participant into account by modelling the correlated errors of these measurements (1, 2). As fetal and childhood growth were defined using different measures (femur length vs. body length and estimated fetal weight vs. measured weight), we constructed best fitting models for fetal and childhood growth separately. First, we constructed linear models for fetal and childhood

growth characteristics separately and subsequently tested whether adding higher polynomials of (gestational) age to the models improved the fit of the models by checking the goodness of fit (smallest –2 log likelihood). Since higher polynomials of (gestational) age did not considerably improve the fit of the models and to prevent overfitting of the models, we kept the models as simple as possible and did not include higher polynomials of (gestational) age to the models. To model the correlated errors, a compound symmetry covariance structure was assumed for the models for fetal growth, indicating that measurements are correlated equally. For the childhood models, we assumed an autoregressive covariance structure, given that measurements closer in time tend to be correlated more strongly. Using alternative covariance structures did not change the results.

The final models can be written as:

Fetal length (SDS) = ß0 + ß1 × insulin or C-peptide quartile + ß2 × gestational age + ß3 × insulin or C-peptide quartile × gestational age

Childhood length (SDS) = ß0 + ß1 × insulin or C-peptide quartile + ß2 × age + ß3 × insulin or C-peptide quartile × age

Fetal weight (SDS) = ß0 + ß1 × insulin or C-peptide quartile + ß2 × gestational age + ß3 × insulin or C-peptide quartile × gestational age

Childhood weight (SDS) = ß0 + ß1 × insulin or C-peptide quartile + ß2 × age + ß3 × insulin or C-peptide quartile × age

BMI (SDS)= ß0 + ß1 × insulin or C-peptide quartile + ß2 × age + ß3 × insulin or C-peptide quartile × age

In these models, 'ß0 + ß1 × insulin or C-peptide quartile' reflects the intercept. The intercept reflects the mean growth characteristic value in SDS for each insulin or C-peptide quartile. The term 'ß2 × (gestational) age' reflects the change in growth characteristics per week (fetal models) or month (childhood models). The term 'ß3 × insulin or C-peptide quartile × (gestational) age', reflects the difference in change in growth characteristics per week (fetal models) or month (childhood models) between the different insulin or C-peptide quartiles. For presentation purposes, we obtained point estimates from these models at time points of interest. The regression coefficients for (gestational) age-independent (intercept: ß0 + ß1 × insulin or C-peptide quartile) and (gestational) age-dependent differences (slope: ß3 × insulin or C-peptide quartile × (gestational) age) are given in Supplemental Tables 4 and 5 below.

References

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Supplemental Table 1. Fetal and childhood growth characteristics

	Total group
	n=4328
Second trimester	
Gestational age (weeks), median (95% range)	20.5 (18.6, 23.3)
Femur length (mm), mean (SD)	34 (4)
Estimated fetal weight (g), median (95% range)	364 (246, 624)
Third trimester	
Gestational age (weeks), median (95% range)	30.4 (28.4, 33.1)
Femur length (mm), mean (SD)	58 (3)
Estimated fetal weight (g), median (95% range)	1608 (1175, 2232)
Birth	
Gestational age at birth (weeks), median (95% range)	40.1 (35.8, 42.3)
Birth length (cm), mean (SD)	50.2 (2.3)
Birthweight (g), median (95% range)	3450 (2261, 4474)
6 months	
Age at follow-up (months), median (95% range)	6.2 (5.2, 8.3)
Length (cm), mean (SD)	67.7 (2.6)
Weight (kg), mean (SD)	7.9 (0.9)
BMI (kg/m^2), mean (SD)	17.2 (1.4)
12 months	
Age at follow-up (months), median (95% range)	11.1 (10.1, 125)
Length (cm), mean (SD)	74.4 (2.6)
Weight (kg), mean (SD)	9.7 (1.1)
BMI (kg/m^2), mean (SD)	17.4 (1.4)
24 months	
Age at follow-up (months), median (95% range)	11.1 (10.1, 12.5)
Height (cm), mean (SD)	88.8 (3.7)
Weight (kg), mean (SD)	13.0 (1.6)
BMI (kg/m^2), median (95% range)	16.5 (14.1, 19.6)
36 months	
Age at follow-up (months), median (95% range)	37.7 (35.3, 40.7)
Height (cm), mean (SD)	97.5 (3.8)
Weight (kg), mean (SD)	15.3 (1.9)
BMI (kg/m^2), mean (SD)	16.0 (1.3)

Supplemental Table 1. Fetal and childhood growth characteristics (continued)

	Total group
	n=4328
48 months	
Age at follow-up (months), median (95% range)	45.7 (44.4, 48.4)
Height (cm), mean (SD)	103.3 (4.1)
Weight (kg), mean (SD)	17.0 (2.2)
BMI (kg/m^2), mean (SD)	15.8 (1.4)
72 months	
Age at follow-up (months), median (95% range)	72.6 (68.2, 96.3)
Height (cm), mean (SD), cm	119.7 (6.1)
Weight (kg), median (95% range)	22.6 (17.6, 34.4)
BMI (kg/m^2), median (95% range)	15.9 (13.7, 21.2)

Supplemental Table 2. Non-response analysis of children included and not included in the analyses (n=9395)

	Included in the analyses	Not included in the analyses	p-value[a]
	n=4328	n=5067	
Maternal characteristics			
Age at intake (years), mean (SD)	30.7 (5.1)	29.2 (5.5)	<0.001
Height (SD), mean (SD)	167.7 (7.5)	166.6 (7.3)	<0.001
Pre-pregnancy weight (kg), median (95% range)	64.0 (49.0, 98.0)	63.0 (47.0, 100.0)	0.039
Pre-pregnancy BMI (kg/m^2), median (95% range)	22.7 (18.1, 34.5)	22.6 (17.8, 35.5)	0.684
Highest education completed, n (%)			
Primary	343 (8.7)	565 (13.0)	<0.001
Secondary	1692 (43.1)	2117 (48.7)	
Higher	1887 (48.1)	1662 (38.3)	
Parity, Nulliparous (%)	2299 (55.1)	2709 (55.4)	0.747
Ethnicity, European (%)	2605 (61.9)	2502 (54.5)	<0.001
Folic acid use, yes (%)	2239 (75.3)	2252 (66.5)	<0.001
Smoking during pregnancy, yes (%)	601 (16.0)	837 (19.8)	<0.001
Gestational diabetes, yes (%)	42 (1.0)	57 (1.2)	0.444
Pre-eclampsia, yes (%)	65 (1.8)	103 (2.4)	0.060
Gestational hypertension, yes (%)	155 (4.2)	155 (3.6)	0.167
Child characteristics			
Sex, male (%)	2235 (51.6)	2522 (49.8)	0.074
Gestational age at birth (weeks), median (95% range)	40.1 (35.8, 42.3)	39.8 (35.3, 42.3)	0.038
Birthweight (g), median (95% range)	3450 (2261, 4474)	3400 (2190, 4510)	<0.001
Ever breastfed, yes (%)	3150 (92.5)	2919 (91.4)	0.086
Timing of introduction of solid foods, No. before 6 months (%)	2328 (89.9)	1941 (89.0)	0.300
Ethnicity, European (%)	2730 (64.8)	2686 (58.3)	<0.001
Age at follow-up examination at 24 months (months), median (95% range)	25.0 (23.4, 31.4)	25.0 (23.4, 31.3)	0.815
BMI at the age of 24 months (kg/m^2), mean (SD)	16.6 (1.4)	16.6 (1.5)	0.590
Age at follow-up examination at 48 months (months), median (95% range)	45,8 (44.4, 48.4)	45.8 (44.5, 48.7)	0.004
BMI at the age of 48 months, mean (SD), kg/m^2	15.8 (1.4)	15.9 (1.5)	0.033
Age at follow-up examination at 6 years (years), median (95% range)	6.0 (5.7, 8.0)	6.0 (5.6, 7.6)	<0.001
BMI at the age of 6 years (kg/m^2), mean (SD)	16.2 (1.8)	16.2 (2.0)	0.651

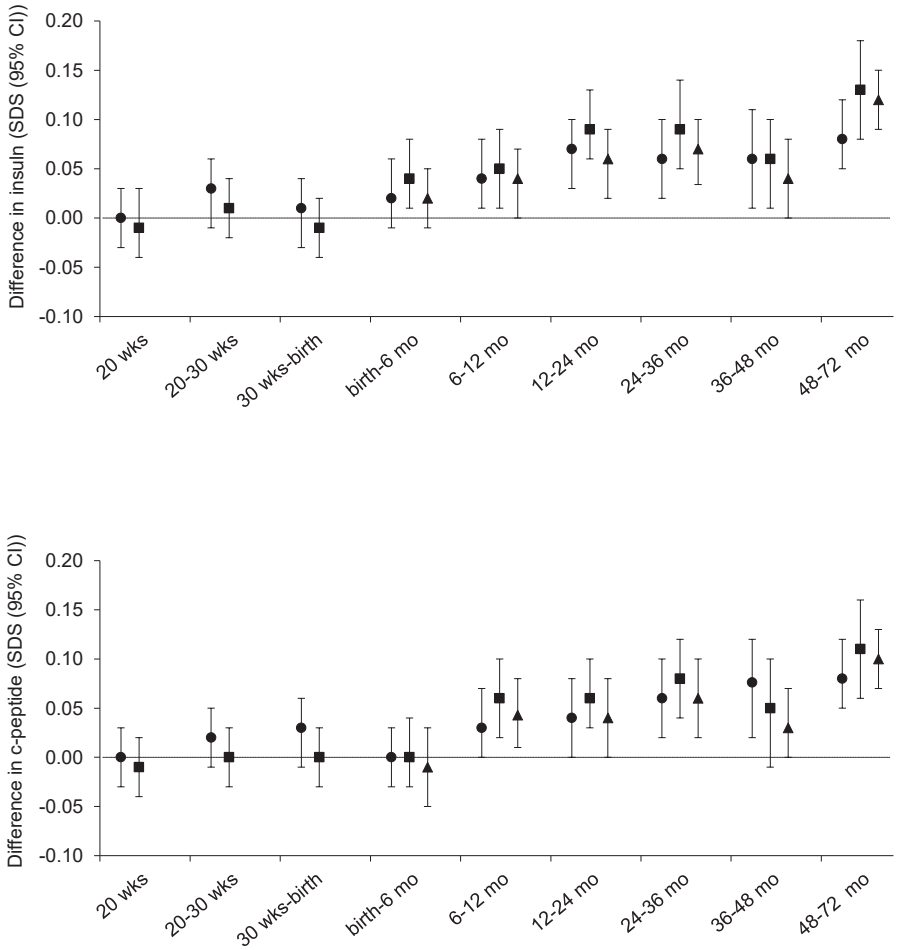
[a] Differences in participant characteristics between the groups were tested using Independent Samples T-tests for continuous variables and Chi-square tests for proportions.

Supplemental Table 3. Associations of birth outcomes with childhood C-peptide levels (n=4321)

		C-peptide (SDS (95% CI))		
Birth outcome	**n**	**Basic model**	**Adjusted model**	**BMI adjusted model**
Gestational age at birth				
Preterm (<37 weeks)	215	0.03 (-0.11, 0.17)	0 (-0.15, 0.16)	-0.04 (-0.19, 0.12)
Term (≥ 37 weeks)	4083	Reference	Reference	Reference
p-value for trend	4298	0.683	0.975	0.647
Birthweight				
Low (≤2500 g)	191	0.09 (-0.06, 0.24)	0.15 (-0.03, 0.32)	0.20 (0.03, 0.37)*
Normal (2500-3999 g)	3503	Reference	Reference	Reference
High (≥4000 g)	627	0 (-0.09, 0.08)	-0.05 (-0.14, 0.04)	-0.10 (-0.19, -0.01)*
p-value for trend	4321	0.465	0.095	0.005
Size for gestational age				
SGA (≤ 10th percentile)	368	0.03 (-0.08, 0.14)	0.05 (-0.06, 0.16)	0.10 (-0.01, 0.21)
AGA (10 - 90th percentile)	3475	Reference	Reference	Reference
LGA (≥90th percentile)	445	0 (-0.10, 0.10)	-0.03 (-0.13, 0.07)	-0.08 (-0.18, 0.02)
p-value for trend	4288	0.730	0.236	0.011

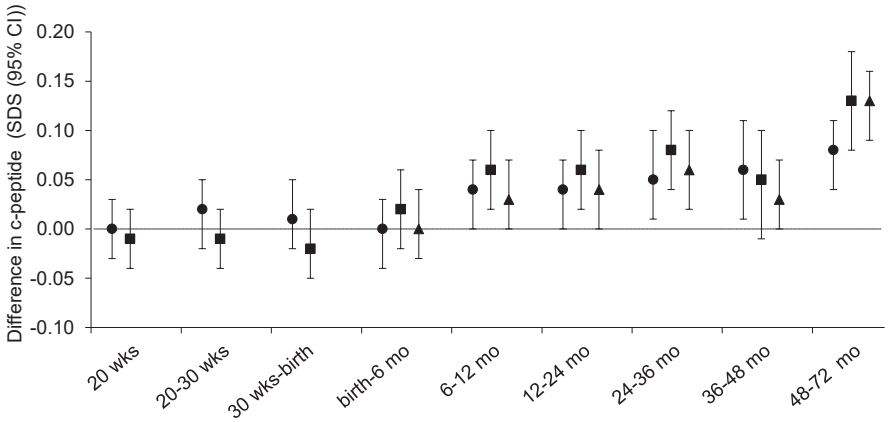
Values are regression coefficients that reflect the differences in C-peptide SDS between the groups of the different birth outcomes. The basic models are adjusted for child's sex and age at insulin and C-peptide measurement. The adjusted models are further adjusted for maternal pre-pregnancy BMI, maternal age, parity, smoking during pregnancy, folic acid use, maternal education level, gestational diabetes, gestational hypertensive disorders, ethnicity of the child, gestational age at birth (for birthweight) and birthweight (for gestational age at birth). The BMI-adjusted models are the adjusted models with additional adjustment for childhood BMI at insulin and C-peptide measurement. P-values for trend were obtained by entering the categorical variables to the models as continuous variables. *p <0.05. AGA, Appropriate for gestational age. LGA, Large for gestational age; SGA: Small for gestational age

Supplemental Figure 2. Associations of fetal and childhood growth conditional on prior measurements with childhood insulin and C-peptide levels (n=4328)



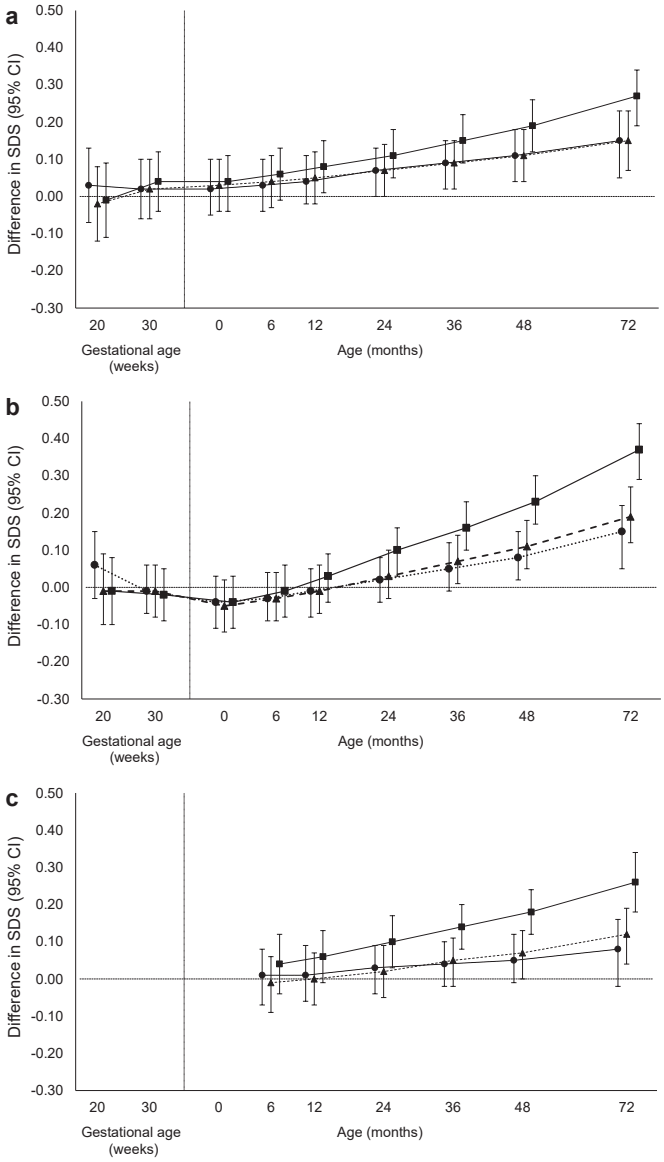
Values are regression coefficients representing differences in childhood insulin or C-peptide SDS per standardised residual change in growth characteristic in each time interval. The standardised residuals were obtained from models in which the growth measures of interest were regressed on the prior corresponding growth measures. For models presented for length and weight, the initial measure of size (starting point) was at 20 weeks of gestation (femur length and estimated fetal weight), and for BMI at 6 months of age. The models are adjusted for child's sex and age at insulin and C-peptide measurement. Circles, length; squares, weight; triangles, BMI; wks, weeks; mo, months.

Supplemental Figure 3. Associations of fetal and childhood growth conditional on prior measurements with childhood C-peptide levels (n=4303)



Values are regression coefficients representing differences in childhood C-peptide SDS per standardised residual change in growth characteristic in each time interval. The standardised residuals were obtained from models in which the growth measures of interest were regressed on the prior corresponding growth measures. For models presented for length and weight, the initial measure of size (starting point) was at 20 weeks of gestation (femur length and estimated fetal weight), and for BMI at 6 months of age. The models are adjusted for child's sex, age at insulin and C-peptide measurement, maternal pre-pregnancy BMI, maternal age, parity, smoking during pregnancy, folic acid use, maternal education level, gestational diabetes, gestational hypertensive disorders, ethnicity of the child, gestational age at birth, breastfeeding, timing of introduction of solid foods and time watching television. Circles, length; squares, weight; triangles, BMI; wks, weeks; mo, months.

Supplemental Figure 4. Fetal and childhood growth patterns according to C-peptide quartile (n=4303)



Results are based on repeated linear regression models and reflect the differences in SDS of (a) length (based on 39,086 measurements), (b) weight (based on 42,692 measurements) and (c) BMI (based on 23,310 measurements) growth in children with C-peptide levels in the second, third and fourth quartiles (C-peptide levels 0.67-0.95, 0.96-1.28 and 1.29-3.69 nmol/l, respectively), compared with those with C-peptide levels in the first quartile (0.11-0.66 nmol/l). The reference value is an SDS of 0. The models were adjusted for child's sex, maternal pre-pregnancy BMI, maternal age, parity, smoking during pregnancy, folic acid use, maternal education level, gestational diabetes, gestational hypertensive disorders, ethnicity of the child, gestational age at birth, breastfeeding, timing of introduction of solid foods and time watching television. Circles/dotted line, second C-peptide quartile; triangles/dashed line, third C-peptide quartile; squares/solid line, fourth C-peptide quartile.

Supplemental Table 4. Longitudinal associations between insulin levels and growth characteristics (n=4293)

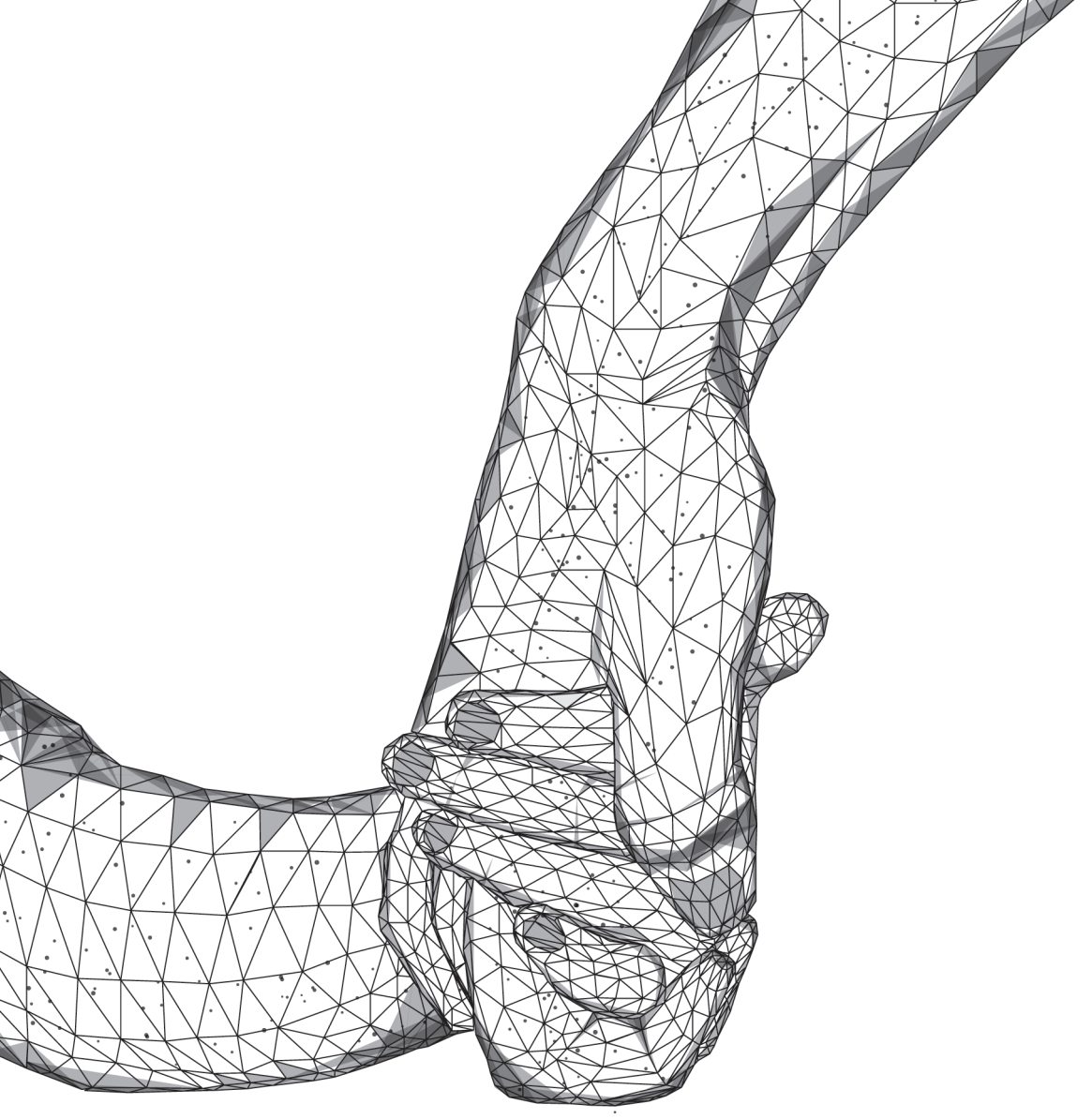
Childhood insulin levels	Intercept (SDS)	p-value	Slope (SDS (95% CI))	p-value
Height				
Fetal				
1st insulin quartile	-0.287	0.27	Reference	
2nd insulin quartile	-0.273	0.90	0.0013 (-0.0059, 0.0086)	0.72
3rd insulin quartile	-0.437	0.18	0.0048 (-0.0024, 0.0119)	0.19
4th insulin quartile	-0.273	0.90	0.0012 (-0.0060, 0.0084)	0.74
Childhood				
1st insulin quartile	-4.426	<0.001	Reference	
2nd insulin quartile	-4.358	0.07	0.0005 (-0.0004, 0.0017)	0.24
3rd insulin quartile	-4.395	0.41	0.0014 (0.0007, 0.0028)	<0.001
4th insulin quartile	-4.337	0.02	0.0022 (0.0016, 0.0037)	<0.001
Weight				
Fetal				
1st insulin quartile	-0.508	0.02	Reference	
2nd insulin quartile	-0.345	0.08	-0.0024 (-0.0080, 0.0031)	0.38
3rd insulin quartile	-0.547	0.68	0.0003 (-0.0052, 0.0058)	0.90
4th insulin quartile	-0.440	0.46	-0.0018 (-0.0073, 0.0037)	0.52
Childhood				
1st insulin quartile	-3.455	<0.001	Reference	
2nd insulin quartile	-3.421	0.34	0.0010 (0.0001, 0.0020)	0.03
3rd insulin quartile	-3.490	0.32	0.0027 (0.0017, 0.0037)	<0.001
4th insulin quartile	-3.463	0.78	0.0060 (0.0050, 0.0068)	<0.001
BMI				
1st insulin quartile	-1.821	<0.001	Reference	
2nd insulin quartile	-1.865	0.71	0.0008 (-0.0006, 0.0021)	0.26
3rd insulin quartile	-1.871	0.62	0.0012 (-0.0002, 0.0251)	0.08
4th insulin quartile	-1.865	0.50	0.0035 (0.0021, 0.0048)	<0.001

Values are regression coefficients obtained from linear repeated measurement models and reflect the (gestational) age independent differences (intercepts) and the (gestational) age dependent differences (slopes: change in growth characteristics SDS per week (fetal models) or per month (childhood models) per insulin quartile, compared with the reference group (1st insulin quartile). The models were adjusted for child's sex, maternal pre-pregnancy BMI, maternal age, parity, smoking during pregnancy, folic acid use, maternal education level, gestational diabetes, gestational hypertensive disorders, ethnicity of the child, gestational age at birth, breastfeeding, timing of introduction of solid foods and time watching television. P-values reflect the significance levels of the regression coefficients

Supplemental Table 5. Longitudinal associations between C-peptide levels and growth characteristics (n=4303)

Childhood C-peptide levels	Intercept (SDS)	p-value	Slope (SDS (95% CI))	p-value
Height				
Fetal				
1st C-peptide quartile	-0.279	0.29	Reference	
2nd C-peptide quartile	-0.220	0.60	-0.0012 (-0.0085, 0.0059)	0.73
3rd C-peptide quartile	-0.394	0.30	0.0046 (-0.0026, 0.0118)	0.21
4th C-peptide quartile	-0.398	0.29	0.0052 (-0.0021, 0.0125)	0.16
Childhood				
1st C-peptide quartile	-4.406	<0.001	Reference	
2nd C-peptide quartile	-4.383	0.54	0.0018 (0.0007, 0.0028)	<0.001
3rd C-peptide quartile	-4.375	0.42	0.0016 (0.0006, 0.0027)	<0.01
4th C-peptide quartile	-4.406	0.32	0.0032 (0.0021, 0.0042)	<0.001
Weight				
Fetal				
1st C-peptide quartile	-0.562	0.01	Reference	
2nd C-peptide quartile	-0.375	0.05	-0.0065 (-0.0120, -0.0010)	0.02
3rd C-peptide quartile	-0.556	0.95	-0.0006 (-0.0062, 0.0049)	0.83
4th C-peptide quartile	-0.554	0.93	-0.0009 (-0.0065, 0.0047)	0.75
Childhood				
1st C-peptide quartile	-3.440	<0.001	Reference	
2nd C-peptide quartile	-3.482	0.24	0.0026 (0.0017, 0.0035)	<0.001
3rd C-peptide quartile	-3.486	0.20	0.0033 (0.0024, 0.0043)	<0.001
4th C-peptide quartile	-3.482	0.25	0.0057 (0.0048, 0.0067)	<0.001
BMI				
1st C-peptide quartile	-1.872	<0.001	Reference	
2nd C-peptide quartile	-1.872	0.92	0.0011 (-0.0002, 0.0024)	0.10
3rd C-peptide quartile	-1.897	0.56	0.0019 (0.0006, 0.0033)	<0.01
4th C-peptide quartile	-1.852	0.63	0.0033 (0.0020, 0.0047)	<0.001

Values are regression coefficients obtained from linear repeated measurement models and reflect the (gestational) age independent effects (intercepts) and the (gestational) age dependent effects (slopes, change in growth characteristics SDS per week (fetal models) or per month (childhood models) per C-peptide quartile, compared with the reference group (1st C-peptide quartile). The models were adjusted for child's sex, maternal pre-pregnancy BMI, maternal age, parity, smoking during pregnancy, folic acid use, maternal education level, gestational diabetes, gestational hypertensive disorders, ethnicity of the child, gestational age at birth, breastfeeding, timing of introduction of solid foods and time watching television. P-values reflect the significance levels of the regression coefficients.



4.3 Metabolite concentrations in pregnant women and their children

Voerman E, Jaddoe VWV, Uhl O, Shokry E, Horak J, Felix JF, Koletzko B*, Gaillard R*. A population-based resource for intergenerational metabolomics analyses in pregnant women and their children: the Generation R Study.

**Denotes shared last authors*

Adapted from: Metabolomics. 2020;16(4):43.

ABSTRACT

Introduction: Adverse exposures in early life may predispose children to cardio-metabolic disease in later life. Metabolomics may serve as a valuable tool to disentangle the metabolic adaptations and mechanisms that potentially underlie these associations.

Objectives: To describe the acquisition, processing and structure of the metabolomics data available in a population-based prospective cohort from early pregnancy onwards and to examine the relationships between metabolite profiles of pregnant women and their children at birth and in childhood.

Methods: In a subset of 994 mothers-child pairs from a prospective population-based cohort study among pregnant women and their children from Rotterdam, the Netherlands, we used LC–MS/MS to determine concentrations of amino acids, non-esterified fatty acids, phospholipids and carnitines in blood serum collected in early pregnancy, at birth (cord blood), and at child's age 10 years.

Results: Concentrations of diacyl-phosphatidylcholines, acyl-alkyl-phosphatidylcholines, alkyl-lysophosphatidylcholines and sphingomyelines were the highest in early pregnancy, concentrations of amino acids and non-esterified fatty acids were the highest at birth and concentrations of alkyl-lysophosphatidylcholines, free carnitine and acyl-carnitines were the highest at age 10 years. Correlations of individual metabolites between pregnant women and their children at birth and at the age of 10 years were low (range between $r = -0.10$ and $r = 0.35$).

Conclusion: Our results suggest that unique metabolic profiles are present among pregnant women, newborns and school aged children, with limited intergenerational correlations between metabolite profiles. These data will form a valuable resource to address the early metabolic origins of cardio-metabolic disease.

INTRODUCTION

Cardio-metabolic diseases are of major public health concern (1-3). The pathogenesis of these cardio-metabolic diseases involves adaptations in metabolic pathways. Thus far, studies mainly focused on a small set of conventional biomarkers to assess metabolic status and pathways. Recent developments in high-throughput technologies and analytical methods have enabled the application of metabolomics for detailed characterization of an individual's metabolic status on a large scale (4-6). Metabolomics measures a large number of low molecular weight metabolites in biological tissues and fluids. The metabolome is the most downstream component of biological processes and closely linked to the phenotype. It carries information about gene expression as well as lifestyle- and environmental factors (5, 6). Metabolomics has already been successfully applied in large-scale epidemiological studies, mainly in adult populations, to identify new biomarkers of cardio-metabolic disease status, development and progression, as well as the underlying pathophysiological mechanisms (7-9).

Accumulating evidence suggests that cardio-metabolic diseases might originate in early life. Adverse exposures in early life may lead to developmental adaptations in organ structure or function, which may predispose these children to later cardio-metabolic disease (10). Early-life developmental adaptations in metabolic pathways may underlie these associations. Only a limited amount of metabolomics studies on the early origins of cardio-metabolic disease have been performed. Most of these studies were small and mainly assessed cross-sectional relationships (11, 12). Also, it is unclear whether metabolite profiles correlate between mothers and their children. The application of metabolomics in longitudinal birth cohort studies may serve as a valuable tool to identify biomarkers of metabolic status, in order to disentangle the mechanisms linking adverse exposures in early life to cardio-metabolic disease later in life (11).

Therefore, in a population-based cohort from early pregnancy onwards among 994 mother–child pairs from Rotterdam, the Netherlands, we obtained serum concentrations of a range of metabolite groups involved in energy metabolism, including amino acids (AA), non-esterified fatty acids (NEFA), phospholipids (PL), and carnitines (Carn) in maternal blood in early-pregnancy, and child's (cord-) blood at birth and at age 10 years. We provide a detailed description of the data acquisition, processing and data structure and examined the relationships between metabolite profiles of pregnant women and their children at birth and in childhood.

METHODS

Study population

The Generation R Study is a multi-ethnic population-based prospective cohort study from fetal life until adulthood in Rotterdam, the Netherlands, described in detail previously (13).

The study was approved by the Medical Ethical Committee of the Erasmus Medical Center, University Medical Center, Rotterdam (MEC 198.782/2001/31). Written informed consent was obtained from all mothers at enrollment in the study. Measurement of conventional biomarkers of metabolic status in pregnancy and childhood has been described previously (14-16). For metabolomics, 2,395 blood samples were analyzed from a subsample of 1041 Dutch mother–child pairs who had their blood drawn at birth (cord blood) and at least 1 other time point: early pregnancy (mother) or at the age of 10 years (child). A number of blood samples (n = 157) was excluded during data acquisition (e.g. low sample volumes, hemolytic samples) and processing (e.g. duplicate samples, high proportion of missing values, missing or non-Dutch ethnicity), leaving a total of 2,238 blood samples from 994 mother–child pairs available for analysis. Of these 994 mother–child pairs, a total of 814 mothers had early pregnancy data available, and 921 and 503 children had data available at birth and at the age of 10 years, respectively. Of all mothers included, 10 had a twin pregnancy. Metabolomics data was only available for one of the twins. Therefore, mothers with twin pregnancies were included only once in the dataset.

Sample collection and processing

Maternal early-pregnancy non-fasting blood samples were obtained at enrollment in the study [median gestational age: 12.8 weeks (95% range 9.9, 16.9)] by research nurses at one of the dedicated research centers (17). Umbilical venous cord blood samples were collected directly after birth [median gestational age at birth: 40.3 weeks (95% range 36.6, 42.4)] by a midwife or obstetrician. Child's non-fasting blood samples were obtained by research nurses at the 10-year follow-up visit to the research center [median age: 9.8 years (95% range 9.1, 10.6)]. All blood samples were transported to the regional laboratory (STAR-MDC), spun and stored at – 80°C for further studies within a maximum of 4 h after collection. For metabolite measurements, blood serum samples were transported on dry ice to the Division of Metabolic and Nutritional Medicine of the Dr. von Hauner Children's Hospital in Munich, Germany.

Metabolite measurements

A targeted metabolomics approach was adopted to determine serum concentrations (µmol/L) of AA, NEFA, PL and Carn (18). Detailed information is given in **Supplemental Text 1 and Supplemental Table 1**. Briefly, AA were analyzed with 1100 high-performance liquid chromatography (HPLC) system (Agilent, Waldbronn, Germany) coupled to a API2000 tandem mass spectrometer (AB Sciex, Darmstadt, Germany) (19). IUPAC-IUB Nomenclature was used for notation of AA (20). NEFA, PL and Carn were measured with a 1200 SL HPLC system (Agilent, Waldbronn, Germany) coupled to a 4000QTRAP tandem mass spectrometer from AB Sciex (Darmstadt, Germany) (21, 22). The analytical technique used is capable of determining the total number of total bonds, but not the position of the double bonds and the distribution of the carbon atoms between fatty acid side chains. We used the following

notation for NEFA, PL and Carn.a:X:Y, where X denotes the length of the carbon chain, and Y the number of double bonds. The 'a' denotes an acyl chain bound to the backbone via an ester bond ('acyl-') and the 'e' represents an ether bond ('alkyl-'). For analyses, we categorized metabolites in to general metabolite groups based on chemical structure (AA, NEFA, PC.aa, PC.ae, Lyso.PC.a, Lyso.PC.e, SM, Free Carn and Carn.a) and in detailed metabolite subgroups based on chemical structure and physiological and biological relevance (AA: BCAA, aromatic amino acids (AAA), essential AA, non-essential AA; NEFA, PC.aa, PC.ae, Lyso.PC.a, Lyso. PC.e and SM: saturated, mono-unsaturated, poly-unsaturated; Carn.a: short-chain, medium-chain, long-chain).

Quality control and pre-processing

To assess the precision of the measurements, six quality control (QC) samples per batch were consistently measured between study samples. After exclusion of outliers, the coefficients of variation (CV; SD/mean) for each batch (intra-batch) and for all batches (inter-batch) of the QC samples were calculated for each metabolite. In line with previous studies (23-26), for each metabolite we excluded batches with an intra-batch CV higher than 25%. Data on complete metabolites were excluded for metabolites with inter-batch CV higher than 35% or if less than 50% of the batches passed the QC (i.e. had an intra-batch CV lower than 25%). To correct for batch effects, the participant data at each time point were median corrected by dividing the metabolite concentration by the ratio of the intra-batch median and the inter-batch median of the QC samples (26). In line with previous studies, metabolites and participants with more than 50% of missing values were excluded (26, 27). Missing values in other participants were imputed using the Random Forest algorithm (R package *missForest*), which has been shown to perform well with MS data (28).

Statistical analysis

First, we calculated the sum of individual metabolite concentrations per general and detailed metabolite group and per time point. In order to explore the variability of the metabolites between participants and between time points, we obtained the median (95% range) for the individual metabolites and the summed metabolite concentrations per general and detailed metabolite group per time point. To enable comparison between time points, only metabolites that were present at each time point were included in the summed variables. Second, we explored the dimensionality of the data, by conducting principal component analyses (PCA) at each time point separately. As log transformations did not sufficiently normalize the metabolite concentrations, we used square root transformations to normalize metabolite concentrations. These normalized metabolite concentrations were subsequently standardized by calculating standard deviation scores [SDS; (observed value – mean)/SD]. Third, as we considered PCA not informative for describing the information contained in our dataset, we further explored the correlation structure of the data by calculating pair-

wise Pearson's correlations coefficients between all individual metabolites within each time point and between individual metabolites at different points. These correlations within and between time points were visualized using two circos plots (R package *circlize*) (29, 30). To facilitate presentation, the first plot only includes correlation coefficients < –0.15 and > 0.15. To display correlation coefficients that are at least of weak magnitude, the second plot displays correlation coefficients < –0.30 and > 0.30 (31). To obtain further insight in possible metabolic pathways, we additionally presented correlations between metabolites within a time point as correlation networks, as correlations between metabolites were strongest within time points (32). To provide a numerical summary of the strength of the correlations, we additionally constructed heatmaps of the median absolute correlation coefficients within general and detailed metabolite groups and between general and detailed metabolite groups at each of the time points separately. We calculated the correlation coefficients for correlations between individual metabolites at different time points. Correlations of 0–0.29, 0.3–0.49, 0.5–0.69, 0.7–0.89, and 0.9–1.0 were considered to be very low, low, moderate, high and very high, respectively (31). As sex differences in metabolite concentrations may exist (33), we repeated steps one and three stratified by child's sex. The statistical analyses were performed using R version 3.3.4 (R Foundation for Statistical Computing) (34).

RESULTS

Description of the study population

Table 1 provides general characteristics of the study population. Of the 994 mother–child pairs with data available, 125 (12.6%), 494 (49.7%) and 375 (37.7%) had data available at 1, 2, or 3 time points, respectively.

Variability

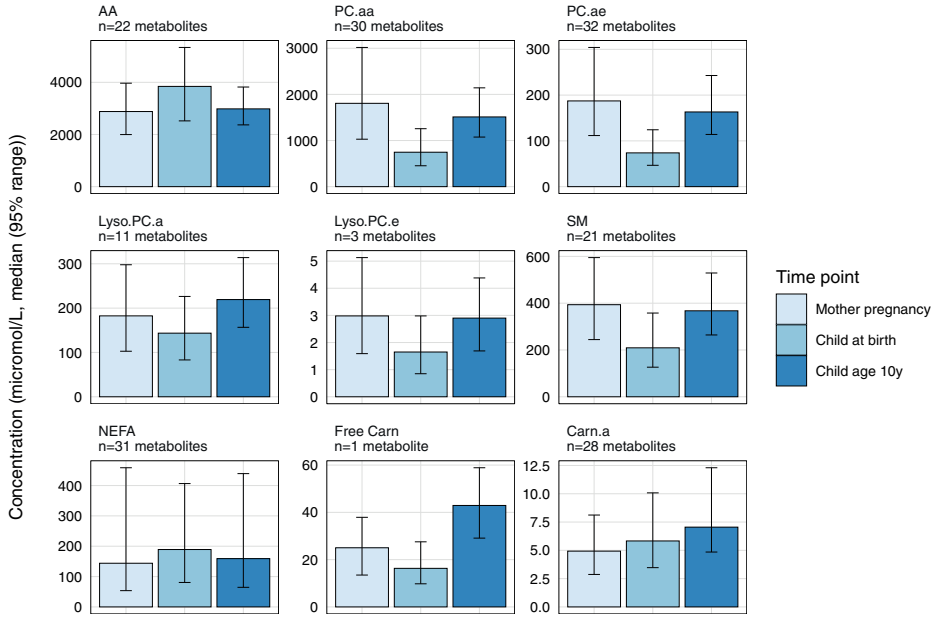
Data was available on a total of 196 metabolites, of which 195 metabolites in early pregnancy, 194 metabolites at birth and 181 metabolites at child's age 10 years. Descriptive information is provided in **Supplemental Table 2**. **Figure 1** shows that the summed metabolite concentration for each general metabolite group varied considerably by time point. Summed concentrations of PC.aa, PC.ae, Lyso.PC.e and SM were highest in maternal blood in early pregnancy, compared to the other time points. Summed concentrations of AA and NEFA were highest in children at birth, whereas summed concentrations of Lyso.PC.a, Free Carn and Carn.a were highest in children of age 10. **Supplemental Table 2** gives the summed concentrations of the detailed metabolite subgroups, which followed similar patterns. **Supplemental Figure 1** shows that the summed metabolite concentrations did not differ by child's sex.

Table 1. General characteristics of the study population

	Time point			
	Total sample	**Mother early pregnancy**	**Child at birth**	**Child age 10 years**
	n=994	**n=814**	**n=921**	**n=503**
(Gestational-) age at blood sample, median (95% range), weeks/years	NA	12.8 (9.8, 16.9)[a]	40.3 (36.6, 42.4)[a]	9.8 (9.1, 10.6)[b]
Maternal characteristics				
Age, mean (SD), years	31.5 (4.2)	31.4 (4.1)	31.5 (4.1)	31.9 (3.9)
Education level, n (%)				
Primary	21 (2.1)	15 (1.9)	20 (2.2)	6 (1.2)
Secondary	342 (34.7)	285 (35.2)	324 (35.4)	165 (32.9)
Higher	623 (63.2)	509 (62.9)	570 (62.4)	330 (65.9)
Pre-pregnancy BMI, median (95% range), kg/m^2	22.5 (18.5, 33.3)	22.6 (18.5, 33.3)	22.5 (18.5, 33.5)	22.4 (18.6, 33.4)
Early pregnancy glucose, mean (SD), mmol/l	4.4 (0.8)	4.4 (0.8)	NA	NA
Early pregnancy total cholesterol, mean (SD), mmol/l	4.9 (0.8)	4.7 (0.8)	NA	NA
Early pregnancy triglycerides, median (95% range), mmol/l	1.2 (0.7, 2.5)	1.3 (0.7, 2.5)	NA	NA
Early pregnancy HDL-cholesterol, mean (SD), mmol/l	1.8 (0.3)	1.8 (0.3)	NA	NA
Early pregnancy LDL-cholesterol, mean (SD), mmol/l	2.5 (0.7)	2.5 (0.7)	NA	NA
Child's characteristics				
Gestational age at birth, median (95% range), weeks	40.3 (36.4, 42.4)	40.3 (36.1, 42.4)	40.3 (36.6, 42.4)	40.3 (37.1, 42.4)
Birth weight, median (95% range), grams	3,545 (2,465, 4,546)	3,550 (2,470, 4,549)	3,548 (2,500, 4,560)	3,560 (2,591, 4,509)
Sex, Male (%)	532 (53.5)	441 (54.2)	497 (54.0)	259 (51.4)
Body mass index at age 10 years, median (95% range), kg/m^2	16.7 (14.0, 22.2)	NA	NA	16.6 (14.1, 21.8)
Glucose at age 10 years, mean (SD), mmol/l	5.3 (0.9)	NA	NA	5.3 (0.9)
Total cholesterol at age 10 years, mean (SD), mmol/l	4.3 (0.6)	NA	NA	4.3 (0.6)
Triglycerides at age 10 years, median (95% range), mmol/l	0.9 (0.4, 2.4)	NA	NA	0.9 (0.4, 2.4)
HDL-cholesterol at age 10 years, mean (SD), mmol/l	1.5 (0.3)	NA	NA	1.5 (0.3)
LDL-cholesterol at age 10 years, mean (SD), mmol/l	2.3 (0.6)	NA	NA	2.3 (0.6)

Values represent mean (SD), median (95% range) or number of participants (valid %). [a] Represents gestational age in weeks. [b] Represents age in years. NA: not applicable.

Figure 1. Median metabolite concentrations by metabolite group and time point



Values represent the median (95% range) of the sum of the individual metabolite concentrations in each of the metabolite groups, by time point. Sums only include metabolites with data at all time points, and therefore do not include concentrations of lyso.PC.a.C20.2, PC.aa.C32.3, PC.aa.C34.5, PC.aa.C36.0, PC.aa.C38.2, PC.aa.C40.3, PC.ae.C34.4, SM.a.C30.1, SM.a.C35.0, SM.a.C37.1, SM.a.C38.3, SM.a.C39.2, SM.a.C40.5, SM.a.C42.4, SM.a.C44.6, SM.e.C36.2, and SM.e.C40.5. SM includes SM.a plus one SM.e.
AA: amino acids, PC.aa: diacyl-phosphatidylcholines, PC.ae: acyl-alkyl-phosphatidylcholines, Lyso.PC.a: acyl-lysophosphatidylcholines, Lyso.PC.e: alkyl-lysophosphatidylcholines, SM: sphingomyelines, NEFA: non-esterified fatty acids, Free Carn: free carnitine, Carn.a: acyl-carnitines.

Dimensionality

Table 2 shows the number of components (PCs) required to explain percentages of cumulative variance at each time point. At each time point, a relatively high number of PCs was needed to explain > 85% of the variance. The obtained PCs did not clearly represent specific metabolic pathways (**Supplemental Figures 2–4**).

Table 2. Number of components required to explain percentages of cumulative proportions of variance at each time point

		Number of PCs				
Time point	**Number of metabolites**	**50%**	**75%**	**85%**	**95%**	**99.5%**
Mother early pregnancy	195	3	15	35	88	163
Child at birth	194	4	21	46	101	169
Child age 10 years	181	6	27	50	98	157

Values represent the number of principal components (PCs) derived from principal component analyses required to explain 50, 75, 85, 95, and 99.5 percent, respectively, of the variances of the data at each of the time points

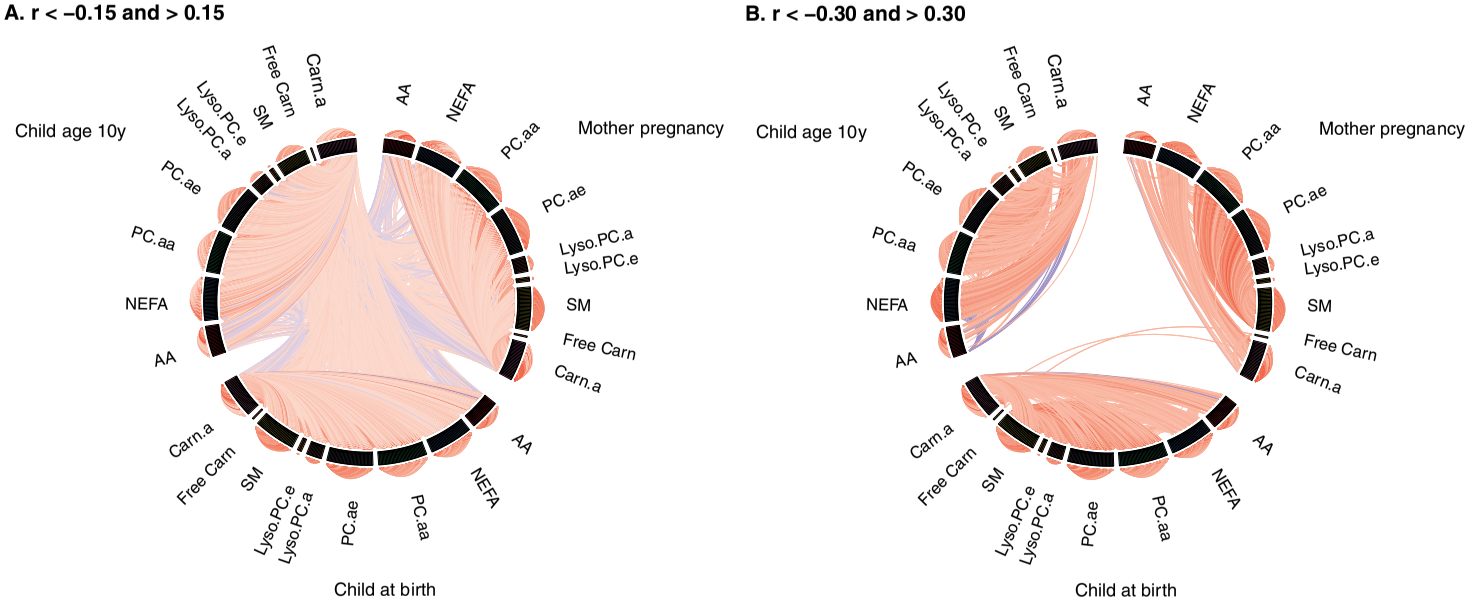
Correlation structure

Figure 2 provides an overview of the correlations between individual metabolite concentrations within general metabolite groups (outer circle), between metabolites concentrations in different general metabolite groups (inner circle) and metabolite concentrations at different time points (lines going through the middle of the circle). **Figure 2a** shows all correlations lower than – 0.15 or higher than 0.15, whereas **Figure 2b** shows all correlations lower than – 0.30 or higher than 0.30. At all time points, relatively high correlations were observed of individual metabolites within general metabolite groups and between individual metabolites from the different PL groups (PC.aa, PC.ae, Lyso.PC.a, Lyso.PC.e, and SM), between AA and Carn.a, and between NEFA and Carn.a. These correlations were mainly of positive direction, except some of the correlations between AA and Carn.a. In children of age 10 years only, some of the AA were negatively correlated with NEFA. Presentation of these correlations within pregnant women, children at birth and children at age 10 years as correlation networks showed the strongest correlations for individual metabolites within general metabolite groups (**Supplemental Figure 5**).

To provide further insight into the strength of these correlations, **Figure 3 a–c** summarizes the correlations as the median absolute correlations of individual metabolites within general and detailed metabolite groups (diagonal) per time point. The median absolute correlations between general and detailed metabolite groups per time point are shown off-diagonal. Median absolute correlations within general and detailed metabolite groups at the same time point were low to high, and ranged between $r = 0.27$ and $r = 0.92$. The strength of these within-group median correlations differed by detailed metabolite subgroup, with BCAA, mono-unsaturated NEFA, mono-unsaturated PC.aa, mono-unsaturated PC.ae, saturated Lyso.PC.e, mono-unsaturated SM and long-chain Carn.a generally having the highest median correlations within their respective general groups. Median absolute correlations between subgroups of different metabolite groups were very low, except for correlations between NEFA detailed subgroups and medium-chain Carn.a in early pregnancy (r ranging between 0.24–0.34) and at age 10 years (r ranging between 0.23–0.44), between BCAA and AAA and short-chain Carn.a in early pregnancy ($r = 0.26$ and $r = 0.33$, respectively) and at age 10 years ($r = 0.30$ and $r = 0.25$, respectively), and between BCAA and short-chain Carn.a ($r = 0.33$) at birth.

Table 3 shows correlations of individual metabolites between each of the time points. For presentation purposes, this table only gives the 30 strongest correlations at each combination of time points, all correlations given in **Supplemental Table 3**. Correlations between early pregnancy and child's metabolites at birth mainly included Free Carn, and Carn.a, and some long chain- and very long chain NEFA and some mainly non-essential AA. Correlations between early pregnancy and child age 10 years included a few AA and some PC.aa. In children, metabolites correlated between birth and age 10 years mainly included phospholipids. Almost all correlations were very weak, except the correlations between early pregnancy and birth Free Carn ($r = 0.35$) and Carn.a C9:0 ($r = 0.32$). **Supplemental Figures 6 and 7** show that the correlations between individual metabolites and median absolute correlations, respectively, were similar for boys and girls.

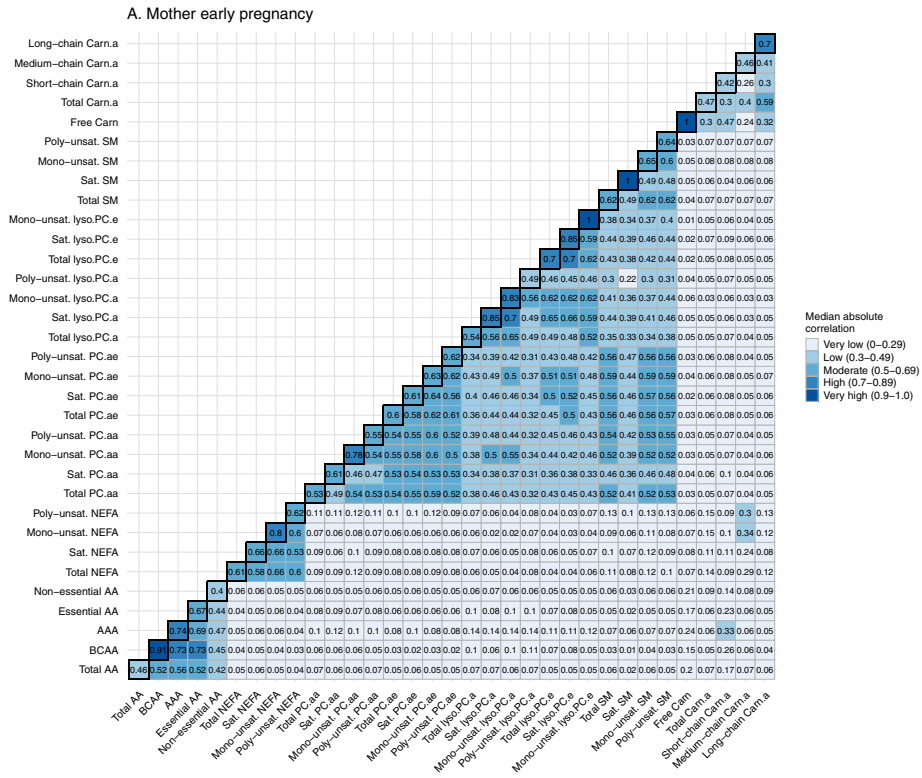
Figure 2. Circos plots of correlations between individual metabolite concentrations



Lines represent Pearson's correlation coefficients between the individual metabolite concentrations within metabolite groups (outer circle), between metabolite groups (inner circle) and between time points (lines going through the middle of the circle). Red lines represent positive correlations and blue lines represent negative correlations. The brightness of the lines indicates the strength of the correlations, with brighter colors for stronger correlations. Figure 2A shows only correlation coefficients lower than -0.15 and higher than 0.15 and Figure 2B shows only correlation coefficients lower than -0.30 and higher than 0.30.

AA: amino acids, NEFA: non-esterified fatty acids, PC.aa: diacyl-phosphatidylcholines, PC.ae: acyl-alkyl-phosphatidylcholines, Lyso.PC.a: acyl-lysophosphatidylcholines, Lyso.PC.e: alkyl-lysophosphatidylcholines, SM: sphingomyelines, Free Carn: free carnitine, Carn.a: acyl-carnitines.

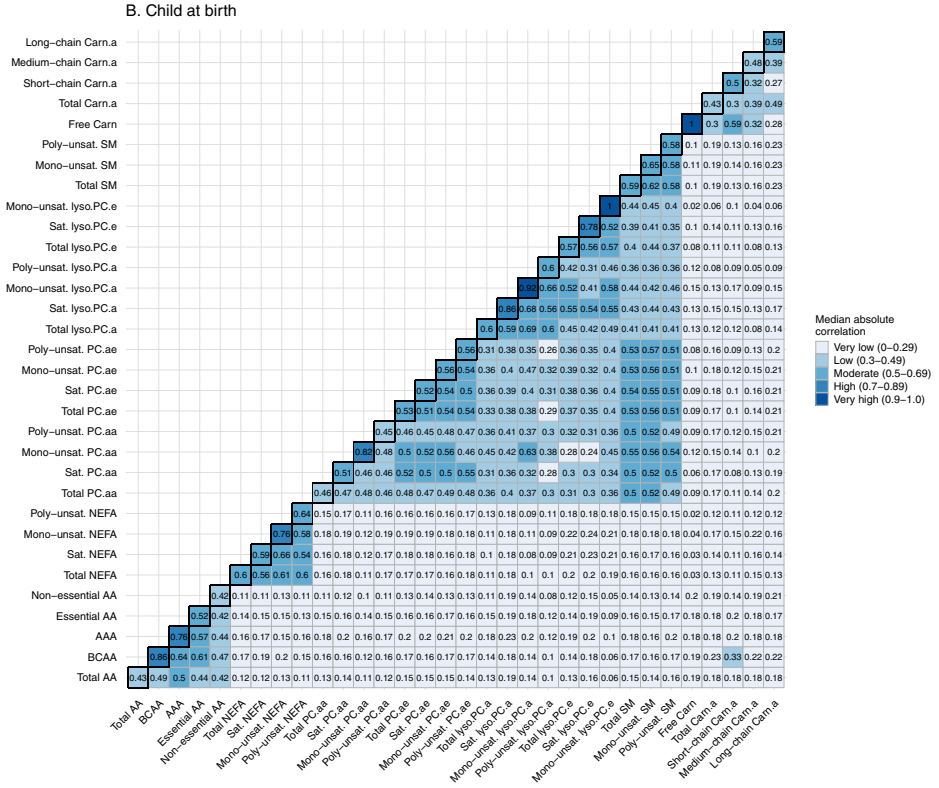
Figure 3. Heatmaps of median absolute correlation of individual metabolites within and between metabolite groups by time point



Values represent median absolute correlation coefficients of individual metabolite concentrations within metabolite groups (diagonal) and between metabolite groups (off-diagonal) by time point. Mono-unsaturated lyso.PC.e, saturated SM and Free Carn include 1 metabolite, resulting in a correlation coefficient of 1 for within-group correlations. For child at birth, no data on saturated SM is available.

AA: amino acids, BCAA: branched-chain amino acids, AAA: aromatic amino acids, NEFA: non-esterified fatty acids, PC.aa: diacyl-phosphatidylcholines, PC.ae: acyl-alkyl-phosphatidylcholines, Lyso.PC.a: acyl-lysophosphatidylcholines, Lyso.PC.e: alkyl-lysophosphatidylcholines, SM: sphingomyelines, Free Carn: free carnitine, Carn.a: acyl-carnitines, Sat.: Saturated, Unsat.: Unsaturated.

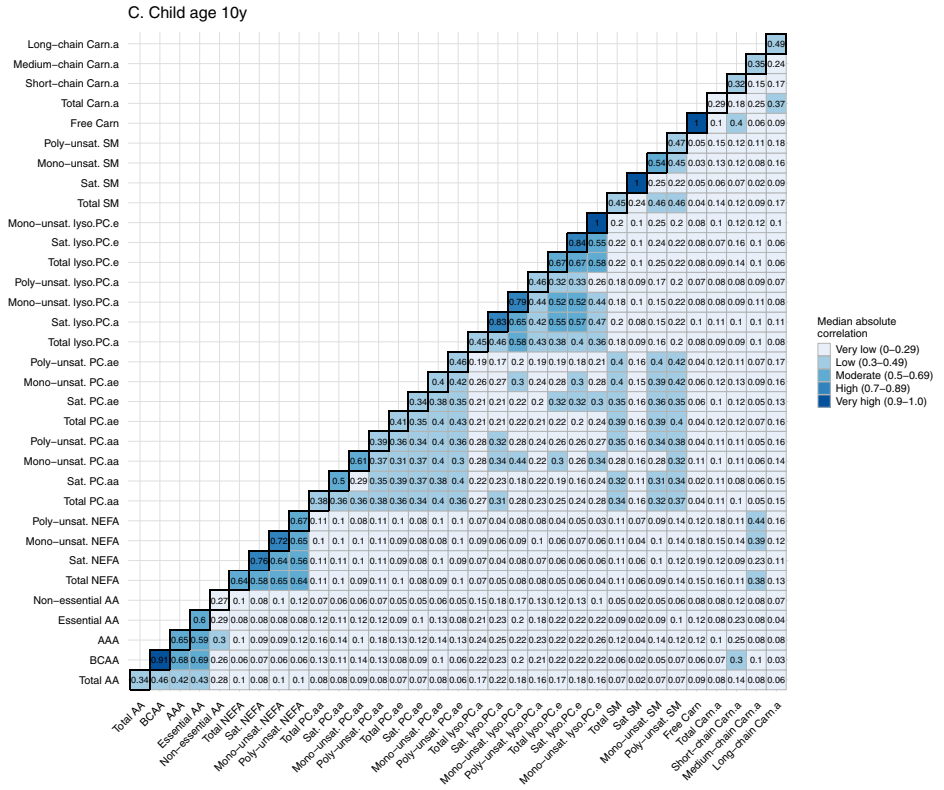
Figure 3. Heatmaps of median absolute correlation of individual metabolites within and between metabolite groups by time point (continued)



Values represent median absolute correlation coefficients of individual metabolite concentrations within metabolite groups (diagonal) and between metabolite groups (off-diagonal) by time point. Mono-unsaturated lyso.PC.e, saturated SM and Free Carn include 1 metabolite, resulting in a correlation coefficient of 1 for within-group correlations. For child at birth, no data on saturated SM is available.

AA: amino acids, BCAA: branched-chain amino acids, AAA: aromatic amino acids, NEFA: non-esterified fatty acids, PC.aa: diacyl-phosphatidylcholines, PC.ae: acyl-alkyl-phosphatidylcholines, Lyso.PC.a: acyl-lysophosphatidylcholines, Lyso.PC.e: alkyl-lysophosphatidylcholines, SM: sphingomyelines, Free Carn: free carnitine, Carn.a: acyl-carnitines, Sat.: Saturated, Unsat.: Unsaturated.

Figure 3. Heatmaps of median absolute correlation of individual metabolites within and between metabolite groups by time point (continued)



Values represent median absolute correlation coefficients of individual metabolite concentrations within metabolite groups (diagonal) and between metabolite groups (off-diagonal) by time point. Mono-unsaturated lyso.PC.e, saturated SM and Free Carn include 1 metabolite, resulting in a correlation coefficient of 1 for within-group correlations. For child at birth, no data on saturated SM is available.

AA: amino acids, BCAA: branched-chain amino acids, AAA: aromatic amino acids, NEFA: non-esterified fatty acids, PC.aa: diacyl-phosphatidylcholines, PC.ae: acyl-alkyl-phosphatidylcholines, Lyso.PC.a: acyl-lysophosphatidylcholines, Lyso.PC.e: alkyl-lysophosphatidylcholines, SM: sphingomyelines, Free Carn: free carnitine, Carn.a: acyl-carnitines, Sat.: Saturated, Unsat.: Unsaturated.

Table 3. Correlations of individual metabolite concentrations between time points, subset of 30 strongest correlations

A. Mother early pregnancy – Child at birth				B. Mother early pregnancy – Child age 10 years				C. Child at birth – Child age 10 years			
Metabolite	**n**	**r**	**P-value**	**Metabolite**	**n**	**r**	**P-value**	**Metabolite**	**n**	**r**	**P-value**
Free Carn	749	0.35	<0.001	Free Carn	413	0.24	<0.001	Cit	457	0.24	<0.001
Carn.a C9:0	749	0.32	<0.001	PC.aa C36:6	413	0.23	<0.001	SM.a C34:2	457	0.21	<0.001
Carn.a C8:1	749	0.28	<0.001	Carn.a C14:2	413	0.22	<0.001	lyso.PC.a C22:6	457	0.20	<0.001
Carn.a C4:0	749	0.26	<0.001	Cit	413	0.21	<0.001	SM.a C42:6	457	0.20	<0.001
NEFA C26:0	749	0.24	<0.001	Orn	413	0.21	<0.001	His	457	0.19	<0.001
Gly	749	0.22	<0.001	Asn	413	0.17	<0.001	Carn.a C4:0	457	0.19	<0.001
Carn.a C10:1	749	0.22	<0.001	PC.aa C38:4	413	0.17	0.001	PC.aa C38:6	457	0.18	<0.001
Carn.a C15:0	749	0.22	<0.001	PC.aa C38:6	413	0.17	<0.001	PC.ae C32:2	457	0.18	<0.001
Carn.a C3:0	749	0.21	<0.001	NEFA C24:4	413	0.16	0.001	SM.a C32:2	457	0.18	<0.001
Cit	749	0.20	<0.001	PC.aa C38:0	413	0.16	0.001	Free Carn	457	0.18	<0.001
NEFA C20:5	749	0.20	<0.001	PC.aa C36:5	413	0.15	0.002	PC.aa C36:5	457	0.17	<0.001
Carn.a C8:0	749	0.20	<0.001	PC.ae C34:3	413	0.15	0.002	PC.aa C38:0	457	0.17	<0.001
NEFA C22:6	749	0.19	<0.001	NEFA C20:5	413	0.14	0.004	PC.ae C34:1	457	0.17	<0.001
Carn.a C2:0	749	0.19	<0.001	NEFA C24:5	413	0.14	0.004	Orn	457	0.16	<0.001
Carn.a C14:2	749	0.19	<0.001	PC.aa C40:6	413	0.14	0.003	PC.aa C43:6	457	0.16	0.001
His	749	0.18	<0.001	PC.aa C43:6	413	0.14	0.005	PC.ae C32:0	457	0.16	0.001
Carn.a C10:0	749	0.18	<0.001	PC.ae C36:5	413	0.14	0.004	NEFA C26:1	457	0.15	0.001
Carn.a C18:2	749	0.18	<0.001	PC.ae C40:0	413	0.14	0.005	NEFA C26:2	457	0.15	0.001
Carn.a C20:3	749	0.18	<0.001	SM.a C35:1	413	0.14	0.005	PC.ae C38:6	457	0.15	0.001
Carn.a.C20.4	749	0.18	<0.001	Carn.a C4:0	413	0.14	0.004	PC.ae C42:3	457	0.15	0.001
Pro	749	0.17	<0.001	Carn.a C15:0	413	-0.14	0.004	SM.a C33:1	457	0.15	0.001
PC.ae C42:4	749	0.17	<0.001	Carn.a C16:0	413	0.14	0.004	Carn.a C8:1	457	0.15	0.002
lyso.PC.a C22:6	749	0.17	<0.001	Ala	413	0.13	0.007	NEFA C20:5	457	0.14	0.003
Carn.a C12:0	749	0.17	<0.001	Thr	413	0.13	0.007	NEFA C24:1	457	0.14	0.003
NEFA C26:1	749	0.16	<0.001	PC.ae C30:0	413	0.13	0.007	NEFA C24:4	457	0.14	0.003
PC.aa C44:12	749	0.16	<0.001	PC.ae C38:0	413	0.13	0.009	PC.ae C42:5	457	0.14	0.002
Ala	749	0.15	<0.001	PC.ae C40:1	413	0.13	0.009	PC.ae C42:6	457	0.14	0.003
Phe	749	0.15	<0.001	PC.ae C42:5	413	0.13	0.008	SM.a C32:1	457	0.14	0.004
PC.aa C38:6	749	0.15	<0.001	Carn.a C16:0.Oxo	413	0.13	0.008	SM.a C36:2	457	0.14	0.002
lyso.PC.a C20:5	749	0.15	<0.001	Gln	413	0.12	0.018	NEFA C22:3	457	0.13	0.006

Values represent Pearson's correlation coefficients (r), and corresponding p-values and number of participants for correlations between metabolites at different time points. For presentation purposes, only the 30 strongest correlations at each combination of time points were presented. A complete list of correlations is given in **Supplemental Table 3**.

DISCUSSION

We described the data acquisition, processing and structure of the metabolomics data available in the Generation R Study and assessed the relationships between metabolite profiles of pregnant women and their children at birth and in childhood. Metabolite concentrations vary considerably between pregnant women and their children at birth and at the age of 10 years. The individual metabolites correlate within groups of metabolites with similar chemical structures, but to a lesser extent between groups of metabolites with different chemical structures. The correlations of individual metabolites between pregnant women and their children at birth and age 10 years are relatively low.

Interpretation of main findings

Metabolomics studies targeting cardio-metabolic diseases have already been successfully applied in adults (7-9), but only a limited number of metabolomics studies have been performed on the early origins of these diseases (11, 12). We obtained intergenerational metabolomics data at three different time points during pregnancy and postnatal life, that may provide more detailed insights in the early origins of cardio-metabolic disease, the underlying mechanisms and identify potential novel biomarkers.

Maternal metabolic profile during pregnancy might influence fetal metabolic profile, either directly through placental transfer, or indirectly by influences on hormone levels or placental function (11). Maternal blood metabolite concentrations generally tend to decrease across pregnancy, likely reflecting increased circulating volume, tissue biosynthesis and placental uptake (24). Fetal metabolite concentrations are the result of both placental transfer and endogenous synthesis. Concentrations of AA, Carn and NEFA, particularly long-chain polyunsaturated fatty acids (LC-PUFA), tend to be higher in fetal blood than in maternal blood (35-37). This might be indicative of an active transport mechanism across the placenta or increased fetal synthesis. Although the large time differences between the metabolite measurements in our study should be noted and preclude direct conclusions about placental transfer, our observation that the summed concentrations of AA, NEFA and Carn.a were higher in cord blood than in maternal early pregnancy blood is in line with these previous studies. The lower PL concentrations observed in cord blood in comparison to maternal early pregnancy blood might be explained by the fact that PL do not cross the placenta, but are hydrolyzed to NEFA that in turn cross the placental barrier (35, 38, 39). Relatively high correlations between individual metabolites within known general and detailed metabolite subgroups in pregnant women as well as in cord blood were observed, as expected from the shared precursors and biosynthesis pathways. However, correlations of individual metabolites between these two time points were relatively weak. These results are in line with those from a multi-ethnic study among 1600 participants that showed mostly weak correlations of these metabolites between maternal blood at 28 weeks of gestation and cord blood

(40). In our study, there is a large time difference between the metabolite measurements in mothers and newborns. Therefore, the relatively low correlations between maternal and cord blood metabolites might result from changes in metabolism in both pregnant women and the fetus that occur throughout pregnancy (24, 38). In addition, placental transfer of nutrients throughout pregnancy is tightly regulated by various transport mechanisms to ensure stable fetal metabolite concentrations at the expense of variations in maternal metabolite concentrations (41, 42). The relatively high correlations for carnitines in our study might be explained by the main source of carnitines for the fetus being placental transfer, rather than endogenous synthesis (43). Thus, individual metabolite concentrations correlate within mothers and newborns, but barely between mothers and newborns. This might result from changes in maternal and fetal metabolism throughout pregnancy and from tightly regulated active trans-placental transport mechanisms resulting in distinct metabolite profiles in pregnant women and their children at birth.

Less is known about the metabolite profiles from birth throughout childhood and the influence of maternal metabolite profiles in pregnancy on these profiles. A study among 127 children from Sweden showed that concentrations of conventional lipids, including total cholesterol, LDL cholesterol and HDL cholesterol increased between the age of 6 months and 4 years, whereas triglyceride concentrations decreased (44). A study among 500 children and adolescents aged 0 to 19 years observed that concentrations of AA, NEFA, and Carn.a dropped after the neonatal period. However, some of these Carn.a increased again from the age of 7 years and returned to neonatal concentrations at age 19 years (45). A large familial resemblance in metabolite concentrations has been suggested, which seems to be largely genetic (46-48). In cross-sectional studies, correlations of metabolites between parents and their offspring vary strongly, ranging from weak to relatively strong (33, 44, 49). Partly in line with these previous studies, we observed that AA and NEFA concentrations were lower in childhood as compared to cord blood samples, whereas concentrations of PL and Carn were higher in childhood. However, the correlations between individual metabolite concentrations of children at birth and at the age of 10 years as well as between mothers in early pregnancy and their children at the age of 10 years were very weak. This might be explained by the large timespan between the measurements. Also, previous research has indicated that metabolite concentrations are highly influenced by nutritional factors, physical activity and the gut microbiome (23, 50-53). Differences in these factors between mothers and their children and over time might explain the weak correlations between different time points. Previous studies observed sex differences in metabolite concentrations in both children and adults (33, 45). We did not observe metabolite concentrations to vary between the sexes. This could be explained by the relatively young age of the participants, as sex differences in metabolite concentrations have been shown to be more pronounced in adolescence and adulthood (33, 45). Thus, correlations between individual metabolites between pregnant women and their children at school-age and within children over time are very low. This

might suggest strong influences of external factors and limited intergenerational correlations of metabolite profiles.

We provided the first explorative analyses of a unique large longitudinal dataset consisting of metabolomics data of pregnant women and their children at birth and in childhood, and studied correlations between a large number of metabolites at these different time points. Not much is known yet about the correlations of metabolites between pregnant women and their children and the metabolite profiles in children from birth until childhood. We observed relatively low correlations of metabolite concentrations between time points. We explored whether offspring sex affected these correlations as this is an important baseline characteristic which has been suggested to influence metabolite profiles in children and adults, but this did not affect our findings. Other maternal and childhood factors are likely to influence metabolite profiles in pregnant women, and the development of metabolites profiles from birth until childhood. Further studies are needed to obtain detailed insight into the influence of maternal and offspring socio-demographic, lifestyle and physical factors on the stability of metabolites profiles in pregnancy and from birth throughout childhood. Future studies using these data should take into account the correlations of metabolites within the same metabolite group. PCA, a data reduction approach commonly used in metabolomics, showed that the data were highly dimensional. This indicates that the variability in the data is difficult to capture in a lower number of components and that each metabolite contributes unique information. In addition, the obtained components did not describe specific metabolic pathways. Therefore, we do not consider the PCs informative in describing the information contained in this dataset. Given the high dimensionality of the data and the relatively high correlation of metabolites within metabolite groups, it seems that future studies focused on relating these data to exposures and outcomes of interest should analyze the data per individual metabolite and per metabolite group with structural, physiological and biological relevance. In addition, correlation networks based on correlations between individual metabolites or more advanced pathway analysis may be useful for identifying metabolic pathways involved in these associations. Due to the longitudinal nature of the data and the large amount of data on relevant exposures and outcomes available in the cohort, these data will form an important population-based resource for future metabolomics analyses on the developmental origins of cardio-metabolic disease.

Methodological considerations

We obtained metabolomics data in a subgroup of the cohort, which consists of Dutch, relatively high educated and healthy participants, as compared to the full cohort (13). This may affect the generalizability of our sample to the full cohort and the general population. We adopted a targeted metabolomics approach, which enabled us to study absolute metabolite concentrations of metabolites known a priori to be relevant for obesity and cardio-metabolic disease. However, the targeted design might also be a limitation in future association studies,

as relevant biological pathways might be missed. The blood samples used in our study were non-fasting and taken during non-fixed times of the day for logistic and ethical reasons (relatively young age of the children). Metabolite concentrations are dependent on fasting status. Fasting blood samples are usually preferred, as they are more reliable over time (54). The use of non-fasting blood samples in our study might influence precision and power to detect associations of interest. However, non-fasting blood samples appear to be more informative of metabolic status throughout the day. Also, non-fasting lipids have been shown to perform equally or even better than fasting lipids in predicting the risk of cardiovascular disease (55). We therefore still consider non-fasting metabolite concentrations to be of interest. Due to the longitudinal design of the study, we were able to measure metabolite concentrations at 3 different time points during pregnancy and early postnatal life. However, due to the large time intervals between the blood samples and differences in the nature of the blood samples, small differences in procedures and handling of the blood samples may exist. As previous studies showed that different pre-storage temperatures and durations only minimally affected measured concentrations of most metabolites, we consider it unlikely that this strongly influenced our results.

Conclusions

Metabolite concentrations vary between pregnant women and their children at birth and at the age of 10 years. Correlations of individual metabolites between pregnant women and their children at birth and in childhood are relatively low. This may suggest that unique metabolic profiles are present among pregnant women, newborns and school aged children, with limited intergenerational correlations between metabolite profiles. These data are an important population-based resource for future metabolomics analyses to address the early origins of cardio-metabolic disease.

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SUPPLEMENTAL MATERIAL

Supplemental Text 1. Metabolite measurements

A targeted metabolomics approach was adopted to determine serum concentrations (µmol/L) of AA, NEFA, PL and Carn, as described previously (1). Proteins of 50 µL serum were precipitated by adding 450 µL methanol including internal standards: labeled amino acid standards set A (NSK-A-1, Cambridge Isotope Laboratories (CIL), USA), 15N2-L-asparagine (NLM-3286-0.25, CIL, USA), indole-D5-L-tryptophan (DLM-1092-0.5, CIL, USA), U-13C16-palmitic acid (CLM-409-MPT-PK, CIL, USA), D3-acetyl-carnitine (DLM-754-PK, CIL, USA), D3-octanoyl-carnitine (DLM-755-0.01, CIL, USA) and D3-palmitoyl-carnitine (DLM-1263-0.01, CIL, USA), tridecanoyl-2-hydroxy-sn-glycero-3-phosphocholine (855476, Avanti Polar Lipids, USA) and 1,2-dimyristoyl-sn-glycero-3-phosphocholine (850345, Avanti Polar Lipids, USA). If sample volume was less than optimal, the concentrations were corrected by the respective factor. Sample volumes less than 25 µL were not used and considered missing. After centrifugation the supernatant was split into aliquots. AA were analyzed by liquid chromatography tandem mass spectrometry (LC-MS/MS) as described previously (2). An aliquot of the supernatant was used for the derivatization to AA butylester with hydrochloric acid in 1-butanol. After evaporation, the residues were dissolved in water/methanol (80:20; (v/v)) with 0.1% formic acid. The samples were analyzed with 1100 high-performance liquid chromatography (HPLC) system (Agilent, Waldbronn, Germany) equipped with 150 x 2.1 mm, 3.5 µm particle size C18 HPLC column (X-Bridge, Waters, Milford, USA) and 0.1% heptafluorobutyric acid as and ion pair reagent in the mobile phases A and B (A: water, B: methanol). Mass spectrometric (MS) detection was performed with an API2000 tandem mass spectrometer (AB Sciex, Darmstadt, Germany) equipped with an atmospheric pressure chemical ionization (APCI) source operating in positive ion ionization mode. IUPAC-IUB Nomenclature was used for notation of the AA (3).

NEFA, PL and Carn were measured with a 1200 SL HPLC system (Agilent, Waldbronn, Germany) coupled to a 4000QTRAP tandem mass spectrometer from AB Sciex (Darmstadt, Germany) (4, 5). NEFA were analyzed by injection of the supernatant to a LC-MS/MS operating in negative electrospray ionization (ESI) mode where they were separated by gradient elution on a 100 x 3.0 mm, 1.9 µm particle size Purusuit UPS Diphenyl column from Varian (Darmstadt, Germany) using 5 mM ammonium acetate in water as mobile phase A and acetonitrile/ isopropanol (80:20; (v/v)) as mobile phase B. NEFA species were quantified using GLC-85 reference standard mixture (Nu-Chek Prep, USA). PL were analyzed by flow-injection analysis (FIA) with LC-MS/MS coupled with ESI (6). The system was run in positive ionization mode with 5% water in isopropanol as mobile phase A and 5% water in methanol as mobile phase B. The analysis was performed for diacyl-phosphatidylcholines (PC.aa), acyl-alkyl-phosphatidylcholines (PC.ae) , acyl-lysophosphatidylcholines (Lyso.PC.a), alkyl-lysophosphatidylcholines (Lyso.PC.e) and sphingomyelines (SM)). Carn (Free carnitine

(Free Carn) and acyl-carnitines (Carn.a)) were analyzed by flow-injection analysis of the supernatant into a LC-MS/MS system using an isocratic elution with 76% isopropanol, 19% methanol and 5% water. The mass spectrometer was equipped with electrospray ionization and operated in positive ionization mode. PL and acyl-carn were quantified using aliquots of a commercial available lyophilized control plasma (ClinChek®, Recipe, Germany), where the concentrations have been determined by AbsoluteIDQ p150 Kit from Biocrates®, a previous published LC-MS/MS method (7) and by in-house quantification with various standards. The calibrators used are given in **Supplemental Table 1**. The analytical technique used is capable of determining the total number of total bonds, but not the position of the double bonds and the distribution of the carbon atoms between fatty acid side chains. We used the following notation for NEFA, PL and Carn.a: X:Y, where X denotes the length of the carbon chain, and Y the number of double bonds. The 'a' denotes an acyl chain bound to the backbone via an ester bond ('acyl-') and the 'e' represents an ether bond ('alkyl-').

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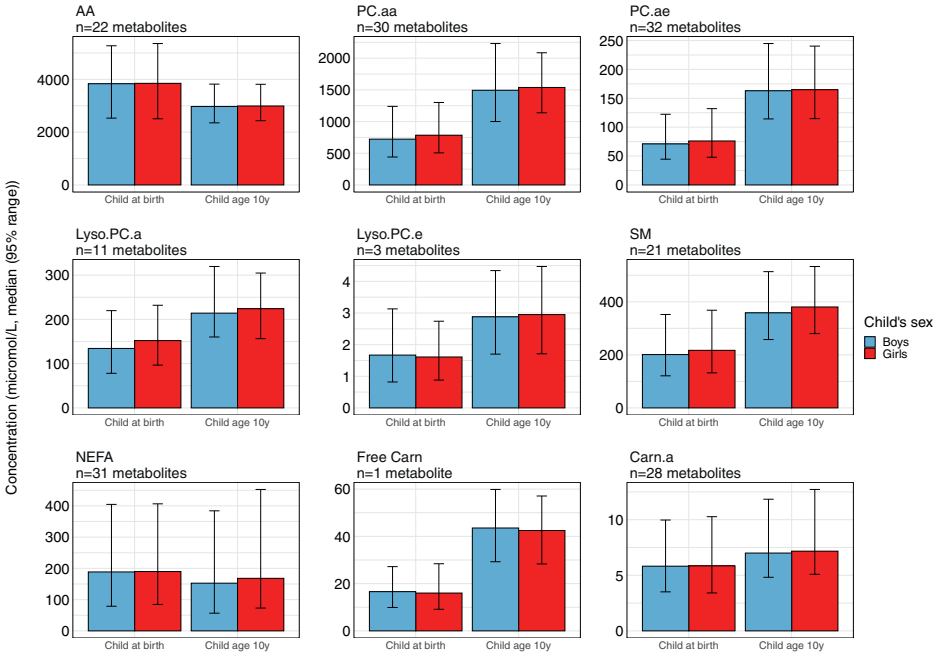
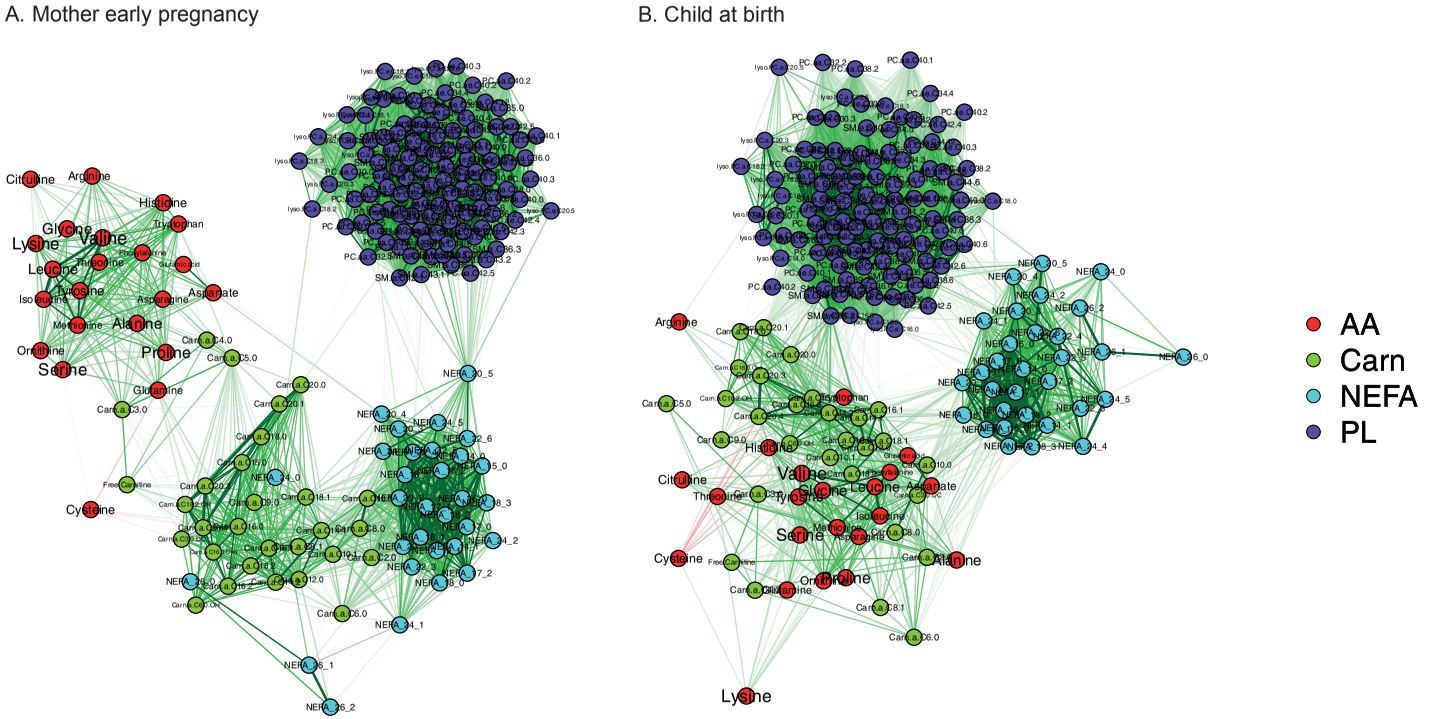
Supplemental Tables 1-3 and Supplemental Figures 2-4. Can be found online.

Supplemental Figure 1. Median metabolite concentrations by metabolite group and time point, stratified by child's sex



Values represent the median (95% range) of the sum of the individual metabolite concentrations in each of the metabolite groups, by time point and child's sex. Sums only include metabolites with data at all time points, and therefore do not include concentrations of lyso.PC.a.C20.2, PC.aa.C32.3, PC.aa.C34.5, PC.aa.C36.0, PC.aa.C38.2, PC.aa.C40.3, PC.ae.C34.4, SM.a.C30.1, SM.a.C35.0, SM.a.C37.1, SM.a.C38.3, SM.a.C39.2, SM.a.C40.5, SM.a.C42.4, SM.a.C44.6, SM.e.C36.2, and SM.e.C40.5. SM includes SM.a plus one SM.e. AA: amino acids, PC.aa: diacyl-phosphatidylcholines, PC.ae: acyl-alkyphosphatidylcholines, Lyso.PC.a: acyl-lysophosphatidylcholines, Lyso.PC.e: alkyl-lysophosphatidylcholines, SM: sphingomyelines, NEFA: non-esterified fatty acids, Free Carn: free carnitine, Carn.a: acyl-carnitines.

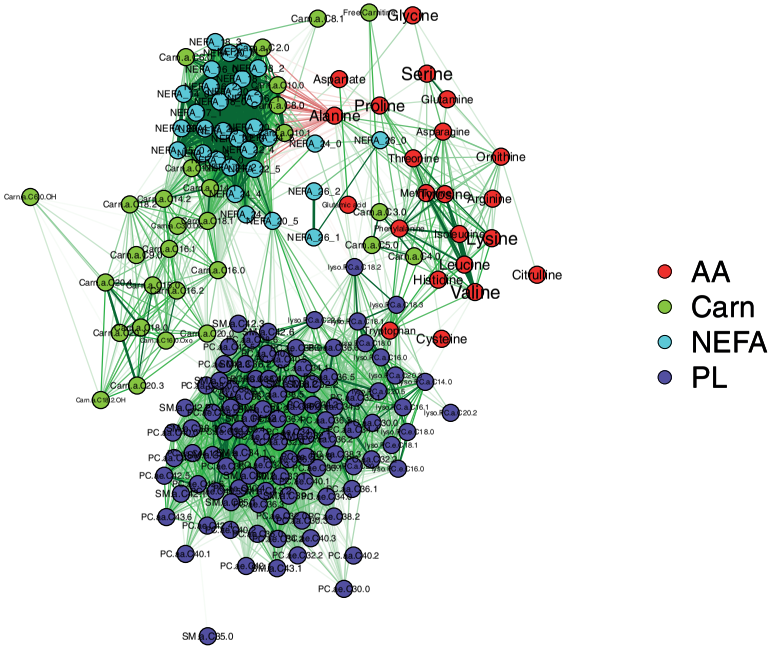
Supplemental Figure 5. Correlation networks for correlations between individual metabolite concentrations by time point



Lines represent Pearson's correlation coefficients between the individual metabolite concentrations within metabolite groups within and between metabolite groups. Green lines represent positive correlations and red lines represent negative correlations. The thickness of the lines indicates the strength of the correlations, with thinker lines for stronger correlations. Only correlation coefficients lower than -0.30 and higher than 0.30 are shown.
AA: amino acids, Carn: carnitines, NEFA: non-esterified fatty acids, PL: phospholipids.

Supplemental Figure 5. Correlation networks for correlations between individual metabolite concentrations by time point (continued)

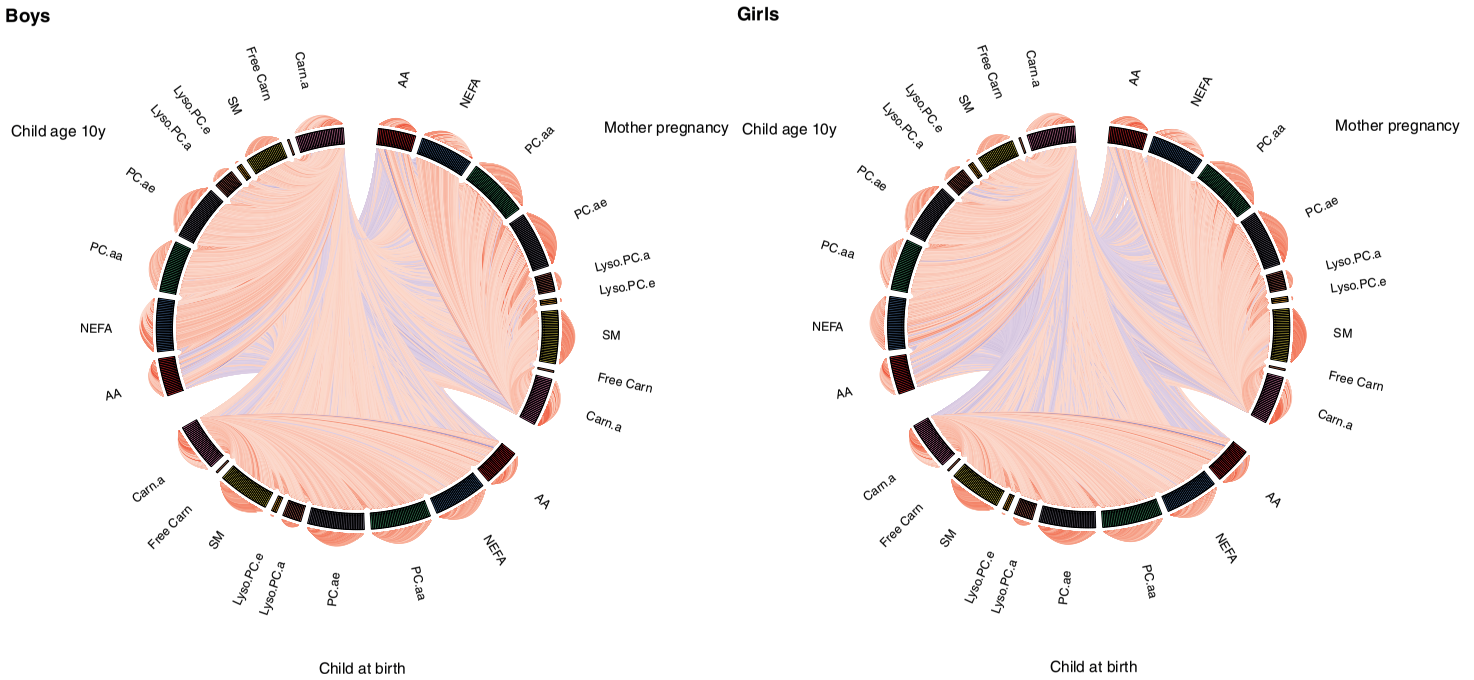
C. Child age 10 years



Lines represent Pearson's correlation coefficients between the individual metabolite concentrations within metabolite groups within and between metabolite groups. Green lines represent positive correlations and red lines represent negative correlations. The thickness of the lines indicates the strength of the correlations, with thinker lines for stronger correlations. Only correlation coefficients lower than -0.30 and higher than 0.30 are shown.
AA: amino acids, Carn: carnitines, NEFA: non-esterified fatty acids, PL: phospholipids.

Supplemental Figure 6. Circos plots of correlations between individual metabolite concentrations, stratified by child's sex

A. r < –0.15 and > 0.15

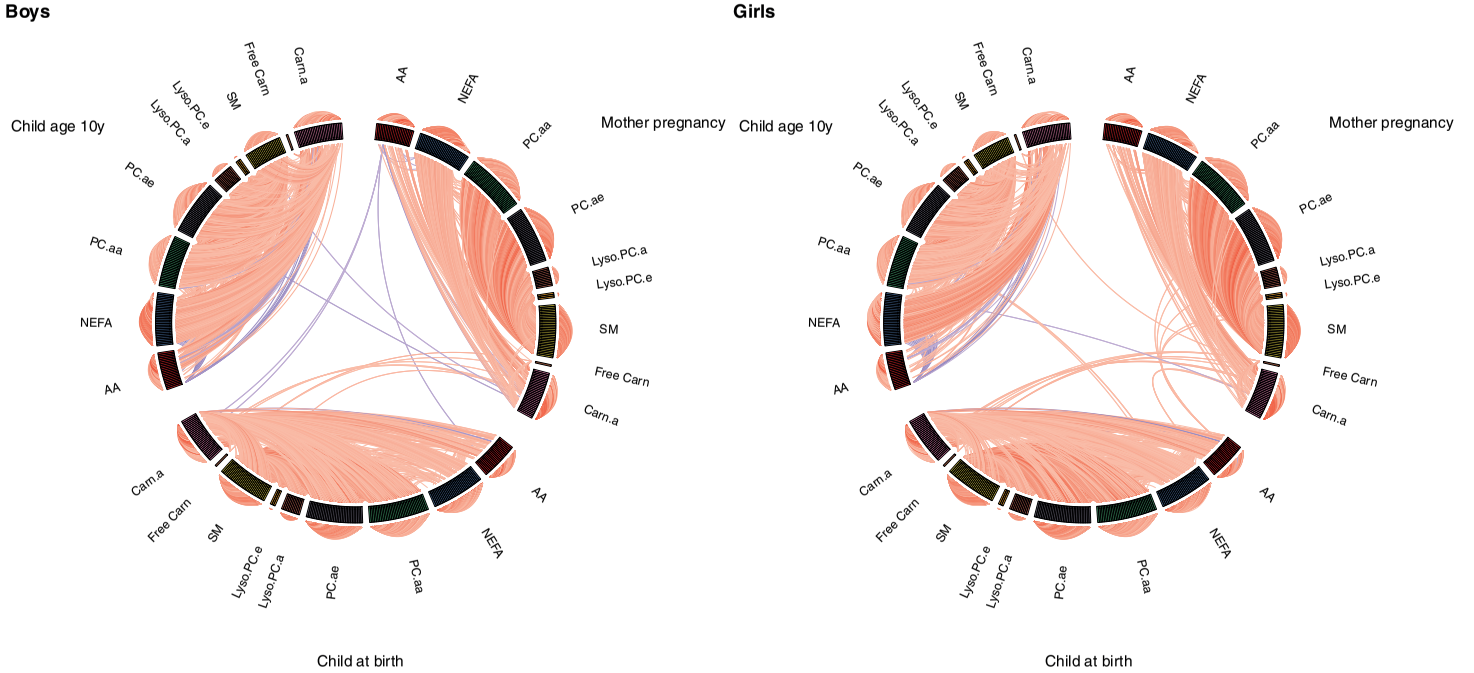


Lines represent Pearson's correlation coefficients between the individual metabolite concentrations within metabolite groups (outer circle), between metabolite groups (inner circle) and between time points (lines going through the middle of the circle), stratified by child's sex. Red lines represent positive correlations and blue lines represent negative correlations. The brightness of the lines indicates the strength of the correlations, with brighter colors for stronger correlations. Figure 2A shows only correlation coefficients lower than -0.15 and higher than 0.15 and Figure 2B shows only correlation coefficients lower than -0.30 and higher than 0.30.

AA: amino acids, NEFA: non-esterified fatty acids, PC.aa: diacyl-phosphatidylcholines, PC.ae: acyl-alkyl-phosphatidylcholines, Lyso.PC.a: acyl-lysophosphatidylcholines, Lyso.PC.e: alkyl-lysophosphatidylcholines, SM: sphingomyelines, Free Carn: free carnitine, Carn.a: acyl-carnitines.

Supplemental Figure 6. Circos plots of correlations between individual metabolite concentrations, stratified by child's sex (continued)

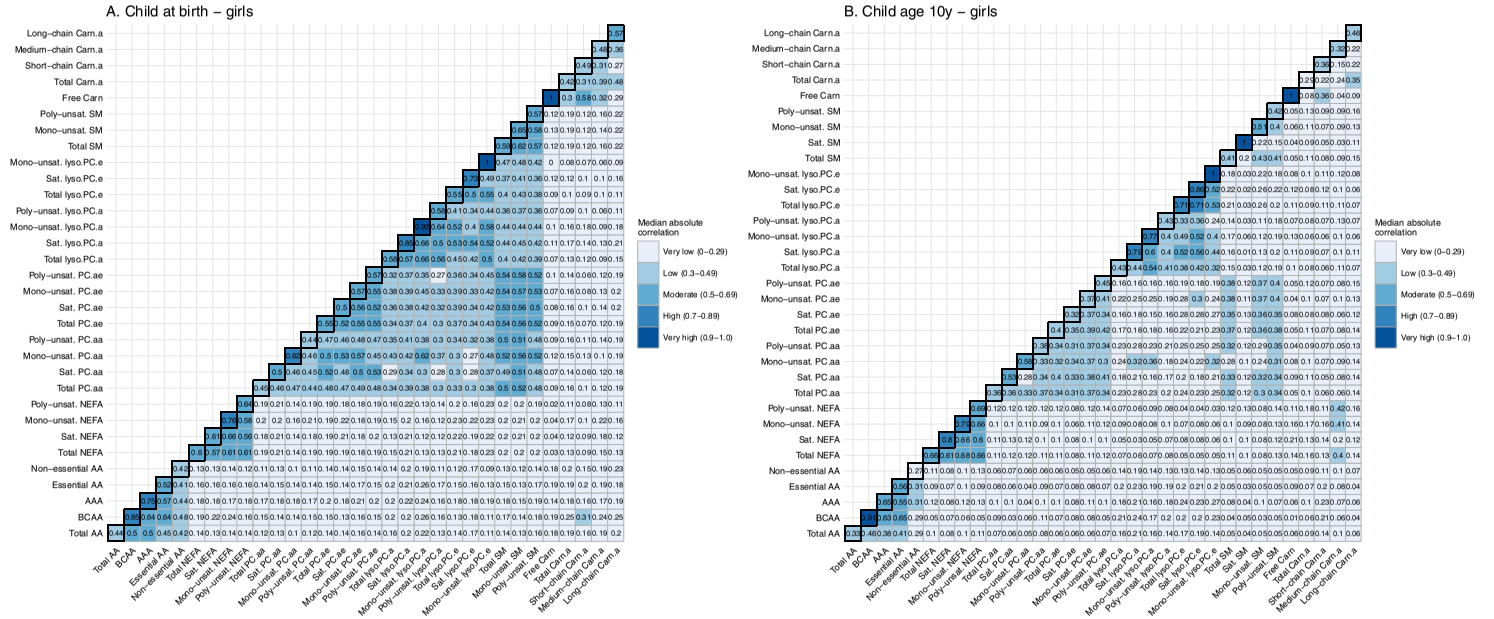
B. r < –0.30 and > 0.30



Lines represent Pearson's correlation coefficients between the individual metabolite concentrations within metabolite groups (outer circle), between metabolite groups (inner circle) and between time points (lines going through the middle of the circle), stratified by child's sex. Red lines represent positive correlations and blue lines represent negative correlations. The brightness of the lines indicates the strength of the correlations, with brighter colors for stronger correlations. Figure 2A shows only correlation coefficients lower than -0.15 and higher than 0.15 and Figure 2B shows only correlation coefficients lower than -0.30 and higher than 0.30.

AA: amino acids, NEFA: non-esterified fatty acids, PC.aa: diacyl-phosphatidylcholines, PC.ae: acyl-alkyl-phosphatidylcholines, Lyso.PC.a: acyl-lysophosphatidylcholines, Lyso.PC.e: alkyl-lysophosphatidylcholines, SM: sphingomyelines, Free Carn: free carnitine, Carn.a: acyl-carnitines.

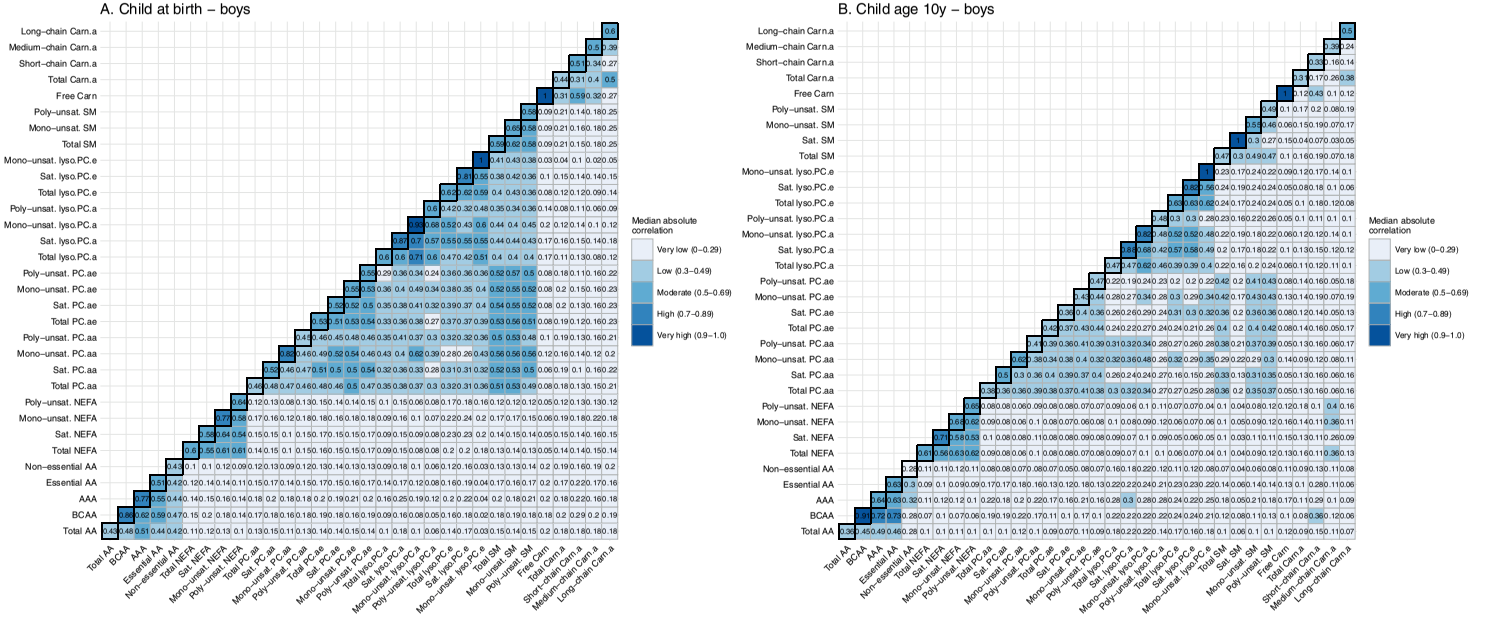
Supplemental Figure 7. Heatmap of median absolute correlation of individual metabolites within and between metabolite groups by time point, stratified by child's sex



Values represent median absolute correlation coefficients of individual metabolite concentrations within metabolite groups (diagonal) and between metabolite groups (off-diagonal), stratified by child's sex. Mono-unsaturated lyso.PC.e, saturated SM and Free Carn include 1 metabolite, resulting in a correlation coefficient of 1 for within-group correlations. For child at birth, no data on saturated SM is available.

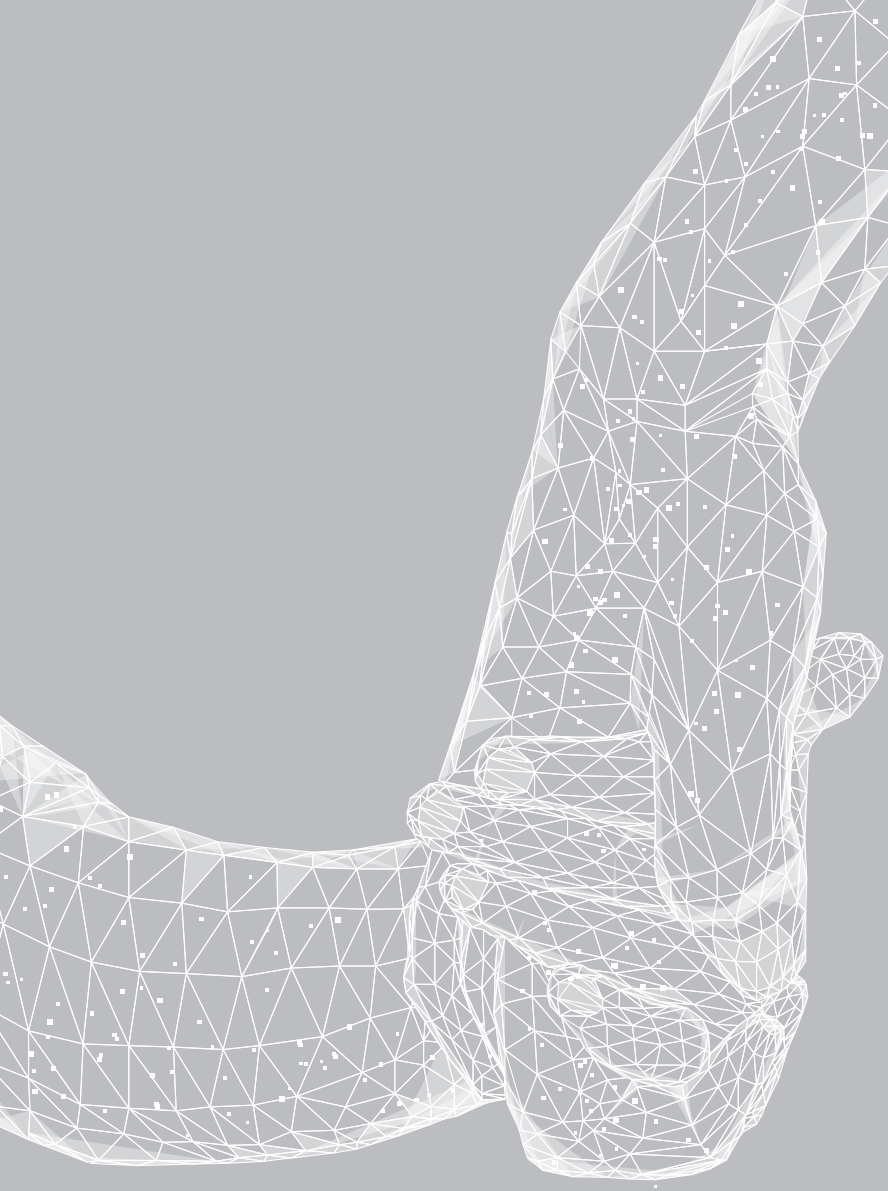
AA: amino acids, BCAA: branched-chain amino acids, AAA: aromatic amino acids, NEFA: non-esterified fatty acids, PC.aa: diacyl-phosphatidylcholines, PC.ae: acyl-alkyl-phosphatidylcholines, Lyso.PC.a: acyl-lysophosphatidylcholines, Lyso.PC.e: alkyl-lysophosphatidylcholines, SM: sphingomyelines, Free Carn: free carnitine, Carn.a: acyl-carnitines, Sat.: Saturated, Unsat.: Unsaturated.

Supplemental Figure 7. Heatmap of median absolute correlation of individual metabolites within and between metabolite groups by time point, stratified by child's sex (continued)



Values represent median absolute correlation coefficients of individual metabolite concentrations within metabolite groups (diagonal) and between metabolite groups (off-diagonal), stratified by child's sex. Mono-unsaturated lyso.PC.e, saturated SM and Free Carn include 1 metabolite, resulting in a correlation coefficient of 1 for within-group correlations. For child at birth, no data on saturated SM is available.

AA: amino acids, BCAA: branched-chain amino acids, AAA: aromatic amino acids, NEFA: non-esterified fatty acids, PC.aa: diacyl-phosphatidylcholines, PC.ae: acyl-alkyl-phosphatidylcholines, Lyso.PC.a: acyl-lysophosphatidylcholines, Lyso.PC.e: alkyl-lysophosphatidylcholines, SM: sphingomyelines, Free Carn: free carnitine, Carn.a: acyl-carnitines, Sat.: Saturated, Unsat.: Unsaturated.



5 General discussion

INTRODUCTION

Childhood overweight and obesity are of major public health concern globally. The prevalence of overweight and obesity in children increased extensively in the last couple of decades (1). Childhood overweight and obesity are associated with adult overweight and obesity and are risk factors of a variety of health problems, including cardio-metabolic diseases, asthma, osteoarthritis, mental health problems and premature death (2). Obesity is defined as an excess of body fat, and is usually based on the body mass index (3, 4). Body mass index is a measure of weight adjusted for height, and is therefore not an exact measure of body fat. More direct measures of body fat mass and its distribution seem to be more strongly linked to metabolic disturbances and the risks of cardio-metabolic diseases (5, 6).

The etiology of obesity and associated cardio-metabolic diseases is complex and multifactorial. Risk factors include, but are not limited to, genetics, excess energy intake, sedentary behavior, lack or excess of sleep, stress and certain diseases (7). In addition, an accumulating body of research suggest that adverse exposures in fetal and early postnatal life may lead to developmental adaptations in organ structure and function, that may increase susceptibility to obesity and cardio-metabolic disease in later life (8). This might be reflected by different growth patterns from fetal life onwards. It has been observed that both children that grow slow and rapid in fetal life and grow rapidly in childhood are at the highest risk to develop cardio-metabolic diseases in later life (9-12). Identifying the factors related to adverse growth patterns and body fat development and the underlying mechanisms will contribute to the understanding of the early origins of these diseases and will help to target interventions aiming to reduce the burden of these diseases.

The general objective of this thesis was to assess the associations of common maternal dietary factors and maternal adiposity with growth, body fat development and cardio-metabolic risk factors in children, as well as the metabolic mechanisms that might underlie these associations. This chapter provides a general discussion of the main findings of this thesis, discusses general methodological considerations and provides suggestions for future research.

INTERPRETATION OF MAIN FINDINGS

Maternal common dietary factors

Nutrition during pregnancy is important for both the pregnant woman's and her child's health (13). Thus far, the associations of maternal dietary factors during pregnancy, such as diet quality, dietary patterns, total energy intake and macro- and micronutrient intake with offspring health outcomes have been studied extensively (13, 14). In this thesis, we specifically focused on intake of caffeine and milk, that are less studied, but common components of the diet in pregnant women.

Maternal caffeine intake during pregnancy

Caffeine-containing beverages, including coffee and tea, are frequently consumed. Consumption of these beverages seems to be beneficial for the risks of several diseases in non-pregnant adults such as type 2 diabetes and non-alcoholic fatty liver disease (15-20), but might have unfavorable consequences in pregnant women. Previous studies, including one in our own cohort, have observed that maternal caffeine intake was associated with increased risks of fetal death, impaired fetal growth and increased risks of low birth weight (21-23). In addition to these short-term outcomes, previous studies have observed associations of any caffeine intake during pregnancy with higher offspring sum of skinfold thicknesses at age 3 months (24), and with higher risks of obesity and central obesity up until age 15 years (25-27). In contrast, a study among 1986 mothers and their children from the United States did not show consistent associations of maternal serum concentrations of paraxanthine, the primary metabolite of caffeine, during pregnancy with childhood body mass index at ages 4 and 7 years (28). In this thesis, we observed that a high caffeine intake tended to be associated with a lower birth weight, a higher weight gain from birth until the age of 6 years and a higher body mass index from 6 months to 6 years and at the age of 10 years. Children of mothers with a high caffeine intake also had a higher total body fat mass and a higher android to gynoid fat mass ratio at the ages of 6 and 10 years, suggesting that maternal caffeine intake might be related to total body fat mass and a central body fat distribution in childhood. In addition, higher caffeine intake during pregnancy was associated with higher abdominal subcutaneous and visceral fat masses and liver fat fraction at the age of 10 years. The associations of abdominal visceral fat mass and liver fat fraction were independent of child's concurrent total body fat mass. This suggests that maternal caffeine intake throughout pregnancy might differentially affect visceral and liver fat accumulation, rather than the total amount of body fat, and may increase susceptibility to cardio-metabolic disease.

The mechanisms by which maternal caffeine intake during pregnancy might affect offspring growth and body fat development are unclear. Studies in adults have suggested that consumption of caffeine may increase adiponectin concentrations and decrease concentrations of pro-inflammatory cytokines (15, 18). We therefore speculate that these altered concentrations in pregnant women may affect fetal nutrient supply, which may subsequently affect development of the adipose tissue. Also, animal studies have suggested that in-utero exposure to caffeine may overexpose the developing fetus to glucocorticoids, leading to an altered development of the HPA-axis (29, 30). High glucocorticoid concentrations have been related to increased central obesity. In addition, the concentration of glucocorticoid receptors is higher in visceral adipose tissue as compared to other fat depots, possibly resulting in differential fat deposition in these depots (31). The associations might also be explained by confounding by unhealthy lifestyle factors that are shared within families. However, a negative control analysis among 50,943 participants showed stronger associations for maternal caffeine intake during pregnancy with the risk of childhood overweight at the age of 3 years,

as compared to those for paternal caffeine intake (26). This suggests that an intra-uterine programming mechanism might at least partly be involved in these associations.

Maternal milk intake during pregnancy

In contrast to maternal caffeine intake during pregnancy, maternal milk intake during pregnancy seems to be associated with increased fetal growth, resulting in higher birth weights (32-38). However, the long-term effects of maternal milk intake during pregnancy remain unclear. A study from the United Kingdom among 6663 mothers and children, did not observe associations of maternal milk intake in late pregnancy with child's height at the age of 7.5 years (39). Another study among 685 mothers and children from Denmark observed that milk consumption during pregnancy was associated with increased height and increased concentrations of insulin-like growth factor 1 (IGF-1) in the 20-year old offspring (40). In this thesis, we observed that high maternal milk intake during pregnancy was associated with a higher childhood body mass index, a higher total fat mass, a higher lean mass, a higher android to gynoid fat mass ratio, a higher abdominal visceral fat mass and a higher risk of overweight/obesity at age 10 years. No consistent associations were observed for pericardial fat, liver fat, blood pressure, lipids, insulin or glucose concentrations. Thus, maternal milk intake during pregnancy seems to be associated with an adverse body fat distribution in childhood, but not with other cardio-metabolic risk factors.

The biological mechanisms linking maternal milk intake during pregnancy with offspring general and visceral body fat masses is not known. It has been suggested that milk intake increases concentrations of insulin, IGF-1, growth hormone, amino acids and fatty acids in the blood. In pregnant women, this might activate the nutrient sensitive kinase mTORC1 in the placenta. Activation of mTORC1 in the placenta might result in increased placental transfer of amino acids and glucose and subsequent fetal overnutrition, causing fetal mTORC overactivation and stimulation of anabolic processes, cell growth and adipogenesis in the fetus (41, 42). Overactivation of mTORC1 is associated with several cardio-metabolic diseases, including obesity, insulin resistance and type 2 diabetes (41, 43, 44). In addition, microRNAs that are present in milk involved in epigenetic upregulation of genes that are involved in the development of cardio-metabolic diseases might also play a role (45). Further research is needed on the mechanisms linking maternal milk intake during pregnancy to childhood body fat mass.

Conclusions

Both higher maternal caffeine intake during pregnancy and higher maternal milk intake during pregnancy are associated with higher childhood general- and abdominal adiposity. Further research is needed on whether optimizing intake of caffeine and milk during pregnancy is beneficial for reducing childhood adiposity and to define recommendations for caffeine and milk intake based on these long-term offspring associations.

Maternal adiposity

Both pre-pregnancy obesity and excessive gestational weight gain are important risk factors for adverse short- and long-term offspring health outcomes (46-56). We previously observed in the Lifecycle - Maternal Obesity and Childhood Outcomes collaboration that both pre-pregnancy overweight and obesity and excessive gestational weight gain have a high population impact on the risks of adverse pregnancy and birth outcomes, with population attributable risk fractions (PAR) ranging between 1.2% and 31.4%, depending on the outcome. Also, the associations of maternal body mass index and gestational weight gain with these short-term adverse outcomes were present across the full ranges of body mass index and gestational weight gain (57). In this thesis, we showed that both pre-pregnancy obesity and excessive gestational weight gain also have a considerable population impact with respect to the risk of offspring overweight and obesity throughout childhood, with PARs ranging between 10.2 and 21.6%. In addition, we observed that not only maternal overweight and obesity were associated with higher risks of overweight and obesity in childhood, but also that these risks were progressively higher among children of mothers with grade 1, 2, and 3 obesity, respectively. The risks of childhood overweight and obesity increased across the full ranges of maternal body mass index and gestational weight gain. This suggests that these risks are not confined to the extremes of maternal body mass index and gestational weight gain, but increase gradually over the ranges of maternal pre-pregnancy BMI and gestational weight gain. For prevention, insight into the combined effects of maternal body mass index and gestational weight gain is needed. We observed that maternal pre-pregnancy obesity was associated with the highest risks of both short-term maternal and infant outcomes and obesity throughout childhood. Gestational weight gain only added to a limited extend to these risks. This suggests that maternal body mass index might be a more important factor than gestational weight gain with respect to the risks of maternal and offspring adverse outcomes.

Thus far, it is unclear how much weight pregnant women should gain to minimize their risk of adverse pregnancy and offspring outcomes. The optimal gestational weight gain reflects a trade-off between outcomes occurring at both low and high levels of gestational weight gain. Previous studies aimed to identify the optimal gestational weight gain, but these studies differed considerably in study populations, statistical approaches and included

outcomes, and the acquired optimal gestational weight gain ranges varied across studies (58-65). Despite these methodological issues, five of these previous studies were included for the construction of the current guidelines of the US Institute of Medicine (IOM; currently known as the National Academy of Medicine). These guidelines are criticized because of the non-systematic approach, lack of inclusion of maternal pregnancy complications and no consideration of obesity severity (63, 66). Only one previous study among 120251 obese US women defined separate optimal weight gain ranges for obesity grade 1 (4.5-11.3 kg), obesity grade 2 (0-4.1 kg) and obesity grade 3 (<4 kg weight loss), based on four outcomes, including preeclampsia, caesarean delivery, small size for gestational age at birth and large for size gestational age at birth, using data from term births only (63). In this thesis we identified ranges of optimal gestational weight gain associated with the lowest risk of maternal and infant outcomes. In line with previous studies, the optimal gestational weight gain in our study was lower for women with a higher pre-pregnancy BMI. Optimal gestational weight gain further decreased with increasing obesity grades and might even involve weight loss for severely obese women. However, this result needs be interpreted with caution as the safety and effectiveness of weight loss during pregnancy remain to be assessed. In addition, we observed that weight gain outside both the ranges defined in our study and IOM ranges was associated with adverse maternal and infant outcomes, with generally stronger associations for the ranges defined in our study. However, both the ranges defined in our study and IOM ranges had limited ability to distinguish between those with and without adverse outcomes. This suggests that the optimal gestational weight gain ranges defined in this study may be informative for preconception counselling, but also that gestational weight gain guidelines in general might not be useful for individual risk prediction.

The mechanisms underlying the associations of maternal weight before and during pregnancy with offspring outcomes are not fully understood. The observed associations might be explained by genetics (67, 68), estimations of the heritability of obesity go up to 85% (69). The remainder might be explained by developmental programming mechanisms or environmental and lifestyle characteristics shared by mother and child. The fetal over-nutrition hypothesis suggests that increased exposure to several types of nutrients in children of women with obesity or excessive gestational weight gain may lead to persistent adaptations in the structure and function of adipose tissue, appetite regulation and energy metabolism, leading to an increased susceptibility to offspring adiposity (70-72). Also, epigenetic processes may play an important role in these mechanisms (73-75). Increasing evidence suggests that maternal adiposity already exerts effects before conception. Increased concentrations of pro-inflammatory cytokines, hormones and metabolites may accumulate in the ovarian follicular fluid and subsequently affect oocyte maturation and reduce embryo quality by metabolic, mitochondrial and chromosomal alterations (76).

Conclusions

Maternal pre-pregnancy body mass index and gestational weight gain are important risk factors for adverse pregnancy and birth outcomes and childhood overweight and obesity. The optimal gestational weight gain associated with the risks pregnancy and birth outcomes was lower for higher pre-pregnancy body mass indexes. However, the predictive ability of these optimal weight gain ranges was limited. These results suggest that maternal weight before pregnancy might be more important than weight gain during pregnancy with respect to the risks of adverse pregnancy and birth outcomes and childhood overweight and obesity. Also, gestational weight gain guidelines might not be useful for individual prediction of the risks of adverse pregnancy and birth outcomes.

Maternal and childhood metabolism

Several possible mechanisms underlying the associations of adverse exposures in early life with later obesity and cardio-metabolic disease have been proposed, which include, but are not limited to, epigenetics, adaptations in placental function, and hormonal and metabolic changes (77, 78). The possible underlying mechanisms specific for maternal adiposity and dietary factors have been described above. In this thesis, we focused in more detail on potential metabolic mechanisms underlying the early origins of cardio-metabolic disease.

Conventional biomarkers of metabolic status

Thus far, studies have mainly focused on conventional biomarkers of metabolic status, such as glucose, insulin and lipid concentrations. These studies suggest that higher concentrations of these biomarkers, already from early pregnancy onwards, are associated with altered fetal and early postnatal growth trajectories and increased risks of obesity and associated cardio-metabolic diseases (79-82). In this thesis, we observed that maternal glucose concentrations already in early pregnancy were associated with lower HDL cholesterol and higher glucose concentrations in childhood, whereas maternal insulin concentrations were associated with a higher body mass index, systolic blood pressure, total fat mass, android to gynoid fat mass ratio, subcutaneous fat mass and insulin levels in childhood. All associations, except those for childhood glucose and insulin levels, were explained by maternal pre-pregnancy BMI. In addition, adverse exposures in early life might also influence offspring metabolic profiles. For instance, differences in fetal and postnatal growth patterns are related to adverse metabolic profiles and increased risks of cardio-metabolic disease in later life. Previous studies have shown that children born with a low birth weight are at increased risk of developing cardio-metabolic disease in later life (9-12). On the other side of the spectrum, children born with a high birth weight are also at risk of these diseases (9-12). However, in this thesis we observed that children with relatively high insulin and c-peptide concentrations in childhood, do not grow differently in fetal life, but have higher weights and body mass indexes in childhood, as

compared to children with lower insulin and c-peptide concentrations. This might suggest a strong influence of postnatal factors, such as childhood body composition.

Metabolomics

Detailed characterization of metabolic status by metabolomics approaches may provide additional insights in the mechanisms that link early life adverse exposures to later cardio-metabolic disease. Several, mostly small, studies have reported associations of amino acids, fatty acids, acyl-carnitines, several lipid species and vitamins in pregnancy (83-89) and at birth (90-98) with (gestational age adjusted-) birth weight. Only two studies assessed directly measured fetal growth and observed associations of maternal urinary branched-chain amino acid concentrations, taurine, histidine and malonate and fetal weight change between 12 and 34 weeks of gestation and fetal anterior abdominal wall width at 34 weeks of gestation (88, 89). In this thesis, we compared concentrations of amino acids, non-esterified fatty acids, phospholipids, and carnitines in pregnant women, newborns and children. In line with previous studies (99-101), concentrations of amino acids, non-esterified fatty acids and acyl-carnitines were higher in cord blood as compared to maternal early-pregnancy blood, whereas concentrations of phospholipids were higher in maternal early-pregnancy blood. The higher amino acids, non-esterified fatty acids, carnitines in cord blood are indicative of an active transport of these metabolites across the placenta, whereas polar lipids get hydrolyzed to fatty acids before crossing the placenta (99-101). Concentrations of amino acids and non-esterified fatty acids were lower in childhood as compared to cord blood samples, whereas concentrations of phospholipids and carnitines were higher in childhood. Despite these higher exact concentrations, correlations of individual metabolites between pregnant women and their children were relatively low, suggesting that distinct metabolite profiles exist.

In addition, we assessed the associations of maternal and newborn metabolite concentrations with fetal growth from first trimester onwards and the risks of adverse birth outcomes. We observed a few associations of maternal phospholipids, particularly acyl-alkyl-phosphatidylcholines, with femur length and head circumference in third trimester and weight and head circumference at birth. No associations were present for other metabolites or with earlier fetal growth measures, suggesting that maternal early-pregnancy metabolite concentrations are only to a very limited extend related to fetal growth. Contrary, newborn metabolite profiles at birth, mainly concentrations of non-esterified fatty acids and acyl-lyso-phosphatidylcholines, were strongly associated with weight, length and head circumference at birth and the risks of SGA and LGA. Concentrations of several diacyl-phosphatidylcholines, acyl-alkyl-phosphatidylcholines, sphingomyelines, amino acids and acyl-carnitines were associated with the risk of pre-term birth, suggesting that suboptimal size at birth and pre-term birth are characterized by distinct cord blood metabolite profiles. Thus, results from this thesis suggest that cord blood metabolite profiles are strongly associated with growth measures at

birth and the risks of adverse birth outcomes. However, it should be noted that as a result of the cross-sectional design of these analyses we are not able to draw conclusions on the direction of these associations.

Conclusions

Maternal and newborn metabolism might influence offspring growth and cardio-metabolic outcomes in later life. Whether the maternal and offspring metabolite concentrations serve as intermediates in the pathways linking maternal body mass index and other lifestyle related exposures to childhood obesity and cardio-metabolic risk factors remains to be studied.

RELEVANCE AND IMPLICATIONS FOR POLICY AND CLINICAL PRACTICE

The results from this thesis might have implications for policy and clinical practice. Given the high prevalence of childhood obesity and associated cardio-metabolic disease, it is important to effectively target factors related to these diseases. Results from this thesis suggest that in addition to adverse birth outcomes, maternal caffeine and milk intake during pregnancy might influence long-term offspring body fat distribution. Current recommendations for maternal caffeine intake during pregnancy are based on the risks of adverse pregnancy and birth outcomes and range between a maximum of 200 and 300 mg per day (102-104). Currently, no specific recommendations of maternal milk intake during pregnancy exist. Women of reproductive age are generally advised to consume 2-3 glasses of milk or milk products per day, although differences in included products and portion sizes exist between countries (105-107). Results of our and previous studies suggest that associations with childhood outcomes might already be present for lower intakes. Future studies should confirm whether optimizing caffeine or milk intake during pregnancy is beneficial for reducing childhood adiposity before these results could be incorporated in guidelines. However, given the considerable amount of women with caffeine and milk intakes above the current recommendations, awareness to the health effects of these factors and adherence to the current recommendations could be increased.

The studies described in this thesis suggest that the preconception period provides an important opportunity for prevention of offspring short- and long-term adverse outcomes. We therefore strongly suggest that prevention strategies focus on optimizing weight and diet before conception. We acknowledge that this is challenging because women planning to become pregnant are generally not in clinical care and a large proportion of pregnancies is unplanned. Therefore, prevention strategies should both involve individual preconception counselling on a healthy weight before and during pregnancy in women that have a known

pregnancy wish by general practitioners and other health professionals and population-based approaches. Population approaches could involve increasing awareness about the importance of a healthy weight before and during pregnancy with respect to health consequences for both mother and child, or could be part of larger population-based health promotion strategies for the general population. Next to the preconception period, prevention strategies should target women during pregnancy with respect to dietary intake and weight. An advantage of targeting women during pregnancy is that they are easy to target and might be more motivated to make lifestyle changes. Our results suggest that optimal gestational weight gain guidelines are of limited importance with respect to individual prediction of adverse pregnancy and birth outcomes. However, gestational weight gain outside the guidelines was strongly associated with adverse pregnancy and birth outcomes at population level. Also, these results are limited to the outcomes that were included in the definition of the guidelines. Therefore, we consider it important to target and monitor weight gain in pregnant women at population level.

In addition, results from our thesis suggest that maternal and newborn metabolite profiles from metabolomics might influence offspring growth and cardio-metabolic outcomes in later life. Our results serve as a first step in understanding of the biological mechanisms underlying the associations of an adverse intrauterine environment with these outcomes. Therefore, these results are currently mainly of interest from etiological perspective, rather than for clinical practice. However, results from metabolomics studies might potentially be useful for the identification of biological biomarkers or individual risk prediction.

METHODOLOGICAL CONSIDERATIONS

Selection bias

Selection bias is a bias in effect estimates that may occur if the association between the exposure and outcomes of interest is different in those included in the study and those in the target population. Selection bias in cohort studies may arise at baseline, resulting from selective inclusion, or at follow-up, due to selective loss to follow up (attrition bias). Selection can either be differential or non-differential. Non-differential selection refers to the situation that non-participation is dependent on the exposure but independent of the outcome, or vice versa, and is not considered to bias effect estimates. Differential selection bias refers to non-participation that is related to both the exposure and outcome under study, and results in selection bias (108).

Most studies presented in this thesis were embedded in the Generation R Study. A total of 61% of all children that were eligible participated at birth. Compared to the general population of Rotterdam, women included in the study were less often from ethnic minority groups and of lower socio-economic status. Also, pregnancy and birth complications, including

gestational hypertensive disorders, preterm birth and low birth weight were less prevalent (109). This suggests selection towards a more healthy population of higher socioeconomic status. Given the prospective design of the study, it seems unlikely that this selection at baseline is differential and might have led to selection bias. However, it seems likely that this selective participation may have led to lower prevalence rates and reduced statistical power, and a limited generalizability to other populations. Of all children included in the study, about 67.5% and 57.4% participated in the follow-up body composition measurements at age 6 and 10 years, respectively. Reasons for loss to follow up were movement outside study area, non-consent, and death of the child (110). Participation rates were slightly lower for measurements involving venous punctures (44.6% and 39.9%), due to non-consent or unsuccessful venous punctures, and abdominal and liver fat measurements using magnetic resonance imaging at age 10 years (29.2%), due to non-consent. As compared to those lost to follow-up, those included in the analyses described in this thesis were generally higher educated, more often of European descent, and smoked less often. It is difficult to speculate whether this selection to a more healthy, higher educated study population has led to selection bias. It seems unlikely, as loss to follow up was not related to exposure status in most studies.

Two studies presented in this thesis were embedded in the LifeCycle - Maternal Obesity and Childhood Outcomes (MOCO) collaboration, a collaboration of 39 pregnancy and birth cohort studies. As the majority of the cohorts included was prospective population-based, we expect these studies to have selection patterns similar to those of the Generation R Study, and therefore consider the risk of selection bias within these studies small. Selection of studies to participate in the collaboration was based on participation in existing collaborations on childhood health and might not be completely at random. Also, of 50 cohorts invited for participation, 11 did not participate because they were not reached or did not share data. However, it seems very unlikely that non-participation of studies in the collaboration is related to the exposures and outcomes under study and has biased the results described in this thesis.

Information bias

Information bias, or misclassification, is a bias in effect estimates that arises from measurement errors of exposure and outcome measurements. Analogous to selection, misclassification can be either differential or non-differential. Differential misclassification refers to misclassification where the exposure status is dependent on outcome status, or vice versa, and can either result in an under- or overestimation of effect estimates. Non-differential misclassification refers to misclassification were exposure status does not depend on outcome status, or vice versa, and generally results in an underestimation of effect estimates (108).

In the studies described in this thesis, exposure data was collected longitudinally before assessment of the outcomes. Data collectors were blinded to the exposure status when collect-

ing data on the outcomes. Also, both participants and data collectors were not aware of the specific research questions under study. This makes bias due to differential misclassification unlikely. However, non-differential misclassification might be present and might have led to underestimated effect estimates. Fetal growth was assessed using ultrasound measurements in each trimester of pregnancy. Data on crown-rump length or biparietal diameter from the first ultrasound visit were used for pregnancy dating for the majority of women. Although this method is more accurate than pregnancy dating by last menstrual period, it neglects variation in early fetal growth. In offspring of these women, growth variation in second and third trimester might be underestimated. Part of the anthropometrics used for calculation of maternal body mass index, gestational weight gain and childhood body mass index came from self-report or parental report. Information on body mass index by self-report tends to be underestimated (111), which might have led to an underestimation of the effect estimates. Information on maternal caffeine and milk intake during pregnancy was obtained using (food frequency-) questionnaires. As these questionnaires rely on recall, it might be possible that some misclassification has occurred. For caffeine, it is likely that women underestimated their intakes, which might have led to an underestimation of the effect estimates. Milk intake could be either under- or overestimated, leading to an under- or overestimation of the effect estimates. Maternal and childhood blood samples were 30 minutes fasting or non-fasting. This may have resulted in non-differential misclassification and an underestimation of the effect estimates.

Confounding

Confounding is a bias in effect estimates that occurs when the exposure of interest coincides with another factor, that is also related to the outcome but is not an intermediate in the association between exposure and outcome under study. Due to the observational nature, the results described in this thesis might be subject to confounding. The exposures assessed in this thesis, weight, diet and metabolic factors, are likely to cluster with each other, but also with other socio-economic and lifestyle related factors, such as ethnicity, education, physical activity, smoking and alcohol consumption. We had data on many of these factors available, which enabled us to adjust our analyses for many possible confounders. These possible confounders were selected based on prior knowledge from previous studies, their associations with exposures and outcomes of interest, or a change of in effect estimates of more than 10%. Adjustment for confounders only changed the effect estimates slightly in most of the studies, suggesting that the influence of confounding factors is small. However, residual confounding might be present. This may either result from inaccurate adjustment for confounding factors due to measurement errors or from unmeasured or unknown confounders. For example, physical activity would be an important factor to take into account in many of the associations studied in this thesis, but this information was not available and might cause residual confounding.

Causality

The observational nature of this thesis precludes conclusions about causality of the associations assessed. However, from a clinical and preventive perspective, insight in whether the observed associations are causal is of great interest. Sir Bradford Hill's criteria can help assessing the causality of an observed association. The criteria for a causal relationship are: strength, consistency, specificity, temporality, biological gradient, plausibility, coherence, experiment and analogy. The effect estimates of the associations of maternal adiposity with childhood growth and body fat development are relatively large. However, the effect estimates of common maternal dietary factors with these outcomes are smaller. The results of the studies presented in this thesis are consistent with those of previous studies in humans and coherent with the results of animal studies. Exposure assessment took place before assessment of the outcomes and dose-response effects were observed for most of the associations assessed. Plausible potential mechanisms have been suggested for the associations assessed, although some need to be confirmed in further research. Although not assessed in this thesis, the associations might not be specific, as the exposures studied are likely to influence other outcomes. The experiment and analogy criteria were not assessed in this thesis. Thus, there seems to be some evidence for causality, but further studies are warranted to obtain more insight in this.

FUTURE RESEARCH

In this thesis, we described associations of maternal common dietary factors and adiposity with offspring growth, body fat development and cardio-metabolic risks factors as well as potential underlying metabolic mechanisms. However, there are several factors that could be improved and clarified in future studies.

General design

The studies in this thesis are based on results from observational pregnancy and birth cohort studies with follow-up until age 10 years (The Generation R Study) and 18 years (MOCO collaboration). Future studies in other populations should confirm and extend the findings presented in this thesis. These future studies should extend beyond the follow-up durations of the studies in this thesis, as associations might become more apparent at later ages. As results from this thesis as well as from other studies strongly suggest that the preconception period is important for offspring health, future cohort studies should already start before pregnancy. Also, follow-up of multiple generations is of interest to assess whether the intergenerational effects of adverse exposures in early life, for instance the intergenerational cycle of obesity. These studies would ideally include repeatedly assessed information on exposure and outcome data, enabling the assessment of temporal relationships and patterns over

time. In addition, efforts should be made to study relatively rare populations, such as severely obese women and the safety and effectiveness of weight loss during pregnancy in these women.

A number of factors could be improved in data collection in future studies. First, maternal pre-pregnancy body mass index, gestational weight gain and childhood body mass index were partly self- or parent reported, which may have potentially induced non-differential misclassification. Measuring these anthropometrics might reduce this risk. More precise measures of maternal fat mass before and during pregnancy as well as its location will be of interest to obtain further insight into the effects of maternal body composition. It is recognized that measuring fat mass by methods such as dual-energy x-ray absorptiometry and magnetic resonance imaging in pregnant women is not feasible in observational studies due to safety concerns. However, alternative methods such as abdominal ultrasounds early in pregnancy, skinfold thicknesses and bioelectrical impedance might already provide important information. Third, in the studies described in this thesis, we only had data available on caffeine intake from coffee and tea. Although at the time of data collection 80% of all caffeine intake was from coffee and tea (112), future studies should also obtain data on other common caffeine sources, such as cola, energy drinks, chocolate and medications. Maternal caffeine intake during pregnancy and maternal milk intake were assessed using questionnaires. An alternative to assessing caffeine intake by questionnaires is measuring urine concentrations of paraxanthine, which is the primary metabolite of caffeine. This approach is more objective and avoids problems as underreporting and recall bias, but depends on differences in metabolism and excretion rates between participants. Questionnaires for assessing caffeine (regular questionnaire) and milk intake (FFQ) were based on recall about the last 3 months. An alternative to FFQ's would be 24-hours recalls, that are more precise and less prone to recall bias and social desirable answers, but reflect the intake of one specific day rather than habitual intakes. Fourth, 30-minutes fasting or non-fasting blood samples were used and no information was available on the time of the last meal. Use of fasting blood samples would avoid misclassification due to food intakes before blood draw. Also, detailed information on duration and intensity of physical activity needs to be obtained.

Causality

In this thesis, we described associations of common maternal dietary factors and maternal adiposity with offspring growth and development. Drawing conclusions on causality based on observational data is difficult. Despite the fact that we adjusted our analyses for many possible confounding factors and the fact that there is some evidence for causality based on the Bradford Hill criteria, further research is needed to establish causality of these associations.

The gold standard for assessing causality are randomized controlled trials. By randomizing participants to be part of either an intervention or a control group, differences in outcomes

can be attributed to the interventions, rather than confounding factors. In addition, these studies would also provide insights in the effectivity of these interventions and the timing of these interventions. With respect to maternal adiposity, randomized controlled intervention trials have been performed to reduce gestational weight gain in second and third trimester. These intervention trials, focused on improving diet and physical activity, have been moderately successful in reducing gestational weight gain (113-116). However, these interventions did not show any effect on offspring outcomes, such as gestational age at birth, birth weight, or childhood weight (113-116). Results from this thesis suggest that pre-pregnancy weight is a more important determinant of offspring adverse outcomes than gestational weight gain. Thus far, interventions that aim to reduce pre-pregnancy weight are scarce (117). A few intervention studies targeted pre-conception lifestyle, such as alcohol intake, smoking, nutrition (mainly micronutrients), and showed small effects on the risks of adverse birth outcomes, but whether these effects were through pre-pregnancy weight remains unclear (118-120). Thus, randomized controlled interventional trials targeting maternal weight in the preconception period are needed. With respect to maternal caffeine and milk intake, intervention studies would be focused on optimizing intakes of caffeine and milk. Thus far, only one intervention study focused on reducing caffeine intake during pregnancy has been performed. A study from Denmark randomized 1207 pregnant women to replace their usual coffee with either caffeinated instant coffee or decaffeinated instant coffee, but did not show any effect on birth weight or length of gestation (121). Further intervention trials focused on restricting caffeine intake during pregnancy are needed to confirm these findings and to assess the long-term offspring health effects. To the best of our knowledge, no intervention trials focused on maternal milk intake during pregnancy have been performed.

In addition, observational study designs that deal with confounding in a more natural way could help in assessing causality. These study designs include parent-offspring comparison studies, sibling comparison studies and Mendelian Randomization studies. Parent-offspring comparison studies compare the strength of maternal-offspring associations with the strength of paternal offspring associations. Stronger maternal-offspring associations than paternal-offspring associations might suggest that the observed associations can at least partly be explained by intra-uterine programming 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. This approach has been performed for pre-pregnancy body mass index and caffeine intake (26, 122), but results need to be confirmed. Sibling comparison studies compare siblings within families, and therefore control for family based characteristics and genetics. Sibling-comparison studies have been performed with respect to maternal weight before or during pregnancy, and are suggestive of a causal relationship (123-127). However, an important limitation of this study design is that it assumes these family based characteristics to be constant over time, whereas these might change along with the exposures under study (128). Mendelian Randomization studies use a genetic vari-

ant known to be strongly associated with the exposure and not affected by confounding as instrumental variable for the exposure, in order to assess whether the exposure is causally related to the outcome (129). Mendelian Randomization Studies have been performed with respect to the associations of maternal obesity with offspring birth weight and obesity, but results are conflicting (68, 130, 131). Sibling-comparison studies and Mendelian Randomization studies regarding the offspring effects of maternal caffeine intake during pregnancy remain to be performed.

Underlying mechanisms

Unraveling the mechanisms underlying the associations between adverse exposures in early life and health and disease in later life is important to design effective interventions interfering with these mechanisms. In this thesis, we focused on the potential underlying metabolic mechanisms, by looking at maternal, newborn and childhood metabolic profiles. We also showed newborn, and to a much smaller extent maternal, metabolite profiles associated with fetal growth measures. Futures studies should focus on the associations of these metabolomics data with early life adverse exposures and outcomes both short- and long-term. Also, mediation analyses addressing the potential mediating role of metabolites in the associations of early life exposures with the outcome are of interest. We applied a targeted metabolomics approach, which is an efficient and straightforward hypothesis-driven approach that provides exact metabolite concentrations, but might miss important metabolites and pathways (132). Untargeted metabolomics approaches might provide more detailed insights in the metabolic pathways under study. We measured metabolite concentrations in blood serum. Other biological samples, such as plasma, urine, saliva, or breast milk may show different metabolic profiles and might therefore provide additional insights. Next to metabolic mechanisms, other mechanisms could be involved. These mechanisms include, but are not limited to, hormonal changes (alterations in HPA and satiety axes), genetics and changes in gene expression (epigenetic mechanisms), and changes in the microbiome. Further studies are needed to address potential underlying mechanisms as well as their interrelationships, by for example multi-omics approaches (133).

CONCLUSIONS

Maternal common dietary factors and maternal adiposity are associated with offspring growth and body fat development. Changes in metabolite profiles might underlie these associations.

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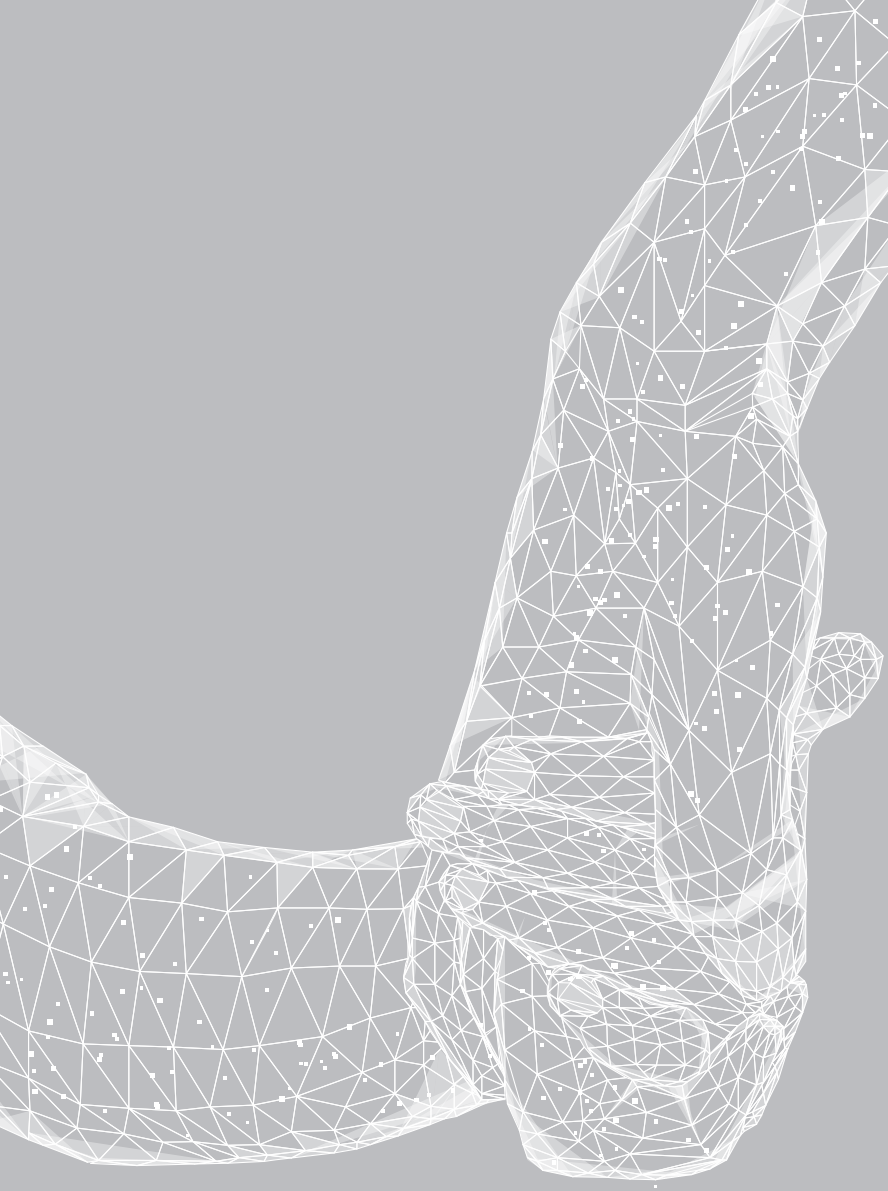


6 Summary Samenvatting

SUMMARY

Chapter 1 describes the background and rationale for the studies presented in this thesis. Childhood overweight and obesity are a major public health problem. Children with overweight and obesity are at risk of a variety of adult diseases, such as overweight and obesity in adulthood, cardio-metabolic disease, asthma, osteoarthritis, mental health problems and premature death. The etiology of obesity and cardio-metabolic disease is complex and multifactorial. It has been suggested that, in addition to well-known risk factors such as genetic predisposition, excess energy intake, and sedentary behavior, susceptibility to these diseases might already be established in early life. This might be reflected by different growth patterns from fetal life onwards. Identifying the factors related to adverse growth patterns and body fat development as well as the underlying mechanisms will broaden the understanding of the early origins of disease and is vital to effectively target interventions aiming to reduce the burden of these diseases. Therefore, the general objective of this thesis was to assess the associations of common maternal dietary factors and maternal adiposity with growth, body fat development and cardio-metabolic risk factors in children, as well as the metabolic mechanisms that potentially underlie these associations. The studies presented in this thesis used data from the Generation R Study, a population-based cohort study from fetal life onwards in Rotterdam, the Netherlands, and the LifeCycle – Maternal Obesity and Childhood Outcomes (MOCO) collaboration, an international collaboration of 39 pregnancy and birth cohort studies from Europe, North-America and Australia.

Chapter 2 describes studies on the influence of common maternal dietary factors on childhood growth, adiposity and cardio-metabolic risk factors. In **Chapter 2.1,** we examined the associations of maternal caffeine intake during pregnancy with childhood growth patterns from birth until the age of 6 years and general adiposity at age 6 years. We showed that a high maternal caffeine intake tended to be associated with a lower birth weight, a higher weight gain from birth until the age of 6 years and a higher body mass index from 6 months to 6 years, and a higher total body fat mass and android to gynoid fat mass ratio at the age of 6 years. This suggests that maternal caffeine intake is related to total body fat mass and a central body fat distribution in childhood. In **Chapter 2.2**, we showed that these associations were also present at the age of 10 years. In addition, higher maternal caffeine intake during pregnancy was associated with higher abdominal subcutaneous and visceral fat masses and a higher liver fat fraction at the age of 10 years. The associations of abdominal visceral fat mass and liver fat fraction were independent of child's concurrent total body fat mass, suggesting that maternal caffeine intake might differentially affect visceral and liver fat accumulation. In **Chapter 2.3** we assessed the associations of maternal milk intake during pregnancy with childhood general- and organ fat measures and other cardio-metabolic risk factors. We observed that a high maternal milk intake during pregnancy was associated with higher childhood body mass index, total body fat mass, lean mass, android to gynoid

fat mass ratio, abdominal visceral fat mass and a higher risk of overweight/obesity at age 10 years. No consistent associations were observed for pericardial fat, liver fat, blood pressure, lipids, insulin or glucose concentrations. Thus, results from this thesis suggest that maternal milk intake during pregnancy seems to be associated with an adverse body fat distribution in childhood, but not with other cardio-metabolic risk factors.

Chapter 3 describes studies on the influences of maternal adiposity before and during pregnancy on offspring adiposity at birth and in childhood. In **Chapter 3.1** we assessed the separate and combined associations of maternal pre-pregnancy body mass index and gestational weight gain with the risks of overweight/obesity throughout childhood and their population impact. We observed that not only maternal pre-pregnancy overweight and obesity are associated with an increased risk of childhood overweight, but that these risks increase gradually across the full range of maternal pre-pregnancy body mass index. Similarly, the risk of childhood overweight/obesity increased across the full range of gestational weight gain. The additional effect of excessive gestational weight gain on the risk of childhood overweight was small among women who are already overweight or obese before pregnancy. In **Chapter 3.2**, we identified ranges of optimal gestational weight gain associated with the lowest risk of maternal and infant adverse outcomes. These ranges were lower for women with a higher pre-pregnancy body mass index. The optimal gestational weight gain decreased with increasing severity of obesity. We also observed that weight gain outside both the ranges defined in our study and the existing guidelines from the US Institute of Medicine (IOM) was associated with adverse maternal and infant outcomes, with generally stronger associations for the ranges defined in our study. However, both the ranges defined in our study and from the IOM had limited ability to distinguish between those with and without adverse outcomes. This suggests that the optimal gestational weight gain ranges defined in this study may be informative for preconception counselling, but also that gestational weight gain guidelines in general might not be useful for individual risk prediction.

Chapter 4 describes studies on the potential metabolic mechanisms linking adverse exposures in early life to later obesity and cardio-metabolic disease. In **Chapter 4.1** we showed that maternal glucose concentrations already in early pregnancy were associated with lower HDL cholesterol and higher glucose concentrations in childhood, whereas maternal insulin concentrations were associated with a higher body mass index, systolic blood pressure, total body fat mass, android to gynoid fat mass ratio, subcutaneous fat mass and insulin levels in childhood. All associations, except those for childhood glucose and insulin levels, were explained by adjustment for maternal pre-pregnancy BMI. In **Chapter 4.2** we aimed to identify critical periods and specific growth patterns from fetal life onwards associated with childhood insulin levels. We showed that, independent of growth in other time intervals, weight growth from 6 months onwards and body mass index growth from 24 months onwards were positively associated with childhood insulin levels, with the strongest associations at the age of 72 months. As compared to children in the lowest quartile of childhood

insulin, those in the highest quartile had a higher length from birth onwards and a higher weight and body mass index from 24 months onwards. These differences increased with age. No associations were observed for fetal growth characteristics. Thus, results from this thesis suggest that rapid length, weight and body mass index growth in childhood, but not during fetal life, is associated with higher insulin levels in childhood. In **Chapter 4.3** we used metabolomics analyses to describe the metabolite profiles in pregnant women, newborns and children as well as their interrelationships. We observed that metabolite concentrations vary considerably between pregnancy women and their children at birth and at the age of 10 years. Correlations of individual metabolites between pregnant women and their children at birth and in childhood are relatively low. This may suggest that unique metabolic profiles are present among pregnant women, newborns and school-aged children, with limited intergenerational correlations between metabolite profiles. In **Chapter 4.4** we examined whether maternal early-pregnancy and newborn metabolite profiles are associated with fetal growth from first trimester onwards and the risks of adverse birth outcomes. We observed only a few associations of maternal phospholipids, particularly acyl-alkyl-phosphatidylcholines, with femur length and head circumference in third trimester and weight and head circumference at birth. Contrary, newborn metabolite profiles at birth, mainly concentrations of non-esterified fatty acids and acyl-lysophosphatidylcholines, were strongly associated with weight, length and head circumference at birth and the risks of SGA and LGA. Thus, results from this thesis suggest that maternal early-pregnancy metabolite are only to very limited extend related to fetal growth, whereas cord blood metabolite profiles are strongly associated with growth measures at birth and the risks of adverse birth outcomes.

Finally, in **Chapter 5** a general discussion of the main findings of this thesis, general methodological considerations and suggestions for future research are provided. In conclusion, maternal common dietary factors and maternal adiposity are associated with offspring growth and body fat development. Changes in metabolite profiles might underlie these associations.

SAMENVATTING

Hoofdstuk 1 beschrijft de achtergrond en rationale voor de studies beschreven in dit proefschrift. Overgewicht en obesitas bij kinderen vormen een groot probleem voor de volksgezondheid. Kinderen met overgewicht en obesitas hebben een hoog risico op ziekten op latere leeftijd, zoals overgewicht en obesitas, cardio-metabole ziekten, astma, osteoartritis, mentale gezondheidsproblemen en vroegtijdig overlijden. De etiologie van obesitas en cardio-metabole ziekten is complex en multifactorieel. Naast bekende risicofactoren als genetische aanleg, overmatige energie inname and sedentaire leefstijl, lijkt ook het vroege leven een rol te spelen in de ontwikkeling van deze ziekten. Dit zou tot uiting kunnen komen door middel van afwijkende groeipatronen al vanaf het foetale leven. Het identificeren van factoren gerelateerd aan afwijkende groeipatronen en de ontwikkeling van lichaamsvet, alsmede de biologische mechanismen die hieraan ten grondslag liggen, zal bijdragen aan een beter begrip van de vroege oorsprong van ziekten en is belangrijk voor de ontwikkeling van interventies gericht op het verminderen van het vóórkomen van deze ziekten. Het doel van dit proefschrift was dan ook het bestuderen van de associaties van veel voorkomende voedingscomponenten en maternale adipositas met groei, de ontwikkeling van lichaamsvet en cardio-metabole risicofactoren bij kinderen, alsmede de potentiële onderliggende metabole mechanismen. Voor de studies beschreven in dit proefschrift is gebruik gemaakt van data van de Generation R studie, een prospectieve cohort studie onder zwangere vrouwen en hun kinderen in Rotterdam, en LifeCycle – Maternal Obesity and Childhood Outcomes (MOCO), een internationale samenwerking tussen 39 zwangerschaps- en geboorte cohorten uit Europa, Noord-Amerika en Australië.

Hoofdstuk 2 beschrijft studies naar de invloed van door zwangere vrouwen vaak geconsumeerde voedingscomponenten, namelijk cafeïne en melk, op groei, adipositas en cardio-metabole risicofactoren bij hun kinderen. In **Hoofdstuk 2.1** hebben we de associatie van maternale cafeïne consumptie tijdens de zwangerschap met groeipatronen vanaf de geboorte tot de leeftijd van 6 jaar en algemene adipositas op de leeftijd van 6 jaar onderzocht. Resultaten van deze studie lieten zien dat een hogere maternale cafeïne consumptie was geassocieerd met een lager geboortegewicht, een hogere gewichtstoename vanaf de geboorte tot de leeftijd van 6 jaar, een hogere body mass index vanaf de leeftijd van 6 maanden tot 6 jaar en een hogere totale lichaamsvetmassa en een hogere ratio van androïde en genoïde vetmassa op de leeftijd van 6 jaar. Dit wijst erop dat cafeïne consumptie door zwangere vrouwen is gerelateerd aan totale lichaamsvetmassa en een centrale vetverdeling bij hun kinderen op de leeftijd van 6 jaar. **Hoofstuk 2.2** laat zien dat deze associaties ook bestaan op de leeftijd van 10 jaar. Daarnaast was een hogere maternale cafeïne consumptie tijdens de zwangerschap ook geassocieerd met hogere abdominale subcutane en viscerale vet massa's en een hogere lever vet fractie bij kinderen van 10 jaar. De associaties van abdominale viscerale vetmassa en lever vet fractie waren onafhankelijk van de totale vetmassa van

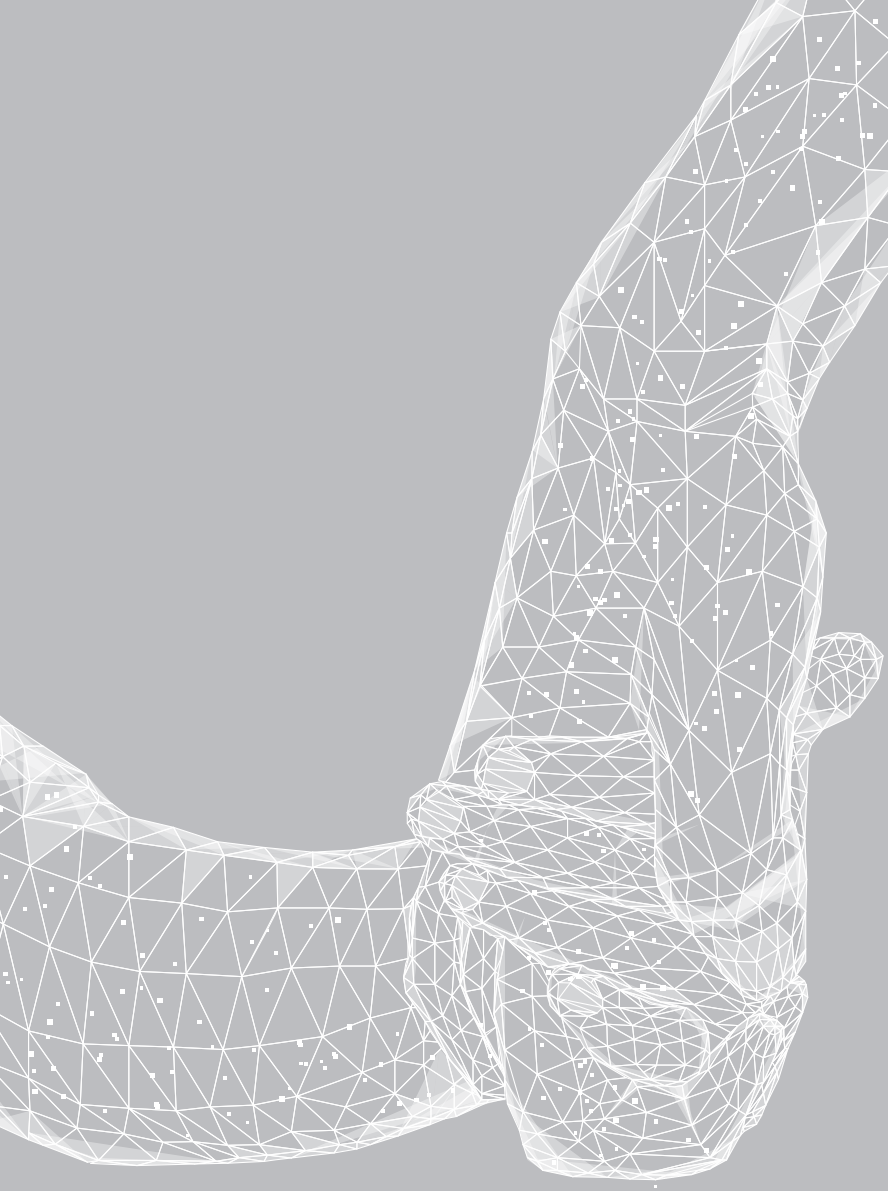
het kind. Dit zou kunnen betekenen dat cafeïne consumptie door zwangere vrouwen in het specifiek de ontwikkeling van visceraal en lever vet bij kinderen beïnvloedt. In **Hoofdstuk 2.3** hebben we de associatie tussen maternale melk consumptie met totaal lichaamsvet en orgaanvet bij hun kinderen onderzocht. In dit hoofdstuk hebben we laten zien dat een hoge maternale consumptie van melk tijdens de zwangerschap was geassocieerd met een hogere body mass index, totale lichaamsvetmassa, vetvrije massa, ratio tussen androïde and genoïde vetmassa, abdominale viscerale vetmassa en een hoger risico op overgewicht of obesitas bij kinderen van 10 jaar. Er waren geen consistente associaties met pericardiaal vet, lever vet fractie, bloeddruk, vetten, insuline of glucose concentraties. Samenvattend kunnen we stellen dat melk consumptie door zwangere vrouwen gerelateerd lijkt te zijn aan een nadelige vetverdeling bij hun kinderen, maar niet aan andere cardio-metabole risico factoren.

Hoofdstuk 3 beschrijft studies naar de invloed van maternale adipositas voor en tijdens de zwangerschap op adipositas bij haar kinderen tijdens de geboorte en in de kindertijd. In **Hoofdstuk 3.1** hebben we de individuele en gecombineerde associaties van maternale body mass index voorafgaand aan de zwangerschap en gewichtstoename tijdens de zwangerschap met het risico op overgewicht en obesitas tijdens de kindertijd onderzocht, alsmede de impact van deze associaties op populatieniveau. De resultaten van dit hoofdstuk laten zien dat niet alleen overgewicht en obesitas voorafgaand aan de zwangerschap zijn geassocieerd met een verhoogd risico op overgewicht bij kinderen, maar dat deze risico's geleidelijk stijgen naarmate de body mass index stijgt. Het risico op overgewicht bij kinderen steeg ook geleidelijk met stijgende gewichtstoename tijdens de zwangerschap. Het effect van overmatige gewichtstoename op het risico op overgewicht bij kinderen was beperkt voor vrouwen die al overgewicht of obesitas hadden voorafgaand aan de zwangerschap. In **Hoofdstuk 3.2** hebben we de optimale gewichtstoename tijdens de zwangerschap bepaald als de gewichtstoename met het laagste risico op nadelige zwangerschaps- en geboorte uitkomsten. De optimale gewichtstoename was lager voor vrouwen die een hogere body mass index hadden voorafgaand aan de zwangerschap. De optimale gewichtstoename was ook lager voor vrouwen met ernstigere obesitas. Zowel gewichtstoename buiten de in dit proefschrift gedefinieerde categorieën als buiten de bestaande richtlijnen van het instituut der geneeskunde (IOM) in de Verenigde Staten was geassocieerd met nadelige zwangerschaps- en geboorte uitkomsten. Deze associaties waren over het algemeen sterker voor de categorieën bepaald in dit proefschrift. Zowel de in dit proefschrift gedefinieerde categorieën als die van het IOM waren echter maar beperkt in staat om onderscheid te maken tussen vrouwen met en zonder nadelige uitkomsten. Dit wijst erop dat de optimale gewichtstoename bepaald in dit proefschrift informatief kan zijn voor preconceptionele counseling, maar dat richtlijnen voor gewichtstoename tijdens de zwangerschap in het algemeen waarschijnlijk niet informatief zijn voor risicopredictie op individueel niveau.

Hoofdstuk 4 beschrijft studies naar de metabole mechanismen die onderliggend zouden kunnen zijn aan de associaties tussen blootstelling aan nadelige factoren vroeg in het leven

en obesitas en cardio-metabole ziekten later in het leven. In **Hoofdstuk 4.1** hebben we laten zien dat maternale glucose concentraties in de vroege zwangerschap geassocieerd waren met een lager HDL cholesterol en hogere glucose concentraties bij kinderen. Maternale insuline concentraties waren geassocieerd met een hogere body mass index, systolische bloeddruk, totale lichaamsvet massa, ratio van androïde tot genoïde vetmassa, subcutane vetmassa en insuline concentraties bij kinderen. Alle associaties, behalve die voor glucose en insuline concentraties bij het kind, konden worden verklaard door verschillen in maternale body mass index voorafgaand aan de zwangerschap. Het doel van **Hoofdstuk 4.2** was om perioden en groeipatronen geassocieerd met insuline concentraties bij kinderen te identificeren. Resultaten van dit hoofdstuk laten zien dat, onafhankelijk van groei in andere perioden, gewichtstoename vanaf de leeftijd van 6 maanden en body mass index toename vanaf de leeftijd van 24 maanden geassocieerd waren met insuline concentraties bij kinderen, met de sterkste associaties op de leeftijd van 72 maanden. Vergeleken met kinderen in het laagste kwartiel van insuline, waren kinderen in het hoogste kwartiel langer vanaf de geboorte en hadden een hoger gewicht en een hogere body mass index vanaf de leeftijd van 24 maanden. Deze verschillen werden groter naarmate het kind ouder werd. Er waren geen associaties voor foetale groei. Deze resultaten wijzen erop dat een snelle groei in lengte, gewicht en body mass index in de kindertijd, maar niet tijdens het foetale leven, geassocieerd is met hogere insuline concentraties bij kinderen. In **Hoofdstuk 4.3** hebben we gebruik gemaakt van metabolomics data om de metaboliet profielen van zwangere vrouwen en hun kinderen bij de geboorte en op de leeftijd van 10 jaar te beschrijven, alsmede de relaties tussen deze profielen. Resultaten van dit hoofdstuk laten zien dat metaboliet concentraties variëren tussen de verschillende tijdspunten. De correlaties van de individuele metabolieten tussen de zwangere vrouwen en hun kinderen waren relatief laag. Dit zou kunnen betekenen dat unieke metaboliet profielen bestaan voor zwangere vrouwen en hun kinderen bij de geboorte en op de leeftijd van 10 jaar, met beperkte correlaties tussen generaties. In **Hoofdstuk 4.4** hebben we onderzocht of maternale metaboliet profielen tijdens de vroege zwangerschap en metaboliet profielen van pasgeborenen gerelateerd zijn aan foetale groei vanaf het eerste trimester en het risico op nadelige geboorte uitkomsten. Resultaten van dit hoofdstuk laten zien dat maar enkele fosfolipiden, vooral alkyl-fosfolipiden, geassocieerd waren met dijbeenlengte en hoofdomtrek van het kind in het eerste trimester van de zwangerschap en gewicht en hoofdomtrek bij de geboorte. Metaboliet profielen van pasgeborenen, vooral niet-veresterde vetzuren en acyl-lysophosphatidylcholines, waren sterk geassocieerd met gewicht, lengte en hoofdomtrek bij de geboorte en met de risico's om zowel te klein als te groot geboren te worden voor de zwangerschapsduur. Dus, de resultaten van dit proefschrift wijzen erop dat de metaboliet profielen van de moeder tijdens de vroege zwangerschap maar in beperkte mate gerelateerd zijn aan foetale groei, terwijl metabolietprofielen van pasgeborenen sterk gerelateerd zijn aan maten van groei bij de geboorte en het risico op nadelige geboorte uitkomsten.

Als laatste geeft **Hoofdstuk 5** een algemene discussie van de belangrijkste bevindingen van dit proefschrift, methodologische overwegingen en suggesties voor verder onderzoek. Concluderend, door zwangere vrouwen vaak geconsumeerde voedingscomponenten en adipositas bij zwangere vrouwen zijn geassocieerd met groei, lichaamsvetonwikkeling en cardio-metabole risicofactoren bij hun kinderen. Veranderingen in metaboliet profielen zouden een onderliggend mechanisme kunnen zijn.



7

Publication list
About the author
PhD portfolio
Dankwoord

PUBLICATION LIST

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[#] *Denotes shared first authors*
** Denotes shared last authors*

ABOUT THE AUTHOR

Ellis Voerman was born on February 2nd 1992, in Alkmaar, the Netherlands. In 2014 she completed her Bachelor of Science in Health Sciences at VU University, Amsterdam, the Netherlands *cum laude*. In the same year, she started the Research Master of Science in Health Sciences, with specialization in Epidemiology at the Netherlands Institute of Health Sciences (NIHES) of the Erasmus University, Rotterdam, the Netherlands. She completed her master's thesis at the Generation R Study Group, Erasmus MC, Rotterdam, the Netherlands. After her graduation in 2016, she extended her research at the Generation R Study Group, Department of Pediatrics into the PhD project 'Diet, Adiposity and Metabolism in Pregnancy and Childhood' under supervision of Prof. dr. V.W.V. Jaddoe (Department of Pediatrics) and Dr. R. Gaillard (Department of Pediatrics). In the final year of her PhD project, she additionally worked as data manager for the international LifeCycle project. The results of her PhD project are presented in this dissertation.

PHD PORTFOLIO

Summary PhD training and teaching activities

Name PhD student:	Ellis Voerman
Erasmus MC Department:	Pediatrics
Research School:	Netherlands Institute for Health Sciences
PhD period:	September 2016 - March 2021
Promotor:	Prof.dr. V.W.V. Jaddoe
Co-promotor:	Dr. R. Gaillard

	Year	Workload (ECTS)
1. PhD training		
Master of Science in Health Sciences (Research), specialization Epidemiology, NIHES, Erasmus University Rotterdam, the Netherlands	**2014-2016**	
Core courses		
Study Design		4.3
Biostatistical Methods I: Basic Principles		5.7
Biostatistical Methods II: Classical Regression Models		4.3
Scientific Writing in English for Publication		2.0
Research Seminars		0.8
Development Research Proposal		2.5
Oral Research Presentation		1.4
Required courses		
Methodologic Topics in Epidemiologic Research		1.4
Principles of Research in Medicine and Epidemiology		0.7
Methods of Public Health Research		0.7
Introduction to Global Public Health		0.7
Primary and Secondary Prevention Research		0.7
Social Epidemiology		0.7
Fundamentals of Medical Decision Making		0.7
Pharmaco-epidemiology and Drug Safety		1.9
Advanced Topics in Clinical Trials		1.9
Advanced Analysis of Prognosis Studies		0.9
Principles of Epidemiologic Data-analysis		0.7
Elective courses		
Clinical Epidemiology		5.7
Epidemiology of Infectious Diseases		1.4
Repeated Measurements in Clinical Studies		1.4
Psychiatric Epidemiology		1.1

Cancer Epidemiology		1.4
Missing Values in Clinical Research		0.7
Women's Health		0.9
Health Economics		0.7
Methods of Health Services Research		0.7
Causal Mediation Analysis		0.7
Survival Analysis for Clinicians		1.9
Maternal and Child Health		0.9
Quality of Life Measurement		0.9
Courses for the Quantitative Researcher		1.4

Other courses

Instellingsgebonden regelgeving en stralingshygiëne niveau 5R, Erasmus MC, the Netherlands	2016	0.7
Research Integrity, Erasmus MC, the Netherlands	2017	0.3
Basic course on R, Postgraduate School Molecular Medicine, Erasmus MC, the Netherlands	2017	1.8
Metabolomics Data Processing and Data Analysis (online course), University of Birmingham, United Kingdom	2018	0.6

Seminars, congresses and presentations

Generation R Research meetings, Erasmus MC, the Netherlands	2016 - 2021	1.0
Maternal and Child Health meetings, Erasmus MC, the Netherlands	2016 - 2021	1.0
Developmental Origins of Health and Disease (DOHaD), Cape Town, South-Africa. *Oral presentation*	2015	1.4
Power of Programming Conference, Munich, Germany. *Poster presentations*	2016	0.7
Generation R Research meeting, Erasmus MC, The Netherlands	2017 + 2019	2.0
Developmental Origins of Health and Disease (DOHaD), Rotterdam, the Netherlands. *Oral presentation*	2017	1.4
Conference on Epidemiological Birth Cohorts and Longitudinal Studies, Oulu, Finland. *Poster presentation*	2018	0.7
Annual conference of the Metabolomics Society. *Poster presentation*	2019	0.7
LifeCycle project General Assembly meetings, Oulu, Finland, Rotterdam, the Netherlands	2018 + 2020	

Other

Vereniging Trustfonds Erasmus Universiteit Rotterdam, several travel grants	2015 - 2020
Reviewed articles for *Plos One, Eur J Epidemiol, Pediatr Obes*	2016 - 2020
Generation R general tasks, including PhD planning for data collection	2016 - 2017
Data manager LifeCycle project	2020 - 2021

2. Teaching

Rama Wahab, PhD student Generation R Study, Clinical Epidemiology, NIHES, the Netherlands. *Maternal glucose metabolism in early-pregnancy and cardio-metabolic risk factors in mid-childhood.*	2018 - 2019	2.0
Mirjam Hulst, Student Nutrition and Dietetics, The Hague, The Netherlands. *Maternal caffeine intake during pregnancy: Associations with offspring abdominal and organ-specific body fat development and awareness among health care professionals and pregnant women.*	2018 - 2019	2.0
Development R exercises Biostatistical Methods I, NIHES, The Netherlands	2018	0.5
Teaching assistant Biostatistical Methods I, NIHES, The Netherlands	2016 + 2018	0.5

DANKWOORD

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